MOLECULAR EPIDEMIOLOGY AND ANTIMICROBIAL SUSCEPTIBILITY OF THERMOPHILIC CAMPYLOBACTERS IN BROILER PRODUCTION IN MOROGORO MUNICIPAL, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF THE DEGREE OF MASTER OF SCIENCE IN ONE HEALTH MOLECULAR BIOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE, MOROGORO, TANZANIA.

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ABSTRACT

Since the discovered of *Campylobacter* in 1913, is known to cause diseases in human. Investigations were done in broiler poultry by culture method, biochemical molecular technique followed by antimicrobial susceptibility of thermophilic *Campylobacter sp* in Morogoro Municipal, Tanzania. The objectives of the study were to establish prevalence of thermophilic *Campylobacter* by molecular and biochemical approach followed by antimicrobial susceptibility at each stage of broiler production. Polymerase Chain Reaction (PCR) was used as the definitive identification method of *Campylobacter*. Out of 580 total samples 58.6% (340) were *Campylobacter* positive by culture. Among 340 isolates, 47.9% (163) were *Campylobacter coli,* 34.4% (117) were *Campylobacter jejuni* and 1.6% (17.6) were *Campylobacter jejuni/coli* with statistical significant P=0.0005. Biochemical test revealed that out of 340 samples, 36% (53) were *C. jejuni* and 64% (74) were *C. coli*. The levels of contamination of *Campylobacter* in feces and carcasses were 70.8% (241) and 23% (99) respectively with statistical significance difference of P=0.0001. Biosecurity and carcasses processing in different farms and stages (evisceration, rinsing, plucking) were found to be the factors for *Campylobacter spp* colonization and contamination between farms with statistical significance difference of P=0.00002. Antimicrobial susceptibility to Ciproflaxin, Tetracycline, Chloromphenical, Azithromycin, Ampicillin, Gentamycin, Nalidixic acid, Amoxycilin and Erythromycin were done on 53 *Campylobacter spp* isolates. High resistance of *Campylobacter spp* isolates was observed in Ciproflaxin and tetracycline with 58% (n=62) and 56% (n=59) respectively, while high sensitivity of *Campylobacter spp* to antibiotic was observed in Chloramphenicol and Azithromycin with 76% (n=81) and 69% (n=73) respectively. Finally it was observed that broiler have high contamination of *Campylobacter* spp and they have high resistance to most antibiotics.

DECLARATION

I, Festo Dominic Mkomba, do hereby declare to Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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LIST OF ABREVIATIONS AND SYMBOLS

Abbreviation	Descriptive meaning
AIDS	acquired immuno-deficiency syndrome
AMP	Ampicillin
AMX	Amoxyclin
AZM	Azithromycine;
Bp	base pair
CFU/ml	Colon forming unit per millitre
CI	confidence interval
Cm	centimeter(s)
DNA	deoxyribonucleic acid
dNTP's	deoxyribonucleoside phosphate
e.g.	Example
et al.	and others
CHL	Chloramphenicol
CIP	Ciprofloxan
EDTA	ethylenediaminetetra acetic acid
ELISA	enzyme linked immuno-sorbent assay
ERY	Erythromycin
Gm	Gramme
GEN	Gentamycine
HCl	hydrochloric acid
HIV	Human immunodeficiency virus
i.e.	that is
IgA	immunoglobulin A

IgG	immunoglobulin G
IgM	immunoglobulin M
IU	International unit
KCl	Potassium chloride
Kg	kilogram(s)
Km	Kilometer
Ltd	Limited
M	Molar (concentration)
Mg	Milligram
mg/l	milligram per litre
ml	Millitre
MM	Millimole
Mm	Millimeter
N	number of samples
$^{\circ}$C	degree Celsius
P	probability value (for statistical significance)
PCR	polymerase chain reaction
Ph	hydrogen ion concentration
Pmoles	Picomoles
RAPD	Random amplified polymorphic DNA
REA	restriction enzyme analysis
RFLP	restriction fragment length polymorphism
RNA	Ribonucleic acid
rpm	revolution per minute

SUA	Sokoine University of Agriculture
TBE	Tris-HCL, boric acid and EDTA
TEMED	N,N,N',N',-tetramethylethylenediamine
U	Unit
UV	ultra violet
V	Volts
v/v	volume by volume
V/w	volume by weight
WHO	World Health Organization
X	Times
NAL	Nalidixic acid
TET	Tetracycline
<	less than
>	greater than
®	registered trade mark
%	Percent

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Campylobacter is a Greek word with 'Campylo' means curved and 'bacter' means rod. The organism were successfully cultured for the first time in 1913 by McFadyean and Stockman from the material of aborted ewes (Skirrow, 2006) and 50 years later (1963) the genus *Campylobacter* were established. But before the name of genus *Campylobacter, Campylobacter* were classified as vibrio species (Sebald and Veron, 1963; Moore *et al*., 2005). Because of the difficult in culturing and isolating bacteria from faecal samples, it was until in 1970s, *Campylobacters* were recognized as an enteric pathogen. The use of improved isolation methods in the culture of faecal samples of patients with enteric symptoms and molecular methods identified *Campylobacter jejuni* and *Campylobacter coli* as important causative agent of human enteric illness (Butzler *et al.*, 1973; Skirrow, 2006) and to date *Campylobacters* are worldwide recognized as a serious reason behind microorganism food-borne zoonotic diseases and major causative agent of bacterial gastroenteritis over the last 20 years (Rivoal *et al.*, 2005; WHO, 2010).

Epidemiological researches have shows that, broiler meat product consumed by human contribute to the high prevalence of human campylobacteriosis (Frost, 2001; Stern *et al*., 2003). In broiler campylobacteriosis does not show clinical sign but when transmitted to human, the diseases appear most in children, old and immunocompromised individuals (Coker *et al.*, 2002) with the incidence being higher than that of Salmonellosis and Shigellosis (Mäkelä *et al.,* 2011).

Transmissions of *Campylobacte*r spp. in broilers mostly occur through horizontal transmission and little by vertical transmission (Newell and Fearnley*,* 2003; Callicott *et al.,* 2006). Therefore horizontal transmission is considered the major route for colonization of housed broilers and is due to environmental where animals surrounding the farm, flies, broiler feed, broiler house water systems and feeding system are considered. Human transmission can be by handling and consumption of contaminated and improper cooked broiler product (Sheppard *et al*., 2009: Guerin *et al.*, 2007).

Campylobacter can be controlled at farm level and during broiler processing, at farm level the use of biosecurity (use of disinfectant before entering in poultry house, control of flies to enter in poultry house are most used). During processing minimize consumer exposure to *Campylobacter* from contaminated environment, good overall hygiene practice, educating the broiler meat vendors, washing carcasses with warm water and chilling of the poultry carcasses which aim in reducing the *Campylobacter* contamination level (Jun, 2009).

Campylobacter infection in human is normally self-limiting but antibiotics treatment is required in immunocompromised patients, severe diseases cases and prolonged *Campylobacter* infection (Allos, 2001). Treatment under these conditions (immunocompromised, prolonged infection and severe diseases cases) and the use of antibiotics in growth promotion, treatment and diseases control causes antimicrobial resistance burden to increase across different antibiotic. Also emergence of new clone's pathogens spreading among animals and human has made antimicrobial resistance as an issue of public health importance. Not only that but also the overuse of antibiotics in treatments of food animal and human have been exacerbated due to increased emergence of bacteria resistance to antibiotics (Kurinčič *et al.,* 2007).

Campylobacter resistance to various antibiotics is a public health concern (Payot *et al,* 2006) and is exacerbated by varying degree of resistance observed across various *Campylobacter* strains to antimicrobials (Ana and Sonja 2012; Wieczorek and Osek 2013).

1.2 Problem Statement and Justification of the Study

In Tanzania, especially in urban centres where the population densities are high, the demand for poultry products including broiler meat has proportionally increased. World health organization reports poultry are easily and accessible animal's protein meat, followed by pork and the third is beef meat and by 2020 more than 70% people will depend on poultry as the source of animal protein due to scarcity of pasture for ruminant as the results of minimal rainfall (WHO Technical Report Series, 2003). Small to large broiler production schemes escalation in many towns to meet the consumer demand has also led to massive increase in the use of antibiotics in these schemes. The use and misuse of antibiotics in Tanzania has been worsened by unregulated selling of substandard drugs mainly done by private veterinary center, poor extension services on broiler productin. Most poultry productions operation are done by unskilled individuals, using poorly built infrastructure and lack of basic biosecurity skills.

In developing countries like Tanzania, human population growth in urban and peri-urban, there are no efforts done to build infrastructure or provision of education which are given to improve farming management, improper way of using antibiotics, and hygiene in the broiler carcasses processing which results to carcasses contamination with zoonotic microbes like *Campylobacte*r, *Salmonella*, *and Proteus*. Not only that but also most animals that are kept by human like pet animals and crown shows high prevalence of *Campylobacter* (Mdegela *et al*., 2006) In broiler farming it is believed that colonization of

Campylobacter starts at different stage of broiler growth with each stage being different. Therefore this study intended to investigate broiler *Campylobacter* contamination, carcasses contamination during slaughter and extent of antibiotics resistance at each stage of broiler growth.

1.3 Study Objective

1.3.1 General study objective

To establish the prevalence and antimicrobial susceptibity of thermophilic *Campylobacter* in broiler poultry at different stages of broiler production

1.3.2 Specific objectives

a. To investigate the prevalence of thermophilic *Campylobacter* species at different stages of broiler production system using molecular approach and biochemical test.
b. To evaluate antimicrobial resistance profiles of *Campylobacter* strains isolated from different stages of broiler production.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Campylobacter*

Thermophilic *Campylobacter* are thermotolerant groups of bacteria which comprise of four species namely, *Campylobacter jejuni, Campylobacter coli, Campylobacter lari* and *Campylobacter upsaliensis.* Among these four species, *Campylobacter jejuni* and *Campylobacter coli* are commonly isolated species from humans with diarrhea (Kalupahana *et al*., 2013) and they accounts up to 95% of all human cases of campylobacteriosis (Koenraad *et al.*, 1995). *Campylobacter jejuni* and *Campylobacter coli* acquired their names from the pathological changes they cause on specific sites of the intestinal tract mucosa of cattle and pigs where *C. jejuni* causes enteritis on the jejunal mucosa of calves hence its name while *C. coli* cause colitis leading to dysentery in pigs (Nonga, 2005).

