UNIVERSITY FOR DEVELOPMENT STUDIES

CHARACTERISATION AND ANTIBIOTIC RESISTANCE PATTERN OF *Campylobacter jejuni* AND NON-*jejuni* sp. ISOLATED FROM POULTRY AND HUMANS IN THE NORTHERN REGION OF GHANA

BY

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DECLARATION

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in the university or elsewhere. Research works that were consulted have been duly acknowledged by way of references.

…….

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ABSTRACT

Campylobacter are known to be a leading cause of human bacterial gastroenteritis. This research to some extent reveals the trend of prevalence and antibiotic resistance among humans and poultry in the sampled area and thus, contributes to reports on *Campylobacter* in the Northern Ghana, which may further form the basis for future policies on management of Campylobacters in the country. The aim of this study was to characterise *Campylobacter jejuni* and Non-*jejuni* sp. isolated from poultry and humans in the Northern Region of Ghana and determine their antibiotic resistance patterns. One thousand and eighty-seven (1,087) samples comprising 346 cloacal swabs from poultry and 741 stool samples from humans were investigated. The poultry samples were sourced from commercial farms and households while human samples came from patients at Tamale Teaching hospital, Tamale Central hospital and healthy individuals in their households. Sampling took place from 25th October, 2017 to 7th May, 2018. Selective agar (Charcoal Cefoperazone Deoxycholate Agar) was used to isolate the *Campylobacter* species under microaerophilic conditions following confirmation with microscopy, catalase test, oxidase test and latex agglutination immunoassay using Thermo Scientific *Campylobacter* Test Kits (Oxoid Ltd., Basingstoke, UK). Lior`s Biotyping Scheme was employed in the detection of the *C. jejuni* biotypes. Kirby-Bauer disk diffusion method was used in determining the resistance profile of the species. Laboratory results and data were entered into excel and analysed with IBM SPSS version 20. Of the total 1,087 samples analysed, 245 (22.5%) were confirmed positive for *Campylobacter* with 149 (43.1%) and 96 (13%) isolates from poultry and humans respectively, with significant difference ($P< 0.05$). However, 105 Campylobacters of the total 245 confirmed isolates were speciated, biotyped and analysed for the susceptibility testing due to viable but non-culturable

challenges pose by the others. The study further identified 68.6% (72/105) *C. jejuni* strains (58 isolates from poultry and 14 in humans) and 31.4% (33/105) Non-*jejuni* sp. (17 strains from poultry and 16 in humans). The biotypes identified were 29 (19 in poultry and 10 in humans) biotype-I, 31 (30 in poultry and 1 in humans) biotype-II, 10 (7 in poultry and 3 in humans) biotype-III and 2 (from poultry) biotype-IV. The *C. jejuni* biotype-II was prevalent in both poultry and humans. Of the 105 *Campylobacter* strains, the highest level of resistance was recorded against tetracycline (100%) while the least resisted antimicrobial was imipenem 6.7% (7/105). The isolates also recorded resistance above the rate of 50% with an observed resistance range of 56.2-93.3% to four of the antibiotics used (ciprofloxacin, erythromycin, ampicillin and ceftriaxone). Multidrug resistance rate of 96.2% (101/105) was recorded across poultry and human *Campylobacter* strains. *Campylobacter* colonisations in poultry and humans along with their high resistance profile to the commonly used antibiotics are major public health issues demanding nationwide attention.

DEDICATION

This thesis is dedicated to the Almighty God for His grace, mercy and guidance throughout my academic pursuit. I also dedicate this thesis to Dr. Courage K. S. Saba who has played a role of more than a father in my life since I met him, may the good Lord richly bless you. I further dedicate this work to my parents Mr. Mark Wilson Kpordze and Mrs. Gladys Ntim, Mr. Antwi Mensah and Miss Vera Arthur for their constructive advice, love and care.

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LIST OF ACRONYMS

AGPs	Antibiotic Growth Promoters
AK	Amikacin
AMP	Ampicillin
AMR	Antimicrobial Resistance
AMU	Antimicrobial Usage
Biotyp	Biotype
C	Chloramphenicol
CBA	Columbia Blood Agar
CDC	Centre for Disease Control and Prevention
CIA	Critically Important Antimicrobials
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standard Institute
CN	Gentamicin
CRO	Ceftriaxone
DNA	Deoxyribonucleic acid
E	Erythromycin
EFSA	European Food Safety Authority
EQAS	External Quality Assurance Systems
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organisation
GBS	Guillain-Barre Syndrome