2.2 Pathogenesis

Campylobacter as other organisms has mechanism that makes them to survive in their host. Chemotaxis, motility and flagella are known to be essential factors in the virulence as they are required for attachment and colonization of intestinal epithelium where adhesion, invasion, toxin production, and subversion of host cell processes are common virulence tools of many enteric prokaryotic pathogens (Crushell *et al.*, 2004). Colonization of *Campylobacter* organisms disturb the normal absorptive processes in the intestine because there is desquamation of epithelium cells by either directly cell invasion, through toxin production, inflammatory response or other immune responses which results in diarrhea (Backert *et al*., 2013).

2.3 Antimicrobial Resistance Mechanisms

Repeated exposure of *Campylobacter jejuni* and *Campylobacter coli* to biocides and antimicrobial compounds results in partially increased tolerance where resistances developed is partially stable. There are several mechanism involved in resistance developments which are, active and adaptive efflux resistance to antibacterial. In active efflux resistance mechanisms, only one type of efflux pump in outer membrane protein are involved where adaptive efflux resistance more than one type of efflux pumps in outer membrane are involved. Adaptive efflux is the one responsible for adaptation of resistance to antibacterial (Ana and Sonja, 2012). Furthermore mutations and horizontal gene transfer causes bacteria to acquire antibiotic resistances (Summers, 2006). Mutations can either be point mutation, deletion mutation or insertion mutation. Example, point mutations in *rpoB*, and *rpsL* gene of *Campylobacter* results in resistance to fluoroquinolone. The horizontal gene transfers are transduction, conjugation and natural transformation. Transduction and conjugation require bacteriphage and cell to cell contact but natural transformation involves the uptake of free DNA from the surrounding environment and incorporation in the organisms (Crushell *et al.*, 2004).

2.4 Antimicrobial Susceptibility

Breakpoint goes in hand with antimicrobial susceptibility and it explains the concept or criteria of resistance and susceptibility to different antibacterial. It is expressed in terms of either concentration of the bacteria (in mg/liter or g/liter) or measured in zone diameter (in cm). Most time criteria can be susceptible, intermediate or resistance for evaluation of the results. The concept is important in choosing dosing rate for antibacterial (pharmacodynamic) (Wikler and Ambrose, 2005). The term susceptibility refers to sensitivity which can either be directly interaction between antibacterial agent and bacteria or extent to which the bacteria respond to antibacterial agent. If the strain is

inhibited by concentration of antibacterial agent that means there is high success of therapeutic. For intermediate bacteria strain is associated with an uncertain therapeutic effect where for resistant bacteria, bacterial strain is not inhibited by a concentration of an antibacterial agent that is associated with a high therapeutic failure (Japanese Society for Chemotherapy, 1993).

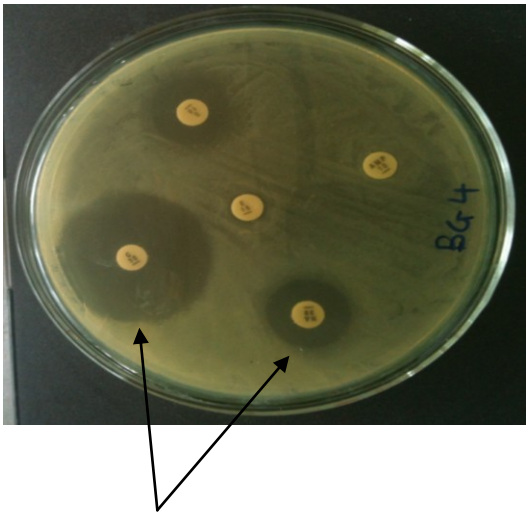

Figure 1: Antimicrobial susceptibility plate showing inhibition zone of *Campylobacter* (susceptible to antibiotic) and area without inhibition zone of *Campylobacter* (Resistant to antibiotic) (Source: Laboratory culture at faculty of Veterinary Medicine)

2.5 Diseases Caused by *Campylobacter*

Thermophilic *Campylobacter spp*. causes a campylobacteriosis in human and animals. In human, causes neurological disorders such as Guillain Barre Syndrome (GBS) and Miller- Fisher syndrome (Hansson *et al*., 2010). In animals *Campylobacter jejuni* causes diarrhoea in dogs, cats, calves and horses (Sandberg, 2002) abortion in sheep and cows, mastitis in cows and avian infectious hepatitis (Hansson *et al*., 2010)

2.6 Clinical Sign of Campylobacteriosis

2.6.1 Broiler

They are known to be healthy carriers of thermophilic *Campylobacter* species in their gastrointestinal tracks though sometimes they can be pathogenically affected. There is no appreciable development of clinical signs in broiler because in most cases, they are healthy carriers of these bacteria (*Mäkelä et al*., 2011). Colonization in broiler refers to non-pathological commensal relationship that exists between host and *Campylobacter.*

For enteric organisms like *C. jejuni* to establish and maintain colonization, a complex interaction of unknown mechanisms between the host and the organism comes into play (Allen *et al*., 2007). *C. jejuni* is introduced and spreads rapidly to virtually all birds in the flock and spread is facilitated by contamination of feed and water (Simon *et al*., 2013). Main sites of colonization of *C. jejuni* are the caeca and the organisms concentrate in the mucus layer of crypts of the villi (Nonga, 2005). At these predilection sites, *Campylobacter* species are usually free-living and have ability to utilize mucin as an energy source (Twan *et al.*, 2009: Summers 2006). Levels of colonization are relatively highly detectable in faeces and concentrations of 5.6×10^4 to 1.2×10^7cfu/g have been reported (Grant *et al*., 1980). Some strains of this species are invasive and/or toxigenic and may cause distention of intestines with contents tend to become foamy, liver abnormalities and diarrhoea (Rao *et al*., 2001). Such strains can also be isolated from spleen, gall bladder and blood of infected chickens (Moore *et al.,* 2005).

2.6.2 Humans

Campylobacteriosis are food-borne bacterial diseases; in human manifest themselves most in children less than five years, the elderly and immunocompromised individuals compared to healthy adults. Incubation periods range between 2 to 5 days. The diseases

are self-limiting and last 1-4 days or up to 10 days at the most (Nonga and Muhairwa, 2009). In human the disease manifest as gastroenteritis, onset of fever associated with malaise and headaches followed with nausea and abdominal cramping resembling the symptoms of acute appendicitis (Olivier *et al.,* 2010). In developing countries patients appear to have less severe symptoms compared to developed countries (Trachoo, 2003). In developed countries the disease is characterized by bloody stool, fever and abdominal pain that is often severe than that observed in *Shigella* and *Salmonella* infections where as developing countries there is watery stool, vomiting, abdominal pain dehydration, and presence of faecal leucocytes. Patients are also often under-weight and malnourished (Coker *et al*., 2002).

In rare cases *C. jejuni* infection lead to bacteremia, arthritis, meningitis, recurrent colitis, acute cholecystitis and Guillain-Barré Syndrome (GBS) other complications. Interestingly, extra-intestinal infections such as pericarditis and myocarditis have been increasingly reported as complications associated with *Campylobacter* infection (Uzoigwe, 2005). GBS is characterized by acute flaccid, rapid progressive symmetric paralysis (Coker *et al*., 2002; Trachoo, 2003).

2.7 Requirements of Thermophilic *Campylobacter* for Growth

They are typically microaerophilic organisms and require concentration of oxygen with 5%, carbondioxide 10% and nitrogen 85% in microaerobic gas incubator or anaerobic candle jars (Kalupahana, 2013). The samples should be incubated for 48 to 72 hours at 42^{o}C to 43^{o}C and pH range of 6.5 – 7.5 for optimal recovery (Williams and Oyarzaba, 2012). Bacterial culture is usually done on either solid media or enrichment broth. Several selective media can be used for isolating the thermophilic *Campylobacters.* They include Skirrow media which is blood-containing media, Campy Blaser which is a

Campylobacter blood agar formulation and blood free media includes, modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA), charcoal based selective medium (CSM) and the blood free semisolid motility medium (SSM) (Botteldoorn *et al.,* 2008). Most of the recommended selective media have one or more antimicrobial agents, mainly cefoperazone, as a primary inhibitor of enteric flora. Most of these media contain growth supplements and antifungal components to inhibit contamination by fungi.

Enrichment methods are intended to isolate *Campylobacter* organisms from samples containing low numbers of organisms. A number of enrichment broths are used to recover *Campylobacter* organisms from samples. These include Preston enrichment broth and *Campylobacter* enrichment broth and other formulations (Dallas *et al*., 2014).

2.8 Identification and Diagnosis

Several methods are involved in identification and diagnosis of *Campylobacter* species. These methods include micromorphology, macromorphology, biochemical tests, serology, and molecular detection techniques. There are difficulties in making routine identification of *Campylobacte*r species by biochemical methods which make these organisms ideal candidates for polymerase chain reaction as the definitive identification methods (Botteldoorn *et al.,* 2008).

2.8.1 Macromophology

The colonial morphology of *Campylobacters* shows some variation within each species (Dallas *et al*., 2014). Thermophilic *Campylobacter spp*. colonies on solid media are low convex or rather flat, grayish in colour and translucent in appearance measuring 2-4 mm in diameter. The colonies are mucoid, sticky and slippery on media making it difficult to take a loopful of colonies for different purposes. The colonies have a swarming

appearance and when on moist fresh agar they tend to coalesce and may extend along the line of inoculation (Skirrow, 2006). When the culture stays for more than 48 hours or when exposed to atmospheric conditions appear as metallic sheen (Holt *et al.,* 1994).

2.8.2 Micromophology

Campylobacter spp and related bacteria are gram negative, vibrioid or bacilli cells with a single polar unsheathed flagellum at one or both ends. The organisms are pleomorphic with different shapes including a curved, slender *S*-shaped or spiral rod with a dimension of 0.2 – 0.5μm wide and 0.5 – 5 μm long. They are non-acid fast, non-spore formers; they stain weakly and need high concentrations of staining reagents. Under wet smear and dark field microscope, the organisms appear as active, motile cells with a characteristic darting and cork screw-like, motion is aided by a single polar flagellum at one end or both ends of the cells (Holt *et al.*1994). When stressed especially aged culture of more than 48 hours, undernourished, exposed to normal atmospheric conditions they develop a coccoidal structure which are regarded as degenerative forms of the bacteria (Park, 2002). The coccoid forms are the minicells formed from the fragmentation of the flagellae and poles of the cells at a late cycle of growth. This form is known as viable but non-culturable state (VBNC), meaning that the organism is unable to grow on media that normally support growth but exhibit metabolism, hence can cause infection (Moore *et al*., 2001).