GMI	Ganoderma Microsporum Immunomodulatory
HCl	Hydrochloric acid
HSPs	Heat Shock Proteins
I	Intermediate
IPM	Imipenem
ISO	International Organisation for Standardization
mCCDA	modified Charcoal Cefoperazone Deoxycholate Agar
CCDA	Charcoal Cefoperazone Deoxycholate Agar
MDR	Multidrug Resistant
MG	Methyl Green
N	Intermediate not Available
NA	Nalidixic acid
No.	Number
NOR	Norfloxacin
OIE	Office International des Epizooties
PCR	Polymerase Chain Reaction
R	Resistant
RNA	Ribonucleic acid
S	Sensitive
SARI	Savannah Agricultural Research Institute
SEA	Southeast Asia
SXT	Sulphamethoxazole/Trimethoprim
TCS	Two-Component Systems
TE	Tetracycline
TSI	Triple Sugar Iron

UDS	University for Development Studies
UK	United Kingdom
USA	United State of America
UV	Ultra Violet
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 Background

Our global society has faced challenges of health care costs, morbidity and loss of productivity through effects from pathogens. Knowing and understanding their prevalence, epidemiology and pathogenicity therefore remains important (Minor *et al*., 2015; Jordan *et al*., 2016). Bacterial infections and aetiology of diseases have globally been recognized with the most shared source of gastroenteritis in humans attributed to *Campylobacter* and its species (Anon, 2012; Kaakoush *et al*., 2015; Acuff and Dickson, 2017). A report by European Food Safety Authority (EFSA) in 2014 identified Campylobacteriosis as the most common bacterial disease among the European Union. In 2010, a rate of 48.56 per 100,000 population was recorded and a confirmed incidence of 212,064 cases were reported (Anon, 2012). Also, an estimation of approximately 9 million human cases of Campylobacteriosis per year in 27 countries in European Union (EU27) has been recorded (Andreoletti *et al.,* 2011; Adley and Ryan, 2016).

Cases usually point to *Campylobacter jejuni* and other non-*jejuni* sp. like *C. coli* as primary sources of human cases of Campylobacteriosis and generally linked to poultry as most important reservoirs for *Campylobacter* despite Campylobacters being commensal in other food producing animals like pigs, sheep, cattle, goats among others (Qin *et al.,* 2011; Szygalski-Biasi *et al.,* 2011; Anon, 2012; Quintana-Hayashi and Thakur 2012; Poly *et al*., 2015; Nohra, 2017; Weis *et al*., 2017).

Poultry and poultry products remain the most imperative sources of *Campylobacter* infection. Well documented studies on their occurrence confirms the bacteria are commonly isolated throughout poultry production, such as rearing and butchery

(Kloska *et al.*, 2017; Abubakar *et al.*, 2019). Estimations from human Campylobacteriosis cases indicate that *Campylobacter jejuni* (*C. jejuni*) is responsible for about 90% of cases, and the remaining attributed to the other non-*jejuni* group with majority related to *Campylobacter coli* (*C. coli*) (Weis *et al.*, 2017; Alarjani, 2019). Naito *et al.* (2010) and Buchanan (2018), share this claim by stating that *Campylobacter jejuni* is the most important cause of bacterial zoonotic enteric infections in industrialized countries.

Individuals such as the elderly, young children and immunocompromised stand most at risk of acquiring Campylobacteriosis with relatively low infectious doses (100-500 cells) reported to be capable of causing the illness with clinical features of fever, abdominal cramps and diarrhoea which often become bloody after one or two days (Basardien, 2012). In severe cases of infection, complications may arise and examples of such are pancreatitis, septicaemia, meningitis, hepatitis, endocarditis, paralytic disease and Guillain-Barré Syndrome (Hu and Kopecko, 2018). However, development of Guillain-Barré syndrome (GBS) which is an autoimmune disease affecting the peripheral nervous system remains the most serious complication of *Campylobacter jejuni* infection though it is thought to occur in approximately 1 in every infected 1000 individuals (Lehmann *et al.*, 2017; Hu and Kopecko, 2018; El-Radhi, 2018). Campylobacteriosis as an ailment is usually self-limiting but treatment with antibiotics is very essential in the severe cases described.

Prevalence of *Campylobacter* sp. varies significantly depending on type of animal production, country as well as the methods used in detection. However, reviews and reports have shown several prevalence rate of *Campylobacter* sp. from various sources in different geographic regions. In Africa, records reveal a range from 2-27.5% prevalence in humans and a percentage range from 2-21% rate shown for children under

five years, while poultry prevalence rate for *Campylobacter* sp. also lies in a range from 14.4-96% (Krumkamp *et al.*, 2015; Nguyen *et al.*, 2016; Karikari *et al.*, 2017; Asuming-Bediako et *al.*, 2019).

From Kaakoush *et al.* (2015), global incidence of *Campylobacter* sp. in humans ranges from 0-41.3% in South America, 4.48-14.9% in Asia and Middle East, and 71.4% in Europe (de Boer et *al.*, 2013). In animals, the review by Kaakoush *et al.* (2015) further revealed prevalence ranges of 32.8-85% rate in pigs and piglets, 6.8-17.5% rate in sheep, approximately 58% in healthy and 97% in diarrhoeal dogs, 16 to ~90% in cattle and 72.9% in poultry (Chaban *et al.*, 2010; Kittl *et al.*, 2013; Food Standards Agency, 2014; Boysen *et al.*, 2014).

There is an observed increment in *Campylobacter* resistance in some studies to quinolones, macrolides and other antimicrobials (Smole-Mozina *et al.*, 2011; Lluque *et al*., 2017). Through foods of animal origin, the resilient isolates are transferred to humans since food-producing animals like poultry, cattle and pigs are possible sources (Quintana-Hayashi and Thakur, 2012; Egger *et al.*, 2012; Alba *et al*., 2018). This is mostly serious with strains that are resistant to quinolones and macrolides which in severe immunocompromised patients and human infections are commonly used for therapeutic purposes (Lluque *et al*., 2017).

1.2 Problem Statement

The commercial poultry and free-living birds are natural reservoirs of thermophilic Campylobacters which have also been isolated from numerous bird species like *Columbiformes*, *Galliformes* and *Anseriformes* that are domestic (Nguyen, 2016). *Campylobacter jejuni* has been found in all areas of commercial poultry production (Agunos *et al.*, 2014; Sahin *et al.*, 2017).

Although, the relationship between Campylobacters and the health of poultry is insignificant yet foodborne gastroenteritis in humans worldwide are predominantly attributed to poultry and poultry products such as contaminated meat which is recognized as the leading cause of human infections (Hermans *et al.*, 2012; Sahin *et al.*, 2017).

Incidence of Campylobacteriosis has taken over Salmonellosis in some countries mostly under the European Union and continues to rise in many other countries (Ketley, 1997; Stingl *et al.*, 2012). According to recent reports by European Food Safety Authority, Campylobacteriosis exceeded *Salmonellosis*, *Escherichia coli* O157:H7, and *Shigellosis* infections in humans (EFSA, 2011). However, providing explanations to this is not very simple.

In several African countries including Ghana, national surveillance reports on Campylobacteriosis outbreaks or prevalence are virtually non-existent, unlike other parts of the world. Surveillance studies on outbreaks of Campylobacteriosis along with other diseases which are done is of key interest to national development and typical instances are that of the EU through their EFSA and the USA also through the Centre for Disease Prevention and Control (CDC).

Antibiotics are not only used in therapeutic treatment of human and animal infections but also, to enhance the growth and performance of food producing animals. They are used in sub-therapeutic doses as Antibiotic Growth Promoters (AGPs) especially on commercial production besides their observed use in local home-based treatments for animals. However, this act of sub-therapeutic use as AGPs and small-scale home-based treatment for animals has contributed enormously to bacteria acquiring resistance to the antibiotics used.

In recent years, resistance against antibiotics in bacteria is of communal health concern and there has been an observational surge in *Campylobacter* resistance, mainly to macrolides (erythromycin), quinolones (ciprofloxacin), as well as to other antimicrobials (Smole-Mozina *et al.*, 2011; Lluque *et al*., 2017). These are the most frequently used drugs in treatment of Campylobacteriosis and also extensively used for therapy in severe human contagions or in immuno-compromised patients (Blaser and Engberg, 2008; Sreeja *et al*., 2017). Food-producing animals are the most targeted home of such resistant strains that are transferred to humans through food of animal source (Quintana-Hayashi and Thakur, 2012; Egger *et al.*, 2012; Economou and Gousia, 2015; Hoelzer *et al*., 2017).

In *Campylobacter*, antibiotic resistance shows high potential threat to consumers since the resistant population is determined by the use of antimicrobials in animals (Ferri *et al*., 2017).

Therapies for *Campylobacter* sp. intestinal infections are usually not required due to their self-limiting nature but exceptions remain in cases of severity of the disease, in patients that are immunocompromised and non-intestinal infections (Vu, 2018).