2.8.3 Biochemical testing

Due to relatively low activity in several conventional metabolic activity test and special growth requirements, species differentiation between *Campylobacte*r species using classical phenotyping methods is rather difficult. Morphology by Gram staining, motility and catalase test are performed in primary isolation (Fitzgerald *et al*., 2008). Further tests

include the hippurate hydrolysis test, oxidase growth at 25ºC, 37ºC and 42ºC, indoxyl acetate hydrolysis and production of hydrogen sulphide (H_2S) (Fitzgerald *et al.*, 2008). Hippurate hydrolysis and Nitrate test are the biochemical tests, which are commonly used to confirm presence of *C. jejuni* and *C. Coli* respectively while catalase and oxidase tests are used to confirm presence of *Campylobacter spp* in the sample (Nakari *et al*., 2008).

2.8.4 Serology and serotyping

Rapid serological and serotyping tests are also used in the diagnosis and identification of *Campylobacters*. The immune response to C*ampylobacter* infection is similar to that of other infectious diseases. Serum IgG and IgM levels rises in response to infection and remain elevated for 3-4 weeks before declining to baseline levels where as serum IgA levels rise during the first few weeks of infection and then fall rapidly (Teunis *et al*., 2012). Antibody mediated agglutination of cellular antigens is also used in diagnosis of *Campylobacter* (Fussing *et al*., 2007). A variety of antibody assays are available for detecting isotype-specific antibodies and these include Enzyme Linked Immunosorbent Assay (ELISA), diffusion-in gel ELISA and immunoblot analysis agglutination tests and complement fixation test (Ang *et al*., 2011). Serotyping is one of the phenotypic tests devised to study the epidemiology of *Campylobacter* infections worldwide and two major serotyping schemes are used that detect heat labile “H” flagella antigens and soluble heat stable “O” (somatic) antigens (Teunis *et al*., 2012; Andrey *et al,* 2000. Bacterial surface antigen is one of the sero-determinants for this sero-typing system and is based on detection of lipopolysaccharide antigens (Patton and Wachsmuth, 1992; Fussing *et al*., 2007). Each system uses latex particles coated with immunoglobulin developed against several *Campylobacter* species.

2.8.5 Molecular detection technique

Polymerase Chain Reaction (PCR) is sometime known as molecular photocopying is a typing method that amplifies target segments of DNA (Engvall *et al.,* 2002). By using oglionucleotides (primers) that bind to the target segments of DNA at the 5′- and 3′-ends, DNA polymerase can be directed to the targeted site for amplification which lead to exponential increase in the total amount of DNA. The technique provides a rapid and highly sensitive method for the detection of species specific DNA sequences. The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA (Dieffenbach and Dveksler, 2003; Abeynayake and Wagenaarb, 2013).

Several variations to the original PCR technique have been developed that are useful in identifying *Campylobacter* spp. These include reverse-transcriptase PCR, multiplex PCR and quantitative real-time (QRT)- PCR (Eberle and Kiess, 2012). Real-time PCR assays are becoming of increasing importance since they assess the level of contamination with a given pathogen (Lübeck *et al.,* 2003). Real-time PCR is based on the principles of conventional PCR but with continuous monitoring of product accumulation (Higuchi *et al.,* 1992). These techniques are easy to reproduce, highly discriminatory and available in most laboratories. Although these techniques can be expensive, they are still one of the most commonly used genotypic methods for typing *Campylobacter* spp (Eberle and Kiess, 2012). An advantage is also the potential use in screening programmes (Lübeck *et al.,* 2003). The presence of inhibitory compounds may affect the PCR reaction and give false negative results. The use of an internal standard as a control of the PCR reaction increases the reliability of the technique (Denis *et al.,* 2001).

2.9 Epidemiology of *Campylobacter*

Prevalence of *Campylobacter* differs from area to area, country to country (Nonga and Muhairwa, 2009). These is due to fact that *Campylobacter* spp. is susceptible to number of environmental conditions such as dry conditions, temperature, presence of oxygen, and other factor like pH, UV and humidity, but may survive in a viable but non-culturable form (VBNC) in the environment (Isohanni and Lyhs, 2009). Even samples of intestinal origin, if allowed to dry or if kept for prolonged periods of time at room temperature, may require enrichment for survival of the organisms (Kalupahana *et al*., 2013), even with fecal samples, some researchers have reported increased detection rates of *Campylobacter* by using enrichment media, whereas other researchers have reported decreased rates of detection or negligible increases over direct culturing procedures (Zweifel *et al*., 2008). All *Campylobacte*r grow at 37ºC, but for the thermophilic species *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis* the optimum temperature is 42ºC and there is no one simple standard method for routine isolation of all *Campylobacter* species. The predominant species *C. jejuni* and *C. coli* grow in a microaerobic condition on selective media (Debruyne *et al*., 2008).

There are several risk factors which suggest the colonization and transmission of the *Campylobacter* to broiler flock. The outside environment of rearing houses is the most important but there are several other factors involved in the transmission of *Campylobacter* to broiler farms. The surrounding environment, rearing house mode and animals management system practices play role in the dynamics of the *Campylobacter* colonization of flocks (Hansson *et al*., 2010). Different farms animals like cattle, pigs and other poultry can be the reservoir of the *Campylobacter* (Backert *et al*., 2013) other factors being humid, moist condition that allow colonization of *Campylobacter*. Transmissions from infected area to non-infected area are often accelerated by vector

such as flies and other insects (Hald *et al*., 2004, Rushton *et al*., 2009). In human sources of infection include contact with or consumption of contaminated carcasses of poultry, raw milk and water (Inglis *et al*., 2004: Bakkenes *et al*., 2011)**.** The broiler carcasses are normally contaminated during slaughter process (Whiley *et al*., 2013).

2.10 Control and Prevention

It is know that to reduce the contamination of *Campylobacter* in poultry automatically will be controlled to human as an important the source of food (Line *et al*., 2008)**.** Control measure of *Campylobacter* in poultry includes; biosecurity measures, hygiene measures although hygiene barriers can be broken through as well as vaccination of chicken against *Campylobacter jejuni* and *Campylobacter coli* which is still under development (Jun, 2009) the use of anti-*Campylobacter* bacteriocin treatment is clearly an effective and feasible strategy to reduce *Campylobacter* load in aged chicken (Line *et al*., 2008). Also control should be directed on the use of different antibiotic to reduce the antibiotic resistance in *Campylobacter* (Svetoch *et al*., 2005). Control of *Campylobacter* needs to target the hazard (Sandberg, 2002), the use of Hazard Analysis of Critical Control Point (HACCP) as the control tool in production, processing and distribution of poultry products may reduce the likelihood of the problem. The strategy of inhibiting growth of microorganism in all stages of food production, processing, distribution and consumption can be the best approach.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Morogoro Municipal, Tanzania (Fig. 2). According to official 2012 census, Morogoro Municipal had a human population of 315 866. Morogoro extends between longitude 35°15' to 38°30' East and latitude 5°15' to 10° 0' South with an elevation of 500 to 600 m above sea level and is about 200km west of Dar es Salaam. The average rainfall ranges between 400 and 1700 mm and ambient temperature between 18 to 29°C. The Municipal has a bimodal rain pattern, with about 83% of the rain falling between February and May, where short rains are between November and January.

The Municipal has one of the highest concentrations of broiler population estimated at 2 100 861 after Dar es Salaam and Arusha and large live-poultry selling capacity (Ministry of Livestock and Fisheries Development, 2010). The Municipal however lack official broiler slaughter places and all the slaughter of finished live broiler birds is done at individual farms which makes it difficult to standardize the hygienic conditions at farm level. This also propels the possibility of easy transmission of meat-borne diseases like Campylobacteriosis to consumers.

Morogoro Municipal has 19 wards with estimated number of total broiler population of 2 100 861 (Source: Morogoro Municipal Livestock and Fisheries Officer 2014). For the purpose of this study, four wards, namely Mazimbu, Mafisa, Kihonda and Msamvu were selected and the numbers of broilers in these areas based on 2013 estimate are 234 500 from Mazimbu, 173 423 from Kihonda, 7432 from Mafisa, 2434 from Msamvu, 267 434) (Source : Morogoro Municipal Livestock and Fisheries Officer).

3.2 Study Design and Sample Size

Longitudinal study was carried out to establish the colonization and prevalence of thermophilic *Campylobacter* species in broiler production in Morogoro Municipal. Sample sizes were calculated using the formula $n=Z^2p\ (1-p)/\ d^2$ (Thrusfield, 1995) where: n is sample size; Z is the multiplier from the normal distribution, p is the expected prevalence and d is the desired absolute precision. The expected prevalence of *Campylobacter* infection (p) are p=69.8% for chickens (Mdegela *et al*., 2006). With Z value of 1.96 at 95% confidence interval (CI) and desired precision (d) of 0.05, the calculated sample sizes (n) are 324 which is minimum sample size.

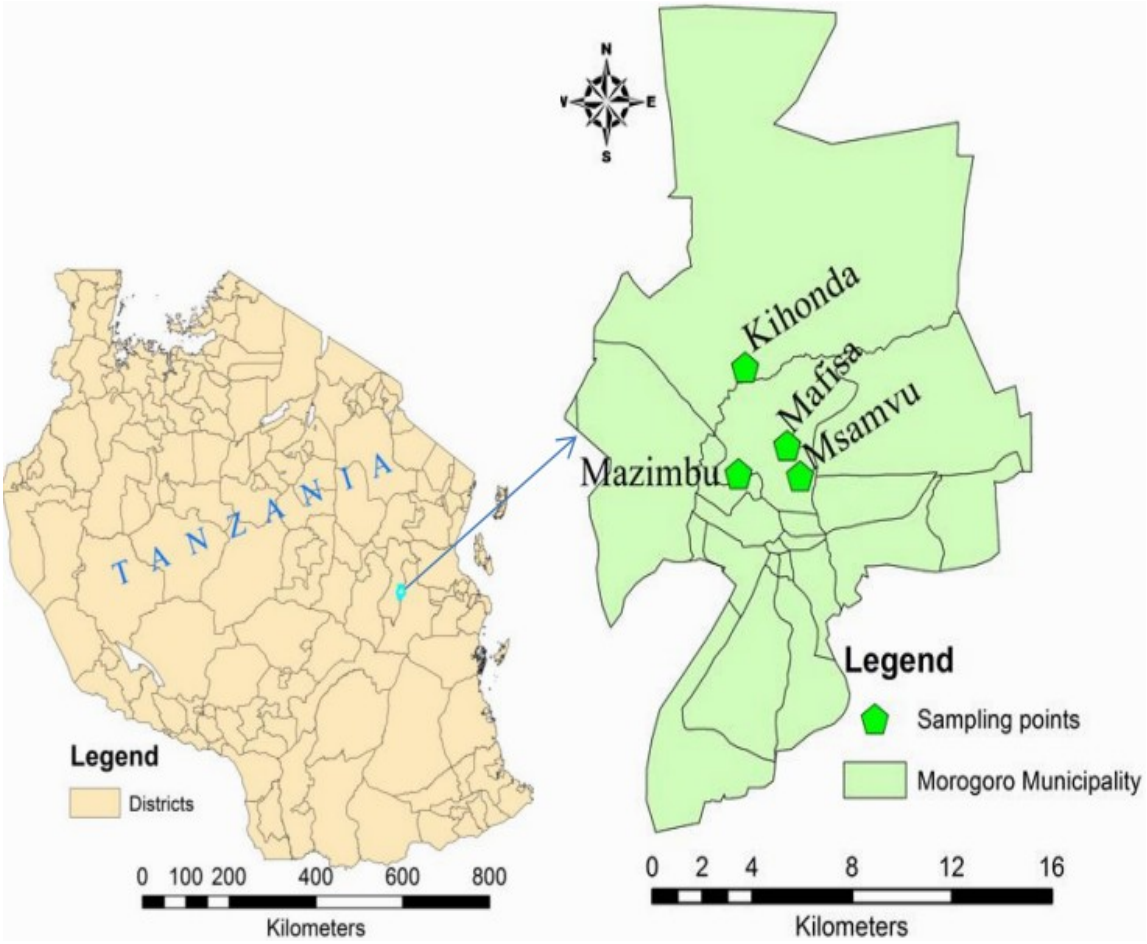

Figure 2: Map of the study area Morogoro Municipality, Tanzania (Source: www. Tanzaniamap.go. tz, Downloaded on 12/3/2014)