Macrolides and fluoroquinolone antibiotics especially erythromycin and ciprofloxacin respectively are frequently used for treatment and prophylaxis (Abbasi *et al*., 2019) but after fluoroquinolone introduction in the 1990`s into veterinary medicine, there is an observed upsurge in resistance to ciprofloxacin in *Campylobacter* strains from humans and animals which may further result in treatment failure (Koga *et al*., 2017; Ge *et al.*, 2019).

In general, knowledge about *Campylobacter* in Ghana is inadequate and most of the few published information describe incidence and antibiotic resistance in

Campylobacter of human source (Abrahams *et al.,* 1990; Lunani, 2007; Krumkamp *et al.,* 2015; Karikari *et al.,* 2017a). There are no surveillance records of incidence and frequency of *Campylobacter* infections in Ghana.

It is therefore of outmost importance to determine the prevalence level of *Campylobacter* sp. in Ghanaian free range and commercial poultry (chicken), and in humans as well.

1.3 Justification

Campylobacteriosis is spread from animals to humans, hence considered a zoonotic illness and researches by Liao *et al*. (2019) and Alonso *et al.* (2011) share the idea that the risk of acquiring Campylobacteriosis from contaminated poultry or chicken greatly increases worldwide, and lies especially in rearing, handling of raw chicken, consumption of an undercooked chicken meat or cross contaminations from variations in food preparation and preferences.

As in other places, it is also uncommon to find households in Ghana, especially in rural and poor communities, rearing poultry or chicken in their homes or back yards on subsistence farming bases with significant contributions to their income (Mtileni *et al.,* 2009). The consumption of poultry especially chicken is observed on a large scale globally where maintaining provisions for minerals, protein and essential vitamins, are considered a cheaper alternative to other meat sources especially in developing countries (Silva *et al.,* 2011; Anon, 2012; Weber and Windisch, 2017). Despite chicken or poultry by-products found naturally to be contaminated with *Campylobacter* in prevalence studies; they are also widely consumed for reasons of special taste, short time needed for preparation and low price (Silva *et al*., 2011; Wong *et al*., 2017; Shrestha, 2018).

Ghana is one of such countries that are into rearing of poultry on household and commercial bases for purposes of social as hobbies, cultural (i.e. rituals, festivals, marriages), economical and nutrition. However, Ghana has limited research reports and no national annual report on *Campylobacter* sp.

In Ghana, the occurrence of *Campylobacter* in human clinical isolates has been identified in some studies; including the first isolation among children with or without diarrhoea in some selected healthcare centres in Accra by Abrahams *et al.* (1990), isolation among outpatients (infants and children) from the Agogo Presbyterian Hospital located in the Asante Akim North municipality in the Ashanti region by Krumkamp *et al.* (2015), isolation among both in-patients and out patients of Komfo Anokye Teaching Hospital in Kumasi by Karikari *et al.* (2017a). Regarding other environmental sectors, Lunani (2007) study on urban water systems in Accra where *Campylobacter* was detected is also acknowledged. Isolation of *Campylobacter* from poultry have also been reported by some researchers in Ghana; thus, Karikari *et al.* (2017b) isolated *Campylobacter* from faecal material and carcasses of commercially produced poultry in Kejetia-Kumasi, Sackey *et al.* (2001) isolated *Campylobacter* from live and dressed poultry in the Accra metropolis, and Abraham et *al.* (1990) also examined and detected *Campylobacter* sp. in local domestic fowls along with other domestic animals like sheep and goat from homes located in rural Ghana.

All these aforementioned researches were based in Southern Ghana. Whereas in Southern Ghana there are few data records, Northern Ghana has paucity of such data.

Based on previous evidence and to attain information concerning existence of Campylobacters in the geographical area of exploration, the current study was piloted to survey frequency of occurrences of Campylobacters from domestic and commercial

poultry, and humans in the Northern part of Ghana. Findings from the study may be of several significance;

i. The findings from this study to some extent will reveal the trend of antibiotic resistance among humans and poultry in the sampled area. Thus, the antibiotic resistance patterns of the *Campylobacter* strains from poultry or chicken and humans will be determined.

ii. Moreover, the study and its findings will provide data that can be used as reference material by regulatory bodies of concern in informing policy. Since the study will contribute to reports on *Campylobacter* in the Northern Ghana, it may form the basis for future policies on Campylobacters development in the country.

iii. Additionally, findings may aid in the development of health programmes; thus in most cases like Campylobacteriosis, concerns aimed at controlling the disease require adequate information on the disease which include its prevalence, causative organism`s antibiotics resistance trend, epidemiology etc.

iv. Finally, the scientific data generated is envisaged to provide an understanding of antimicrobial resistance patterns and diversity, among strains (biotypes) of *Campylobacter jejuni* and non-*jejuni* sp. from humans and poultry.

1.4 Objectives of the Study

1.4.1 General Objective

The aim of this study was characterisation and antibiotic resistance pattern of *Campylobacter jejuni* and Non-*jejuni* sp. isolated from poultry and humans in the Northern Region of Ghana.

1.4.2 Specific Objectives

The specific objectives included:

1. To investigate the prevalence of *Campylobacter jejuni* and Non-*jejuni* sp. in poultry and humans in the Tamale Metropolis and Nyankpala community.
2. To characterize by the identification of different biotypes employing Lior`s Scheme of Biotyping.
3. To investigate the resistance patterns of isolated *Campylobacter* strains to various antimicrobials.

1.5 Research Questions

This study seek out to answer the following questions;

1. How prevalent is *Campylobacter* sp. among poultry and humans?
2. What are the biotypes of *Campylobacter* sp. present in poultry and humans?
3. Are *Campylobacter* isolates from this study resistant to antibiotics?
4. What is the multidrug resistance rate and pattern of the isolates?

1.6 Study Limitation

This study had a limitation and thus, a total of 245 confirmed isolates of *Campylobacter* sp. were enumerated and stored before biotyping and performing of antibiotic susceptibility test. When time was due for the biotyping and antibiotic susceptibility test, some isolates were lost due to power problems under storage of -21 °C. Those that were still viable and confirmed were used for the process. A total of 105 *Campylobacter* isolates were still viable at the time of the tests. Hence, results used in this research for biotyping and antibiotic susceptibility pattern are dependent on the final 105 viable *Campylobacter* isolates. However, results on prevalence rate of *Campylobacter* sp. was done with respect to the initial 250 confirmed *Campylobacter* isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of the Discovery and Early History of *Campylobacter*

Vibrio-like bacteria that are non-culturable and spiral-shaped was first dated to Theodore Escherich who first made such observations in the feaces of dead children who suffered from diarrheic disease (Escherich, 1886; Buchanan, 2018). After Escherich`s observations, several other observations and identification of this spiral-shaped bacteria were also made from terminated bovine foetuses which resulted in the name *Vibrio fetus* subsp. *intestinalis* due to their morphology and origin (Al-neama, 2017). In 1953, similar observations of this shaped bacteria that was affecting the fertility of ewes and cows was made by Florent (Florent, 1953; Debruyne *et al.,* 2008; Butzler, 2018). During sexual contact, the infected cows failed to become pregnant and soon returned to heat after being serviced by the bull. This infectious infertility with a free bull symptom was spread and *Vibrio fetus* subsp. *venerealis* was found to be the organism causing such symptoms. *V. fetus* and its` associated *V. bubulus* were moved to *Campylobacter* the new genus after 1962 (Sebald and Véron, 1963; Michi *et al*., 2016). During 1973, the classification of *Vibrio*-like organisms was published by Veron and Chatelain with the inclusion of four distinct species which were *C. coli*, *C. fetus*, *C. jejuni*, *C. sputorum* subsp. *sputorum* and *C. sputorum* subsp. *bubulus* under the *Campylobacter* genus (Veron and Chatelain, 1973; Paravisi, 2017).

Method of filtration was then applied by Butzler and his associates in 1970's to isolate bacteria that resembled *Campylobacter* from diarrheic stools of humans (Butzler *et al.,* 1973; Morley, 2014). For the isolation of similar organisms at that

time from human faeces, Skirrow in the late nineteen seventies helped describe the use of a selective supplement such as antibiotic supplements (e.g. vancomycin) (Skirrow, 1977; Lévesque *et al.*, 2016).

2.2 Family Campylobacteraceae and Their Characteristics

The genera *Campylobacter*, *Arcobacter*, *Sulfurospirillum* and *Dehalospirilum* are the four main known genera found under the family *Campylobacteraceae*. The family is made up of Gram-negative bacteria, has low G-C content, micro-aerobic/microaerophilic and non-saccharolytic (Vandamme, 2000; Vandamme *et al.*, 2010; On *et al*., 2017).

2.2.1 Genus *Campylobacter*

In 1963, the named *Campylobacter* genus which belongs to the family *Campylobacteriaceae* was proposed by Sebald and Véron (1963). In recent times, the family has an overall species of 23 along with six (6) subspecies described (Silva *et al*., 2011; Zhou *et al*., 2013; García-Sánchez *et al*., 2018).