3.3 Farms Classification

3.3.1 Mazimbu farm

Mazimbu ward in Morogoro Municipal has a human population of 72 527 and 234 500 of broiler (Source: Morogoro Municipal Livestock and Fisheries Officer, 2014). The selected farmer had three poultry houses within the household compound with carrying capacity of about 560 broilers each. The farm was as family business and managed by the household wife while the husband ran piggery farm outside the Morogoro Municipal. Routine husbandry activities were performed by children and maids. Chicks that are kept come from Amadoli Company.

Biosecurity issues were minimal because everyone (mother, father, children and other member of the family) was allowed to manage the birds whenever they are around at home or to perform routine management activities. They use disinfectant although they change after three to four days. The attendant does not use protective gear (gumboot, overcoat and facemask).

Slaughtering is done at their home premises. Slaughtering of the broiler was done between 5 to 7 weeks of age and were done within the household premise. Perforations of the carcasses around the vent were done that allow to removal the visceral organ and cutting of the carcasses into two pieces (hind legs and fore part). Finally the carcasses were rinsed in warm water approximate 30-35°C.

3.3.2 Kihonda farm

Kihonda ward in Morogoro municipal has a human population of 44 424 and 173423 of broiler (Source: Morogoro Municipal Livestock and Fisheries Officer 2014). The farmer selected for this study had two poultry houses within family premises each having capacity of carrying about 430 broilers. The farmer kept other animals within the area including pigs, dogs and local chicken. Routine husbandry activities were done by two house girls. Chicks that are kept come from Dar es Salaam. Biosecurity issues are minimal because everyone (mother, father, children and other member of the family) is allowed to manage the birds whenever they are around at home or to perform routine management activities. They did not use disinfectant. The attendant wore protective gear (gumboot and overcoat) but they did not use facemask. Slaughtering of finished broilers was done within the household premises between 5 to 7 weeks of age. Perforation around the vent were done and visceral organ were removed followed by cutting the carcasses

into two pieces (hind legs and fore part). Finally they rise the carcasses with warm water (approximate 30-35°C).

3.3.3 Mafisa farms

Mafisa ward in Morogoro Municipal has a human population of 13 587 and 7432 broiler (Source: Morogoro Municipal Livestock and Fisheries Officer). The selected farmer in Mafisa area had one poultry house built in his household compound with a capacity of carrying 350 to 400 broilers. Apparently this farmer also keeps local chicken in the same household compound. The full broiler enterprises were managed at all levels by the household wife and chicks come from Dar es Salaam. Biosecurity issues are minimal because everyone (mother, father, children and other member of the family) were allowed to manage the birds whenever they are around at home or to perform routine management activities. They did not use disinfectant. The attendant had only overcoat without gumboot.

3.3.4 Msamvu farm

Msamvu is one of the Morogoro Municipal ward with estimated human population of 5613 people and 3500 of broiler poultry (Source: Morogoro Municipal Livestock and Fisheries Officer 2014). The selected farmer in this ward had five poultry houses with capacity of carrying 650-700 broilers each. Apart from that, the farmer kept goat, cattle, local chicken and turkey in the same premises. Management, processing and selling of the broilers were supervised by household wife and each room attended by one separate attendant. Chicks raised in this farm came from Amadoli Company Dar es Salaam.

3.4 Broiler Selection and Sampling

The age of birds targeted for this study were third, 21, 35 days old and for the purpose of this study they were designated as first, third and fifth weeks old. Birds were also sampled on the day of slaughter. Broilers were kept up to the age of five to seven weeks before slaughter. Litter materials were changed whenever they got wet. Vaccination against Newcastle diseases were done at 10 days followed by booster dose on chicken should be slaughtered at 5-7 week.

3.5 Broiler Fecal Sampling

Fecal sampling was done during the first week, third week and fifth week. Broilers were randomly selected from each flock. The randomization of study birds was carried out in broiler house by means of picking fresh droppings from different places within the broiler house. The total sample collected per each farm were 25, 30, 40 and 50 fecal droppings from 250, 300, 400 and 500 broilers farms respectively per each sampling. Fresh fecal sample were picked by sterile Popsicle stick from the floor and stored in sterile polypropylene tubes with 5ml of Maximum Recovery Diluent (MRD) (Neogen, Lansing, Michigan, USA) and stored in cool box with ice pack (4°C) for processing within 12 hours.

3.6 Carcasses Samples

Sterile swabs were used to swab the carcasses during slaughter time. Carcasses swabs were aseptically swabbed on the fore and hind limb following evisceration and after washing with hot water. The collected samples were placed either in sterile polypropylene tubes with peptone water and transported to the laboratory on ice for processing within 12 hours. Total swabs collected were 25, 30, 40 and 50 carcasses swab from 250, 300, 400 and 500 broiler farms.

3.7 Laboratory Analysis of fecal and carcasses swab Samples

To isolate *Campylobacter* species, approximately 2 grams of fresh fecal droppings were suspended with 9.0 ml of Maximum Recovery Diluent (MRD) (Neogen, Lansing, Michigan, USA) by shaking. Thereafter, 1.0 ml aliquot of the resulting suspension was removed and added to 9.0 ml of Preston broth containing *Campylobacter* growth supplements (CM067, SR048, SR117 and SR232; Oxoid, Hampshire, England) and incubated at 42^{o}C for 48 hours under microaerophilic condition (5% O_2, 10% CO_2 and 85% N_2) generated by microaerophilic candle jars with a lighting candle. After incubation, an inoculum (100 µL) of culture was streaked onto Modified Charcoal Cefaperazone Deoxycolate Agar (mCCDA) (CM 0739, Oxoid) containing the corresponding supplement (SR155E, Oxoid). Plates were thereafter incubated for an additional 48 hours at 42^{o}C under the same microaerophilic conditions. Where available, three presumptive *Campylobacter* colonies from each mCCDA plate were then subcultured onto Muller-Hinton (MH; Difco, Sparks, MD) agar containing *Campylobacter* Selective Supplement, CSS (SR117, Oxoid) and incubated microaerobically at 42^{o}C for 48 h. Pure isolates were then stored at −80˚C in MH broth (Difco, Sparks, MD) supplemented with 80% glycerol (v/v) until further identification and characterization. From the carcass swabs, 50 mL of MRD was added and thoroughly squeezed for 3 minutes. Five milliliter aliquot of the resulting suspension was removed and added into 10 mL of enrichment Preston broth and processed for *Campylobacter* isolation as described in the procedures for isolation from feces.

3.8 DNA Extraction from *Campylobacter* Isolates

Bacterial DNA extracts used in polymerase chain reaction (PCR) were prepared from *Campylobacter* pure cultures by boiling as previously described by Dingle *et al.* (2005). Briefly, a few bacterial colonies from the plates were suspended in 100µL of sterile

RNase/DNase-free water, heated at 95°C for 10 min, cooled, and centrifuged at 13 000x g for 10 minutes. The supernatant containing the nucleic acids was used as template DNA and stored at -20°C until used.

3.9 Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) amplification was performed in a final volume of 25µL containing 3µL of DNA, 12.5µL master mix (5 x DreamTaq buffer (Thermo Scientific); 4 mM $MgCl_2$ magnesium chloride, 200µM deoxynucleoside triphosphates, DreamTaq DNA Polymerase 5 U/µL), 8µl of nuclease free water and 0.75 µl of *Campylobacter jejuni* and *Campylobacter coli* reverse and forward primers (total 1.5µl). The gene targeted for *Campylobacter jejuni* was *mapA* gene with the primer sequence 5'-CTA TTT TAT TTT TGA GTG CTT GTG-3' for forward primer and 5'-GCT TTA TTT GCC ATT TGT TTT ATT A-3' for reverse primer which amplifies a 589 bp fragment. The gene targeted for *Campylobacter coli* was *ceuE* gene with the primer sequence 5'-ATT TGA AAA TTG CTC CAA CTA TG-3'for forward primer and 5'-TGA TTT TAT TAT TTG TAG CAG CG-3' for reverse primer which amplify 462 bp. PCR amplification reactions were carried out in a DNA thermo cycler (Eppendorf, Hamburg, Germany) as following: Initial denaturation for 5 min at 95 °C, followed by 35 cycles each consisting of 2 min at 95 °C, 60 s at 50 °C, 2 min at 72 °C and a final extension step of 5 min at 72 °C for 5 min (Yamazaki-Matsune *et al*., 2007)

3.9.1 Agarose gel preparation

One percent (1%) agarose gel was prepared by mixing 1 gram of agarose powder with 100ml 1X TBE (Tris/Borate/EDTA) buffer (Promega Corporation, Madison, WI, USA). The gel was melted by boiling the solution on a hot plate followed by cooling to about 50°C. Then the gel was stained with 0.5% of ethidium bromide solution in and the molten

agarose poured onto the horizontal electrophoresis gel casting equipment and left at room temperature to solidify for one hour.

3.9.2 Agarose gel electrophoresis

To analyse the PCR amplicons, 12µL of the PCR products were each mixed thoroughly with 2µL of blue/orange 6X loading dye (Promega-Invitrogen CA) on the laboratory Parafilm (Pechiney Plastic Packaging, Menasha, WI, USA). The resultant mixture was loaded into respective wells of the agarose gel. Gel electrophoresis was carried out in a gel tank containing 1XTBE buffer at a constant voltage of 100V for 60 minutes. The DNA bands were visualized using ultraviolet transilluminator. To ensure specific product amplification in all PCR analysis, *C. jejuni* 81–176 (wild-type strain) and *C. coli* (ATCC 33559) were used as positive controls while standard grade laboratory water used as negative controls. Expected PCR amplicons of 857 bp, 589 bp and 462 bp DNA fragments corresponding to the *Campylobacter* genus*, C. jejuni* and *C. coli,* respectively, were determined using a 1Kb plus DNA ladder (Invitrogen, California, USA).