2.2.3 Species of *Campylobacter*

From Vandamme *et al.* (2010), 32 species and 13 subspecies are recognized and named under the genus *Campylobacter*. Under this genus, the *Campylobacter coli* and *Campylobacter jejuni* are mostly noted to cause infections in human whereas, other species such as the *C. fetus, C. lari, C. concisus, C. sputorum, C. hyointestinalis, C. ureolyticus, C. rectus, C. upsaliensis* and *C. gracilis* are also known as being capable of causing human infections (Man, 2011; EFSA, 2014; Liu *et al*., 2016; Cody *et al*., 2017; Aidley *et al*., 2018).

2.3 General and Morphological Characteristics of the *Campylobacter* Genus

According to Kay *et al.* (2011) *Campylobacter* sp. are recognized as Gram-negative, non-spore forming rods, has spirally or S-shaped, curved cells with 0.2 to 0.8 µm wide, and 0.5 to 5 µm long. Generally, cells of *Campylobacter* have at one or both ends the presence of a single polar unsheathed flagellum, and they move in a corkscrew-like motion (On *et al*., 2017).

Generally, *C. coli* and *C. jejuni* are well-thought-out to be livestock commensals, birds and other animals such as domestic pets like cats and dogs. Great number of *Campylobacter* isolations have been recorded in enteritic livestock of their young stages such as lambs, calves and piglets. These organisms have also been found in animals that are very healthy. Many birds and other animals both domestic and wild have their intestinal tracts colonized by *C. jejuni* and *C. coli*, and in foods, humans as well as natural waters, *Campylobacter* have been isolated (Vandamme *et al.*, 2010; Silva *et al.*, 2011; Acke *et al.*, 2011; EFSA, 2014; Matthew-Belmar *et al*., 2015; Pattis *et al*., 2017; Rouby *et al*., 2019).

Campylobacters are motile except the *C. gracillis* (Connerton and Connerton, 2017). Generally, biochemical characteristics of *Campylobacter* involves reducing fumarate to succinate, variable catalase activity, nitrate reduction, negative reaction to methyl red, the indole and acetoin production by majority of the species, oxidase activity presence except in *C. gracilis* and hippurate hydrolysis absence except in *C. jejuni* (Stern *et al.*, 1992). Moreover, Campylobacters lack the capability to ferment carbohydrates and hence through the reduction of tricarboxylic acid intermediates, energy is utilised with amino acids as the main substrates (Smibert, 1984; Vegge *et al*., 2016).

Campylobacters do not require hydrogen as it is importantly needed by its closely related bacteria *Helicobacter pylori* (Perez-Perez and Blaser, 1991). An optimum temperature for *Campylobacter* growth is 42 °C and cells do not possess the ability to grow below 25 °C; hence the name thermophilic Campylobacters (Vandamme *et al*., 2015; Natsos *et al*., 2019). From studies, lecithinase or lipase action is absent and the G-C content of their DNA falls within 29-47% (Debruyne *et al.*, 2008; Vandamme *et al*., 2015).

In the identification of pathogenic *Campylobacter*, hippurate test remain the most widely used biochemical assay and it helps detect *C. jejuni* which possess *hipO* gene whose product hydrolyses hippurate to benzoate and glycine (Oyarzabal and Carrillo, 2017; Nguyen, 2017).

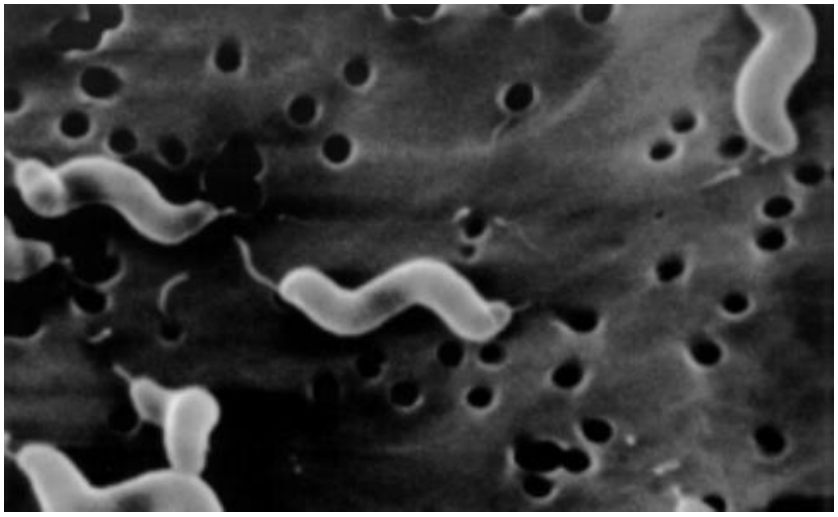


Figure 2. 1: Scanned Electron Micrograph of the Corkscrew Shape and Single Polar Flagellum of *Campylobacter jejuni* (Sean *et al.*, 1999)