3.10 Biochemical Tests

The catalase and oxidase biochemical tests were additionally used to confirm the presence of *Campylobacter spp* while Nitrate test and Hippurate hydrolysis test were used to identify and confirm thermophilic *C. coli* and *C. jejuni* respectively.

3.10.1 Oxidase test

To confirm the presence of *Campylobacter* identity, respective colony to be tested was spread on oxidase detection strip (MB0266A, OXOID LTD) impregnated with *tetramethyl-p-phenylenediamine dihydrochloride (TPD)* by using platinum wire loop. Observation was done for 5 minutes. The principal of reaction is based on the presence of

phenylenedianase which reduces TPD to either oxides (forming color) or reduced (forming color). The presence of deep blue/violet color indicates a positive reaction.

3.10.2 Hippurate hydrolysis test

A 0.3 ml of sterile normal saline was aliquoted in a sterile plastic test tube containing a sodium hippurate (2-4 gm) (Lipfilchem s.r.l, Roseto, d. A. (TE)-Italy) powder and mixed thoroughly. Using a sterile wire loop, one well-isolated colony was inserted in the solution. The tubes were incubated at 37^0C for 24 hours. Then four drops of 7% nihydrin solution were added and a blue purple colouration was indicative of a positive test for *C. jejuni.* The reaction is based on the presence of hippurate hydrolase where end product glycerine is formed and tested by ninhydrin. Formation of blue purple indicates the presence of *C .jejuni.*

3.10.3 Nitrate test

A 0.3 ml of sterile normal saline was added in a plastic test tube containing nitrate (Lipfilchem s.r.l, Roseto, d. A. (TE)-Italy) reagent. Using a sterile wire loop, a well-isolated colony was added in the solution. The tubes were then incubated for 24 hours at 37^0C. Then a drop of *alpha-naphtylamine* and one drop of sulfanilic acid was added and mixed gently for a few minutes. The development of red orange colouration signified a positive test for *C. coli.* The reaction is based on the reduction of Nitrate to Nitrite compound.

3.10.4 Catalase tests

To confirm the presence of *Campylobacter*, A thick smear of each presumptive *Campylobacter* was made on a sterile glass slide to which a drop of 5% hydrogen peroxide (B00AEJ2Z1E, USA) was added; positive catalase reaction was based on

appearance of effervescences within few seconds. The principle of the reaction is based on the presence of catalase enzymes which break down hydrogen peroxide to form water and oxygen.

3.11 Antimicrobial Susceptibility Test

To assess antimicrobial resistance profile, *Campylobacter* isolates (approximately100 colonies) were tested for their susceptibility to a panel of nine antimicrobials using disc-diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS, 2012) guidelines. Disc impregnated with antibiotics (Oxoid Ltd-Basingstoke) and their corresponding concentration were as follows: Ciprofloxacin (CIP: 5 μg); nalidixic acid (NA: 30 μg); Erythromycin (ERY: 15 μg); Gentamycin (GEN: 10 μg); Tetracycline (TE: 30 μg) Amoxicillin (AML: 10 μg), Azithromycin (AZM: 15µg), Chloramphenicol (CHL: 30μg) and Ampicillin (AMP 10: μg).. Briefly, well-isolated colonies were suspended into sterile normal saline and turbidity adjusted equal to a 0.5 McFarland standard using Vitek colorimeter (Lenexa, Kansas, USA). A sterile cotton swab were then dipped into the suspension and spread evenly on the entire surface of a Mueller–Hinton agar (MB0266A, OXOID LTD) supplemented with 5% sheep blood.

After drying the plates for 5 min, antibiotic discs were placed using Disc dispenser (Oxoid, LTD) and incubated under microaerophilic conditions (5% oxygen, 10% Carbondioxide and 85% Nitrogen) for 48 hrs at 42°C. Standard reference strains of *Staphylococcus aureus* (ATCC 25922) *Escherichia coli* (ATCC 29213) were used as quality control organisms in antimicrobial susceptibility determination. Diameters of the zone of inhibition around the disc were measured to the nearest millimeter using a metal caliper, and the isolates were classified as sensitive (S) and resistant (R) using National Committee for Clinical Laboratory Standards (NCCLS, 2012) guidelines. In this study,

Multidrug Resistance (MDR) was defined as resistance to three or more antibiotic classes as previously described by Hakanen *et al*. (2003).

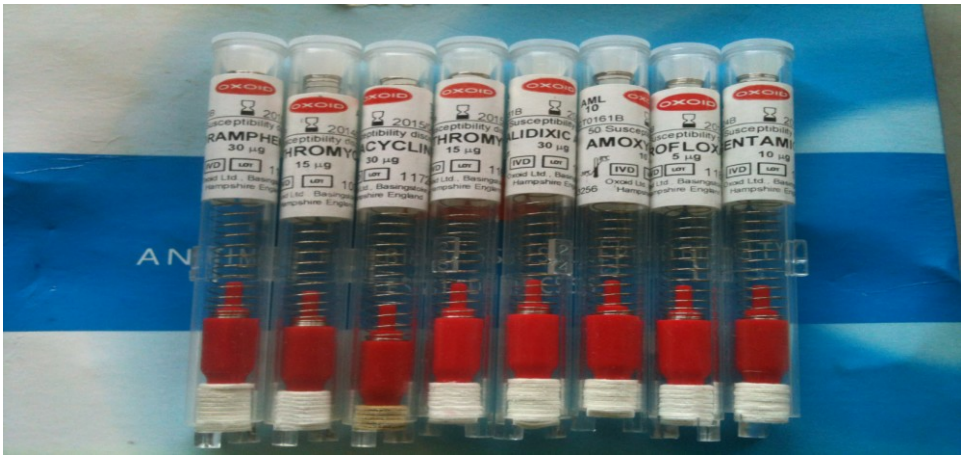

Figure 3: The disk impregnated with different antibiotics that were used for antimicrobial susceptibility test.

3.12 Statistical Analysis

Data collected by Culture, Polymerase chain reaction and biochemical test were stored in a Microsoft Office Access 2003 database and analyzed using MedCal. For prevalence and antimicrobial resistance data descriptive statistics (frequencies and cross tabulations) were computed to determine proportions for different items. Chi-square (χ^2) test for trend and proportion at a critical probability of 0.05 and 95% confidence interval was used. The level of agreement according to precision was expressed as the kappa statistic, defined as the proportion of potential agreement beyond chance exhibited by two tests. Also ANOVA table: (two factors with replication) were used.

CHAPTER FOUR

4.0 RESULTS

4.1 Fecal and swab samples collected in various farms and their *Campylobacter* prevalence

4.1.1 Fecal and swab samples

The number of broiler chicken involved in this study at Msamvu farm was 400 birds. A total of 40 birds were selected at random and fecal samples collected at each visit during week one, three and five. In addition 40 carcass swabs were collected from slaughtered birds making a total of 160 samples collected from this farm. At Kihonda farm, 250 birds were made available for this study and a total of 25 fecal samples were collected at each visit during the first, third and fifth week. In addition 25 carcass swabs were collected from slaughtered birds making a total of 100 samples. Likewise, at Mafisa farm, 300 broiler chickens were involved, of which 30 samples fecal samples were collected per each visit on first week, third week and fifth week and 30 carcass swabs collected at slaughter making 120 total samples. For Mazimbu farm, 500 chickens were available for study, out which, 50 fecal samples were collected at week one, three and five, respectively and during slaughter that makes 200 total samples. Overall, 435 fecal and 145 swabs were collected during this study makes a total of 580 samples

4.1.2 Prevalence of *Campylobacter* in different farms by culture and PCR analysis

All the fecal and swab samples collected in all farms (n=580) were cultured and out of these 340 were *Campylobacter* positive by morphology. In addition all 340 positive samples by culture were confirmed to be positive by PCR analysis. The overall distribution of *Campylobacter* per species in different farms is summarized in Table 1 and

Fig. 4 shows PCR products for *ceuE* and *mapA* genes amplification for positive and test samples.

Overall prevalence of *Campylobacter* in all four farms and at all stages of broiler production were 58.6% (340/580), of which, 241(71%) were *Campylobacter* positive from fresh feces and 99(29%) from broiler carcass swabs (Table 1). PCR identification of *Campylobacter spp* from both fecal and swab samples revealed 47.9% (163/340) has *C. coli* and 34.4% (117/340) has *C. jejuni*. Few samples were *Campylobacter* positive for both ceuE and mapA (*C. jejuni* / *C. coli* co-existing 17.6% (60/340). Out of 163 samples which were *C. coli* positive, 73.6% (n=120) were from feces while 26.4% (n=43) were from carcass swabs. Similarly, out of 117 samples positive for *C. jejuni*, 69.2% (n=81) were from feces and 30.8% (n=36) were from carcass swabs.

The overall prevalence of *C.coli* in all fecal samples collected across different farms (n=435) and confirmed by PCR) was 27.6% (n=120) while in carcass swabs it was 29.77% (n=145). Similarly, the overall prevalence *C. jenuni* was 18.6% (n=81) and 24.8% (n=36) in fecal and swabs samples, respectively.

Between farms under study, the highest prevalence of fecal *C.coli* was found at Mafisa farm (63%) followed by Msamvu farm (55%) and lowest at Kihonda farm (33%) while for *C.jejuni* the highest prevalence was found at Kihonda (40%) and the lowest at Msamvu (29%) (Table 1). For carcass swabs, *C.coli* prevalence was highest at Msamvu farm (65%) followed by Mafisa farm (40%) and the lowest at Kihonda farm (33%). Similar results for *C.jejuni* showed highest prevalence for Mafisa farm (40%) and lowest at Kihonda farm (33%) (Table 1).