2.4 *Campylobacter* Outbreaks and Campylobacteriosis

Campylobacter is indicated to be the causative agent commonly for bacterial gastroenteritis globally. In the EU and other countries, the most unceasingly reported zoonosis which is Campylobacteriosis in humans is mainly caused by the two

Campylobacter species named; *C. jejuni* and *C. coli* (EFSA, 2015; Hald *et al*., 2016; Higham *et al*., 2018; Ocejo *et al*., 2019).

Most available data represent *Campylobacter jejuni* infections since it is projected to be responsible for 90% of Campylobacteriosis cases as the remaining proportion (10%) is attributed to *C. coli* (Janssen *et al.,* 2008; Cody *et al*., 2017; Seguino *et al*., 2018). However, estimated cases of such infections vary from one country to the other with typical example of a range of 12.7 cases per 100,000 in the USA to as high as 396 cases per 100,000 in New Zealand (Baker *et al.,* 2007; Alrubaye, 2018).

Campylobacter is the major cause of gastroenteritis in the United Kingdom and occupies the fifth position of domestically acquired foodborne infections in the United States (CDC, 2011; Barrett and Nic-Fhogartaigh, 2017). When it comes to places like Denmark, Austria, Finland, Germany, Sweden, Italy and Norway, it remains the utmost common reported foodborne disease (Urdaneta-Vargas, 2016; Nijhof, 2017).

According to Anon (2012) and Khan *et al*. (2018), one of the most recognized causes of gastroenteritis in humans from foodborne sources is *Campylobacter* and report from the European Food Safety Authority (EFSA) confirms Campylobacteriosis to be the most common foodborne bacterial disease. In 2013, cases of Campylobacteriosis confirmed in humans were 214,779 with an average of 64.8 per 100,000 of the population of the EU (EFSA, 2015).

Additionally, when it comes to incidence and prevalence of Campylobacteriosis, Kaakoush *et al.* (2015) shares a better picture using a diagram (figure 2.2) for providing information on the epidemiology of Campylobacteriosis globally from literature.