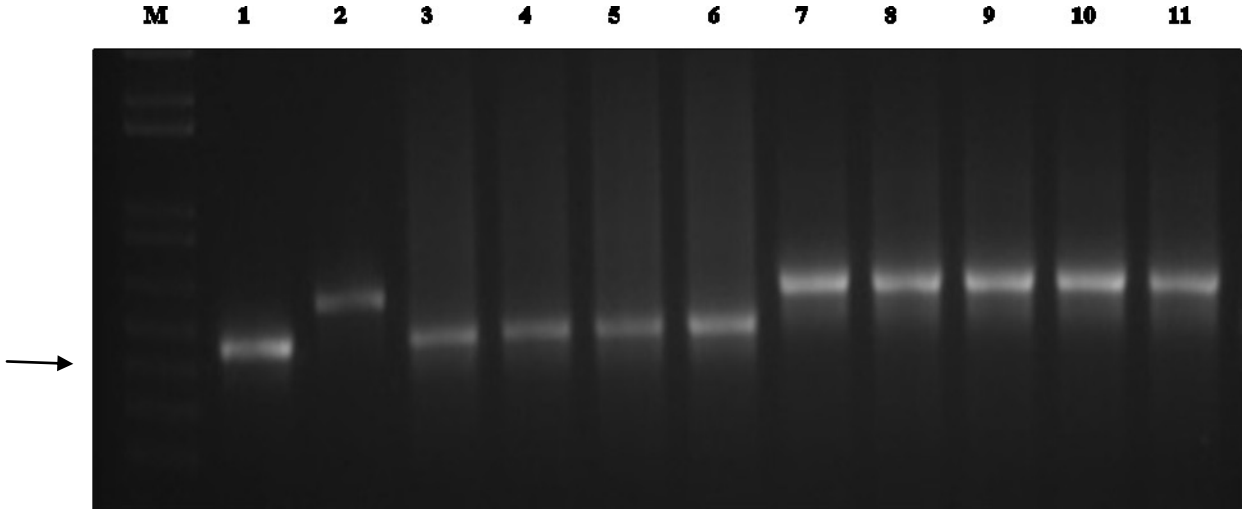

Figure 4: PCR products for *ceuE* and *mapA* genes amplification. Lane 1: Positive control for *C. coli* (462 bp), Lane 2: Positive control for *C. jejuni* (589 bp), Lanes 3 – 6: *C. coli* and Lanes: 7 – 11 *C. jejuni* positive samples. M: 1kb Plus DNA ladder

Table 1: Number of *Campylobacter* isolates from feces and carcasses in four farms

Farm	Total sample	Total isolate from sample	Feces				Carcasses			
			C. coli	*C. jejuni*	*C. coli/C. jejuni*	Total isolates	*C. coli*	*C .jejuni*	*C. coli/C. jejuni*	Total isolates
Msamvu	160	101(63%)	43(55%)	23 (29%)	12(15%)	78(77%)	15(65%)	8(35%)	0(0%)	23(23%)
Kihonda	100	82(82%)	19(33%)	23(40%)	16(28%)	58(71%)	8(33%)	8(33%)	8(33%)	24(29%)
Mafisa	120	63(53%)	27(63%)	16(37%)	0(0%)	43(68%)	8(40%)	8(40%)	4(20%)	20(32%)
Mazimbu	200	94(47%)	31(50%)	19(31%)	12(19%)	62(66%)	12(38%)	12(38%)	8(24%)	32(34%)
Total	580	340(59%)	120(73.6%)	81(69.2%)	40(17%)	241(71%)	43(43.4%)	36(36.8%)	20(20%)	99(29%)

4.1.3 Prevalence of *Campylobacter* in different farms at different age stages

Table 2 shows the distribution of *Campylobacter* spp at different stages of broiler production in all farms under study. As depicted above (Table 2) fecal samples for *Campylobacter* investigation were collected during the first week, third week and fifth week and carcasses swabs were collected when birds were ready for slaughter. This varied between weeks 5-7 depending on the differences in individual bird growth rates. Generally *Campylobacter* infection was absent during week one at Mafisa farm, *C. coli* infection was evident at Kihonda (14%) and Mazimbu (9%) farms, while both *C. coli* (14%) and *C. jejuni* (26%) infections were observed at Msamvu farm during the first week.

In subsequent weeks, *Campylobacter* infection rates showed no clear trends across farms under study (Table 2). The average level of fecal *Campylobacter* colonization ranges from 15% to 50% (P=0.0358) during the third and fifth weeks. During the third week, *C. coli* was found be highest prevalent at Mafisa (43%) and lowest (14%) at Msamvu farms. During week 5 of age, fecal *C. coli* prevalence was highest at Msamvu farm (46%) and lowest at Kihonda farm (29%).

C. jejuni colonization for the 3rd and 5th weeks of age followed a similar unequivocal trend as for *C. coli* across farms. Msamvu farm had highest fecal *C.jejuni* (48%) on week 3 while Mazimbu had lowest prevalence (15%) during the same week. On week 5, highest fecal *C.jejuni* prevalence was observed at Kihonda farm (61%) and lowest at Mafisa farm (15%). Mixed *C.coli* and *C.jejuni* infections were completely absent at Mafisa farm during the entire study period; but were observed on week 5 at Mazimbu farm (prevalence 60%). Similar mixed infection was observed at Kihonda farm at all

weeks (17%, 33%, and 17%) and Msamvu at 3rd and 5th week (33% and 67%, respectively).

The overall level of *Campylobacter* contamination in carcasses at slaughter ranged from 15% to 40% (P=0.687) across farms (Table 2). Contamination level for *C. jejuni* was high for Mazimbu and Mafisa farms with the prevalence of 39% and 33%, respectively. High prevalence of *C. coli* was found in Kihonda farm with 29% (n=8) and Msamvu farm with 26% (n=15) (Table 2).

Table 2: The distribution of *Campylobacter* spp in different stages of broiler production

Farms	*Campylobacter spp*	Sampling stages				Total
		Week 1	Week 3	Week 5	Week 6- 7	
Mazimbu	*C.coli*	4(9%)	8(19%)	19(44%)	12(28%)	43(100%)
	C.jejuni	0(0%)	4(13%)	15(48%)	12(39%)	31(100%)
	C.coli/jejuni	0(0%)	0(0%)	12(60%)	8(40%)	20(100%)
Mafisa	*C.coli*	0(0%)	12(34%)	15(43%)	8(23%)	35(100%)
	C.jejuni	0(0%)	4(17%)	12(50%)	8(33%)	24(100%)
	C.coli/jejuni	0(0%)	0(0%)	0(0%)	4(100%)	4(100%)
Kihonda	*C.coli*	4(14%)	8(29%)	8(29%)	8(29%)	28(100%)
	C.jejuni	0(0%)	4(13%)	19(61%)	8(26%)	31(100%)
	C.coli/jejuni	4(17%)	8(33%)	4(17%)	8(33%)	24(100%)
Msamvu	*C.coli*	8(14%)	8(14%)	26(46%)	15(26%)	57(100%)
	C.jejuni	8(26%)	15(48%)	8(26%)	0(0%)	31(100%)
	C.coli/jejuni	0 (0%)	4(33%)	8(67%)	0(0%)	12(100%)
Total		**28**	**75**	**146**	**91**	**340**

4.2 Proportional of C*ampylobacter* Positive Sample by Biochemical Test

Table 3 shows the prevalence of *Campylobacter spp* based on biochemical tests. Out of 340 samples which were found to be *Campylobacte*r spp by PCR, only 147 were confirmed to be *Campylobacter* positive by Catalase and oxidase tests. About 36% were positive for *C. jejuni* by Hippurate hydrolysis test and 64% were positive for *C. coli* by

nitrate test. Based on biochemical tests alone, the overall highest *Campylobacter* prevalence were found at Mazimbu farms for *C.jejuni* with 35% for *C.coli* while the lowest prevalence was found at Mafisa farm with 19% for *C.jejuni* and 16% for *C. coli*.

Table 3: Campylobacter positive samples by biochemical test

	Mafisa	Kihonda	Msamvu	Mazimbu	Total
C. jejuni	10(19%)	11(21%)	13(25%)	19(35%)	53(36%)
C. coli	12(16%)	17(23%)	19(26%)	26(35%)	74(64%)
Total	22(15%)	28(19%)	32(22%)	45(31%)	147(100%)

4.3 Antimicrobial Sensitivity Profile

4.3.1 General resistance profile

Due to limited resources, 53 *Campylobacter* positive isolates were randomly selected and tested for antimicrobial susceptibility using 9 antibiotics. The antibiotic sensitivity profile was done on PCR confirmed *C. coli* (n=26), *C. jejuni* (n=18) and mixed *C. coli/C. jejuni* (n=9) making a total of 53 isolates. Out of these isolates, 14, 10, 16, 13 isolates were isolated from Mazimbu, Mafisa, Kihonda and Msamvu farms, respectively. Among the 26 *C. coli* tested, six were isolated from Mazimbu farm, five from Mafisa, eight from Kihonda and seven from Msamvu farms, Among 18 *C. jejuni* tested, five were isolated from Mazimbu, four from Mafisa, five from Kihonda and four from Msamvu farms. In regard to nine *C. jejuni/C. coli* isolates tested, three were from Mazimbu and Kihonda, respectively, one from Mafisa and two from Msamvu farms.

The overall resistances of *C. jejuni* and *C. coli* to various antibiotics were Ciproflaxin 58% (n=62) Tetracycline 56% (n=59), Ampicilin 53% (n=56), Gentamycin 52% (n=55), Nalidixic acid 55% (n=58), Amoxicilin 53% (n=56), Erythromycin 36% (n=38), Azithromycin 31%(n=33) and Chloramphenicol 24%(n=25) (Fig 5).

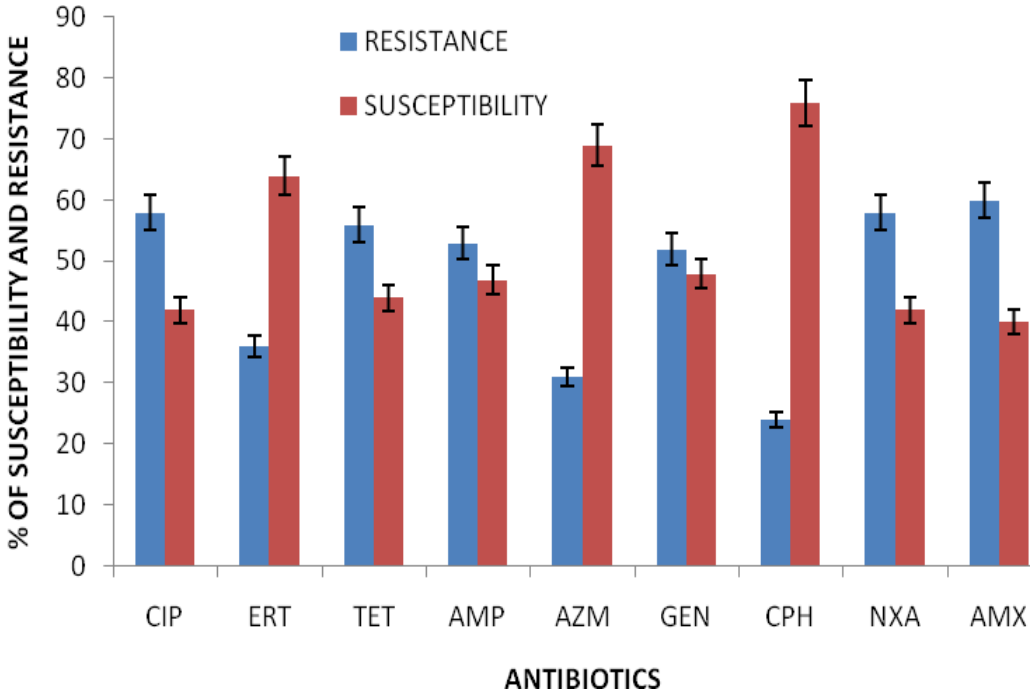

Figure 5: Antibiotic susceptibility and resistance profiles of *Campylobacter* isolates to various antibiotics

4.3.2 Multidrug resistance for *Campylobacter spp* isolates in different farms

Table 4 shows the multidrug resistance profile among *Campylobacter* spp in different farms under study. For *C. coli*, highest Multidrug resistances were found at Mafisa farm with 64% (n=19) followed 61% (n=33) and 60% (n=38) at Mazimbu and Msamvu farms, respectively. The lowest multdrug resistance for *C. coli* 44% (n=32) was found at Kihonda farm. For C. *jejuni* high multdrug resistance was found at Mazimbu farm with 60% (n=27) and the lowest multdrug resistance was found at Msamvu with 33% (n=12). In farms with mixed co infection (*C. coli/C. jejuni*), the highest multdrug resistance of 70% (n=19) was found at Kihonda farm while the lowest was found at Mafisa farm with 22% (n=2).

Table 4: Multidrug resistance of *Campylobacter* spp at different farms

Farm/Spp	Antimicrobial Susceptibility Status		
	Resistance	Susceptibiliy	Total
MAZIMBU			
C.coli	33 (61%)	21(39%)	54 (100%)
C.jejuni	27 (60%)	18(40%)	45(100%)
C.jejuni/C.coli	11 (40%)	16 (40%)	27(100%)
KIHONDA			
C.coli	32(44%)	40(56%)	72(100%)
C.jejuni	22(48%)	23(52%	45(100%)
C.jejuni/C.coli	19(70%)	8 (30%)	27(100%)
MAFISA			
C.coli	29(64%)	29 (36%)	45(100%)
C.jejuni	16(44%)	20 (56%)	36(100%)
C.jejuni/C.coli	2(22%)	7 (78%)	9(100%)
MSAMVU			
C.coli	38(60%)	25 (40%)	63(100%)
C.jejuni	12(33%)	24(67%)	36(100%)
C.jejuni/C.coli	10(55%)	8 (45%)	18(100%)

4.3.3 *Campylobacter spp* susceptability and resistance to various antibiotics

Results from the *Campylobacter spp* isolates checked for their antibiotic resistance, the resistance for C. *coli* varied from Nalidixic acid 50 %, followed by tetracycline and Ampicilin with 46 % each. Antibiotic resistance*s* for *C. jejuni* were 66% for Ciproflaxin, 60% for Tetracycine and 55% for Nalidixic acid. No significant antibiotic resistance differences were observed between *C. jejuni* and *C. coli* isolates examined in this study (Table 5).

Table 5: Comparative resistance between *Campylobacter* spp isolates for different antibiotics

Antibiotic	Proportional of resistant isolates (%)		P-value
	C. coli (n=26)	*C. jejuni (n=18)*	
CIP	42.3	66	0.2149
ERY	34.6	33	0.8309
TET	46	61	0.5013
AMP	46	50	0.9635
AZM	15	44	0.0730
GEN	30.7	33	0.8663
CHL	26.9	33	0.9202
NAL	50	55	0.9584
AMX	30	44.4	0.5096

Key: CIP; Ciprofloxan, ERY; erythromycin, TET; Tetracycline, AZM, Azithromycine; AMP; Ampicillin; GEN;Gentamycine, CHL; Chloramphenicol; NAL; Nalidixic acid; AMX; Amoxyclin

4.3.4 Comparative of antibiotic resistance between those frequentlt used and less used in broiler production

Comparisons of the antibiotics tested to the isolates that were found to be used by the farmers in the broiler production and those which were tested and not commonly used in the broiler. Almost all antibiotics that were found to be used by the farmers in broiler production and other domestic animals were found to have high resistance (more than 50%) compared to those that are not used in the broiler production only Nalidixic acid and Ciproflaxin were found to have more than 50%.

Table 6: Comparative of antibiotic resistance proportion (%) between those used in broiler production in and those not used in Morogoro Municipal, Tanzania

Antibiotics used in broiler and animal production	Proportion of resistance (%)	Antibiotics not used in broiler production	Proportion of resistance (%)
Tetracycline	56.0	Ciproflaxin	58.0
Ampicilin	53.0	Nalidixic acid	58.0
Gentamycine	52.0	Erythromycine	36.0
Amoxicilin	53.0	Azithromycine	31.0
		Chloramphenical	24.0

Ampicillins, Gentamycin and Amoxylin are less commonly used in treatment of chicken. Among the drugs tested, Tetracyclines are commonly used in chicken treatment, but the others are widely used in human medical practices. This compounded with the fact Campylobateriosis is a zoononic problem; birds are also a victim of harbouring very resistant strains of *Campylobacter* emanating from people and their surroundings environment.

CHAPTER FIVE

5.0 DISCUSSION

Molecular epidemiology and the current antimicrobial susceptibility of thermophilic *Campylobacter spp* were investigated in four broiler farms in Morogoro Municipal, Tanzania. This study was driven by the fact that, as the broiler farming has continued to grow fast in the municipal over the recent years, broiler rearing systems and supply of broiler meat to the various food chains as remained trivial, uncontrolled and in most cases done by well-to-do families with limited biosafety and management practices. In most cases the birds are raised in poorly ventilated houses, overstocked to maximize profits and antibiotics use is extensively done as a measure of reducing uncontrolled mortalities. These factors do predispose to the colonization of thermophilic *Campylobacter jejuni/coli* at all broiler production levels (Rushton *et al*., 2009, Lu *et al.,* 2013)

All the households selected in this study raised broiler in their family compounds as a source of extra income, using poorly designed and ventilated housing for chicken, some using rooms designated for human livelihood to raise the birds. Such practices are wide spread and these farms are the major supplier of finished broiler meat in Morogoro Municipality as well as many towns and cities in Tanzania (Nonga and Muhairwa, 2009). The day old chicks are bought from established hatcheries in Dar es Salaam and feeding is done by using both commercial and locally produced feeds whose biosafety is also questionable (Hunters *et al.,* 2009). The birds rearing cycle ranged between 6-7 weeks with an interlude of less than a week in all these farms. All farmers involved in this study sold their finished chicken in local markets ranging from street vendors, established retail shops and catering businesses.

5.1 *Campylobacter* prevalence in Morogoro Municipal, Tanzania

This study has shown that *C. jejuni /C. coli* colonization was high in all farms studied and this mainly commenced in chicks older than one week and continued unabated until slaughter and sold to local customers. General prevailing conditions which were common in all farms which could contribute to the observed high prevalence included poor building structures with also lacked repairs and house building repair, poor hygiene and staff lacked the right working gear, improper cleansing and disinfection regimes of flocks houses, inadequate use of litter material, close vicinity with human premises, human traffic and contaminated water supplies include poorly cleaned water pipes and polluted stored water. It has been shown in other studies done in Morogoro and other areas in Tanzania that *Campylobacter spp* are found in air, water, litter, feed and all domestic animals which surrounds the poultry (Mdegela *et al.,* 2006; Nonga and Muhairwa, 2009; Nonga *et al.,* 2009; Jacob *et al.,* 2011).

It was further observed that the broiler slaughter processes done at farm level in all farms were contributing to the contamination of *Campylobacter* to the carcasses ready to enter the local food chains. Following slaughter, birds were plucked followed by washing/rising of carcasses with warm water as the results farms were confirmed to harbor *Campylobacter*-positive flocks. These concured with other studies which showed that carcasses process contributed to the high prevalence of *Campylobacte*r in both domestic animals and other meat animal's consumer (Allen *et al*., 2007: Alter *et al*., 2005).

5.2 Comparisons of prevalence of *Campylobacter* between farms

*Campylobacte*r contamination differed significantly between farms studied with highest prevalence found in Kihonda farm (82%) followed by Msamvu farm (63%), Mafisa farm (53%) and the lowest in Mazimbu farm (47%). In contrast to Mazimbu farms which only

raised broilers in their premises, in both Kihonda and Msamvu farms, farmers kept also other animals within the premises including pigs, dogs, cattle, goats and local chicken which may compromise biosecurity. Reports from other studies have shown that *Campylobacter* prevalence differ from farm to farm, depending on many factors like sampling locality, seasonality, types of poultry (broiler or layers) and management system (Kazwala *et al.,* 1990; Nonga and Muhairwa, 2009) but integrated farming where various species of animals were raised in the same compound under compromised biosecurity especially at Kihonda and Msamvu farms could also be the factor for high prevalence of *Campylobacter*. Other factors which contribute to the high prevalence of *Campylobacter* are use of manure for gardening outside their home premises and use of ordinary earth floor rather than cemented floor (Cardinale *et al*., 2004; Zweifel *et al.,* 2008). The reasons above could also explain why Kihonda and Mafisa farms had more prevalence of *C. jejuni and C. coli*, than other farms, respectively. The high contamination of *C.coli* observed in broiler carcasses at Msamvu farm (65%) and the lowest at Kihonda farm (33%) showed a corresponding trend observed in *C. coli* prevalence in these two farms. Similar results for *C. jejuni* showed highest prevalence for Mafisa farm (40%) and lowest at Kihonda farm (33%).

Frequency overuse of the same premises and equipment for slaughtering at Msamvu farm may have possibly contributed to high contamination of *Campylobacter* in carcasses compared to other farms. Finding from the present farms, shows that among 340 *Campylobacter* isolates by culture, 57% (n=193) were negative by biochemical test which were confirmed to be positive on polymerase chain reaction and therefore these confirm the low identification and discrimination power of the convectional biochemical tests compared to those DNA-based techniques (Mdegela *et al*., 2006). Therefore DNA-based techniques should be advocated to use after culture.

While a number of studies that have assessed the prevalence *Campylobacter* spp, have shown that *C. jejuni* is frequently isolated than *C. coli* in both carcasses, feces, water and in environment (Nonga 2005; Mdegela *et al.,* 2006; Sheppard *et al,* 2009, Makela *et al*., 2011), these studies have shown a reverse trend. *Campylobacter coli* showed higher prevalence in both feces (73.6%) and carcasses (43.4) across farms under study compared to *C. jejuni* with 69.2% and 36.8% prevalence in feces and carcasses, respectively.

The reasons behind were type of *Campylobacter spp* circulating in the environment, sampling technique, laboratory methodology employed, animal/breed that investigated, area or geographical position, and seasonality (Pezzoti *et al.,* 2002; Pezzoti *et al.,* 2003, William and Oyazaba, 2012; Whiley *et al*., 2013). Apart from above factors, other could possibly be production practice, environment change and increased number of challenges to the biosecurity barrier (Nonga, 2005). Also *Campylobacter* resistance have been found to persist in farms as contaminants in poultry products even in the absence of the use of antibiotics due to poor hygiene practices, insufficient biosecurity measures in the farms (Moore *et al*., 2006). Contamination may also be due to house water distribution system, *Campylobacter* that reside in protozoa which contaminate water distribution system, wild birds and house flies (Snelling *et al.,* 2005; Price *at al*., 2007, Hakanen *et al*., 2003).

5.3 Prevalence of *Campylobacter* spp by Age

The findings from this study have shown with the exception of Mafisa farm that *Campylobacter* colonization started as early as first week in the other three farms. In studies done elsewhere (Sahin *et al.,* 2001; Newell and Feanley, 2003) broiler chicks have been reported to be free of *Campylobacte*r during the first week and colonization become detected after one week because of the presence of maternal immunity or antibodies. Contributing factors to the study could be due to poor hygiene in the poultry

houses and close poor brooding of the chicks during the first week. Use of the same litter material from the previous batch and because of the high cost of feed container and most of them use low cost designed material (wood made container) for feeding their birds (Achen *et al*., 1998; Shreeve *et al*., 2000)

It was further observed that *Campylobacter* colonization peaking in all farms around the fifth week, the age at which finisher birds were starting to be sold. Indeed these results correspond with other reports which have shown that *Campylobacter spp* load and colonization can increase up to 10^7 or 10^{10} colony-forming units (CFU) per gram of feces during the fifth week (Sahin *et al*., 2002; Nonga, 2005; Dhillon *et al*., 2006). In the study farms, hygiene was generally poor; the bird stocking density was high and there was poor ventilation of the houses. These facts made the litter to be wet fast due to excessive moisture in the rooms. There are several reasons which explain the high colonization of *Campylobacter* spp observed during the third and fifth week, which include risk of exposure to the environment, contaminant entering a broiler house which increases over time, cross infection of broiler chicken and cattle by same *Campylobacter* strain and *Campylobacter spp* with different prevalence (Meisens *et al*., 2009, Emer *et al*., 2011 Kazwala *et al,* 1993, Kashoma *et al*., 2014).

5.4 *Campylobacter* in Carcasses

In study farms, all broiler farmers sold dressed carcasses to the street vendors, hotel and others people for different uses. This practice is common to majority of traditional poultry keepers since slaughtering of all species of birds is not centralized in Morogoro Mucipality as well as other places in Tanzania. This is due to the lack of abattoirs, slaughter slabs or defined area for slaughtering the poultry in many places. These practices favored the uncontrolled spread of various forms of infectious agents

including *Campylobacter* species and their contaminations to birds dressed carcasses. The outcome is shown in results from these studies, which shows high carcasses contamination with prevalence of *C. coli* of 42%, followed by *C. jejuni* 35% and co-infection of *C. coli/jejuni* were 23%. Different studies have reported high contaminations of broiler carcasses during defeathering and evisceration which is thought to be an important cross contamination stage (Oosterom *et al*., 1983; Corry and Atabay, 2001; Figueroa *et al*., 2009, Rahimi *et al*., 2010). More than one type of *Campylobacter* spp (*C. coli* and *C. jejuni* and co-infection) was found in carcasses during the study, which is in agreement with Newell *et al*. (2001) that, carcasses contamination with *Campylobacter* strains does not appear as single strain but it appear as multiple because carcasses are in touch with many contaminants.

Also *Campylobacter* contamination comes from many sources and others have reported contaminations from slaughterhouse, environment surrounding broiler houses and from contaminated equipment used during slaughter, rupture of intestine during processing and transport (Davies and Conner, 2007). *Campylobacter spp* following contaminate they persist in carcasses for a long time because *Campylobacter* adhere on skin surface of the host by physicochemical mechanisms, where they create permanent structural bonds which form a biological film like structure which is difficult to remove unless rinsed by clean water immediately after contamination (Sanders *et al*., 2007) .

5.5 Antimicrobial Resistance and Susceptibility

5.5.1 Use of antibiotics that cause resistance

Commercial poultry production system like the one practiced in the study farms advocated the use of mass medication/treatment of poultry whenever the need arises. Antibiotics are mixed in water or feed given to birds daily for the duration prescribed by

the drug manufactures. This approach though easy to practice sometimes results in individual broiler poultry receiving inadequate curative doses, excessive or between excessive and curative but not required doses, even the non suffering one will be medicated (Shea, 2004; Besta and Essack, 2008). It was also observed that treatment of birds to incidental diseases in the study farms was done by households members who had no knowledge of safe handling and use of these drugs, lacked animal health extension services, and the use of drugs bought from Veterinary shops along the Municipal on cases which were unconfirmed. Hence use and misuse of drugs was high which could predispose to the creation of the observed high thermophilic *Campylobacter* resistance or reservoir of antibiotics.

Inadequate knowledge on the use of veterinary drugs for treatments among farmers, poor prescription given to the broiler client by drugs seller or veterinary shops/center dealer, use of drugs without considering the number of days and poor management of antibiotics distribution by local government authority are the factors which have been reported to contribute to the antibiotics resistance in poultry (Gouali`e *et al*., 2009; Haruna *et al*., 2012). Among nine antibiotics tested namely, Ciproflaxin, Tetracycline, Ampicilin, Gentamycin, Nalidixic acid, Amoxicillin, Erythromycin, Azithromycin and Chloramphenicol, only the Teracyclines preparations are commonly used in broiler rearing while others antibiotics tested are used mainly in human medical practices. Among these antibiotics tested highest resistance of *Campylobacter spp* isolates were from tetracycline. Similar observations have been reported by Nonga and Muhairwa 2009, under the study of antibiotics resistance of *Campylobacter* in poultry in Morogoro. The high resistance to tetracycline drugs observed in this study was probably due to the fact that, they are most extensively used in veterinary practice in general not only in Morogoro but the whole parts of Tanzania (Nonga and Muhairwa, 2009). It has been

shown by Nachamkin *et al*., 2000 that, highest resistances of tetracycline are the results of overuse of antibiotic in animals which might cause the *Campylobacter* to acquire new resistance gene for resistance. Tetracyclines on the other hand, have also been used extensively for many years in the treatment of humans infected with *C. jejuni* and *C. coli* which could be the reasons for high resistance observed in this study (Luangtongkum *et al*., 2006; Lance *et al*., 2007; Besta and Essack, 2008).

Amoxycillin and Ampicilin tested are also used in poultry medication. They have an extensive use in both Veterinary and Human practices (but less used in broiler) and coincidentally, birds seem to be at the bottom of this mess, being colonized by already resistant strains of C. *jejuni/coli* emanating from their surroundings shed my animals, humans etc. There was clear evidence from this study which showed that although birds were not routinely treated by many of these antibiotics, they do carry resistant *Campylobacter* strains to many of the common antibiotics used for human and veterinary practices. Hence broiler farms served as one of the easier routes of circulating resistance *Campylobacter* between humans, animals and birds (Aydin *et al.,* 2001).

Campylobacter showed high resistance to Ciproflaxin and Nalidic acid tested with 58% each. These are commonly used antibiotics in human *Campylobacter* treatment. High resistance to Ciproflaxin (58%) observed in these study were caused by topoisomerases mutations in Ciproflaxin resistance determining gene (*gyrA* gene) (Nachamkin *et al.,* 2000; Haruna *et al.,* 2011). Erythromycin, Azithromycin and Chloramphenicol are drug of choice for treatment of Campylobacteriosis in human at any clinical state (acute, subacute and chronic) (Skirrow and Blaser, 2000; Yildirim *et al*., 2005: Taremi *et al*., 2006) but are not commonly used in Campylobacteriosis treatment in poultry (Payot *et al*., 2006).

5.6 Limitation of the Study

Two of the major limitation in this study are

i. The study did not sample other domestics animals kept in the broiler poultry farms. This would be of interest to show the circulating Campylobacter spp, not only that but also within broilers and other domestics animals species

ii. The study did not genotype the Campylobacter spp thus definitive dynamics of transmission with poultry and other domestics animals could not be established.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The present study has shown high prevalence of *C. coli* and *C. jejuni* in both carcasses and feces in broiler poultry in study farms. *Campylobacter* colonization started as early as the first week of broiler chick life and peaks at the time the birds are ready for sale. This resulted in marked *Campylobacter* contamination in broiler dressed carcasses ready to enter the human food chain. The rearing of birds was done by untrained persons as a source of household income and within their home premises, which in some farms also reared number of other animal species including cattle, sheep, goats, pigs, local chicken, cat, dog, turkey and pigeon. This may increases the chance of cross transfer of *Campylobacter* spp among various animal species in the premises including broiler birds.

It was further observed that, all broilers across farms were colonized by *C. jenuni/coli* which showed marked resistance to the antibiotics tested during this study. Observation was of great interest due the fact that apart from Tetracycline which is extensively used in broiler management in Morogoro, other drugs checked for resistance namely Ciproflaxin, Ampicilin, Gentamycin, Nalidixic acid, Amoxicilin, Erythromycin, Azithromycin and Chloramphenicol are not. These drugs are however used in control and treatment of various infectious agents in other animal species as well as humans. These results confirmed that broiler chicken in these farms and possibly in other farms in Morogoro and elsewhere in Tanzania are colonized by already highly multi-drug resistant *Campylobacter* species. The broilers are possibly serving as a vehicle of re-circulating these resistant *Campylobacter spp* to other animals and humans via their contaminated feces and dressed carcasses.

6.2 Recommendations

I. During longitudinal study of *Campylobacter*, when sampling is done in broiler, also all animals that surround the household should be sampled. This will help to understand the type of *Campylobacter* circulating in the region. Moreover, *Campylobacter* genotyping would be of interest to show the dynamic transmission within and between domestic animals.

II. Poultry farmers in general require well organized national and local programs to educate and train them on best practices in poultry management, housing, extension services etc to try to improve management and control major diseases some of which like Campylobacteriosis poses health risks.

III. Since broiler meat is widely consumed in many avenues in Morogoro and in Tanzania in general and broilers are easy to rise and their meat is relatively cheaper than many other protein sources, it is a time now to modernize the broiler finishing process and its entry into the human food chain.

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