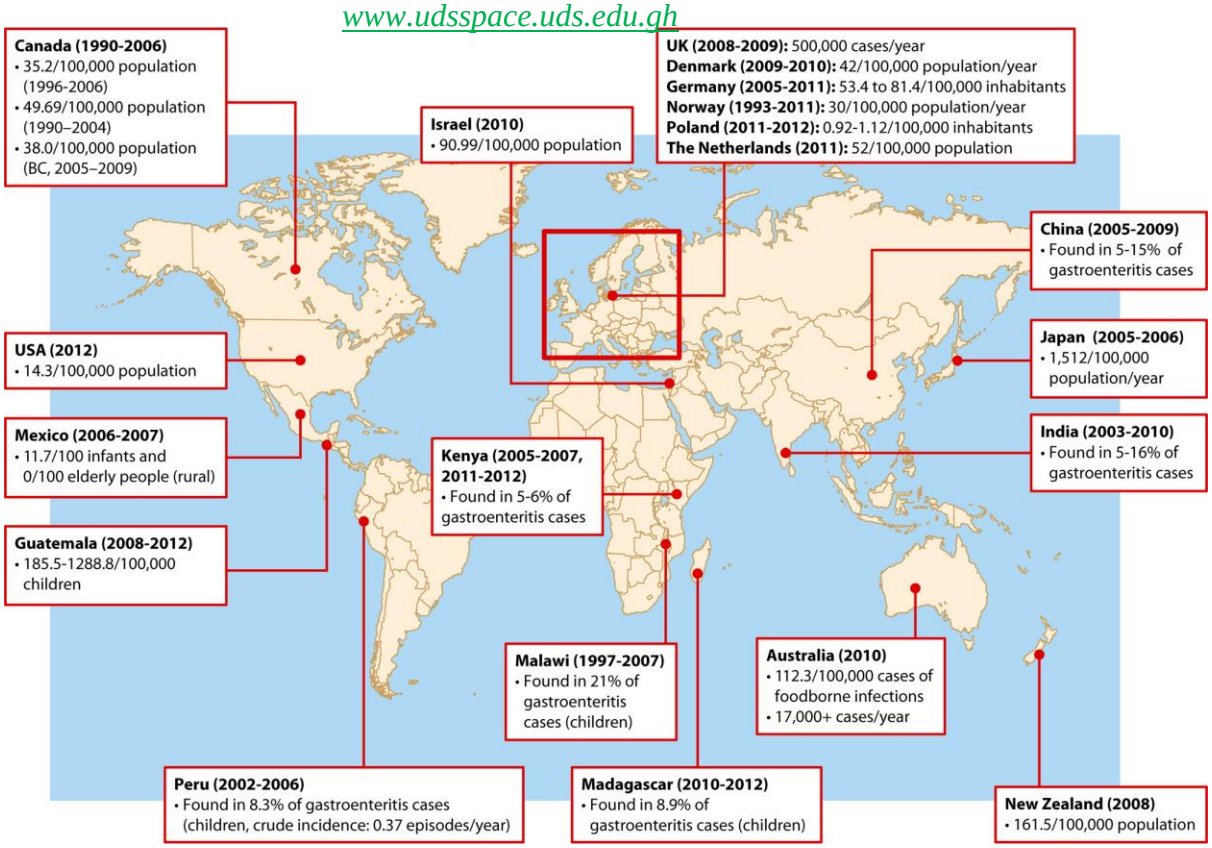


Figure 2. 2: Global Epidemiology from Literatures Giving Incidence and Prevalence of Campylobacteriosis (*C. jejuni/C. coli*) (Kaakoush *et al.*, 2015)

These number of cases are estimated based on reports and confirmation from laboratory tests which aid in projecting an estimation of unreported cases. It is very necessary in this vain because most *C. jejuni* gastroenteritis are self-limiting and as such most cases arising from such infections are usually not reported. Infections especially from *C. jejuni* is normally infrequent which is in contrast to that by other foodborne pathogens like *Salmonella* and *E. coli* O157 as their epidemics are also rarely reported beside eruptions generally linked to either drinking contaminated or untreated water (Kuusi *et al.*, 2005), and consumption of raw milk (Teunis *et al.*, 2005).

Infections from *C. jejuni* are rare and often specific interventions are not required by most patients (Hanada *et al*., 2018). *Campylobacter* infections have global occurrence and in nature, are known to be sporadic (Rahman *et al*., 2018; Upadhyay *et al*., 2019).

2.5 Clinical Presentations of *C. jejuni* and *C. coli* Infections

Acute gastroenteritis through varying degrees of abdominal cramps, diarrhoea and fever remain the very clinical presentation of both the *C. jejuni* and *C. coli* infections in humans whereas in some instance pus, mucous or fresh blood may be present in stool samples (Teunis *et al*., 2018; Dennehy, 2019). The established minimum infectious dosage in clinical research range from bacterial cells of 500-800 (Black *et al.*, 1988; Moffatt *et al*., 2017), with a typical 3-5 days period of incubation and an even longer periods of 10 days.

More so, *Campylobacter* causes diarrhoea as well as abortion in most animals and in humans, it causes gastroenteritis which ranges from mild type of diarrheal disease to severe ones (Sethi, 2017; Poly *et al*., 2019). Most often bloody diarrhoea along with other symptoms such as fever, abdominal pain and cramping especially after exposure in 2-5 days with symptoms lasting for a week (Addis and Sisay, 2015; Dennehy, 2019). Severe complications of *Campylobacter* infections range among Guillain-Barre syndrome, meningitis, reactive arthritis, endocarditis, hemolytic uraemic syndrome, osteomyelitis and neonatal sepsis; that are serious health issues and can even lead to death (Fica *et al.,* 2011; Kuwabara, 2011; Pires, 2014; Tejan *et al*., 2018).

From several complications of *Campylobacter* infections, the Guillain-Barré syndrome (GBS) condition proves to be the serious disorder caused by *C. jejuni* in the long-term (El-Zamkan and Hameed, 2016; Kowalcyk *et al*., 2018). Guillain-Barré syndrome (GBS) which is an autoimmune disease occurs during immunity where the antibodies generated is as a result from immune response to *C. jejuni* and *C. coli*`s lipo-oligosaccharide (Al-Banna *et al*., 2018; Poly *et al*., 2019). The antibodies do not differentiate human Ganoderma Microsporum Immunomodulatory (GMI) ganglioside

and the lipo-oligosaccharride of these Campylobacters; later in long term after infections are cleared, the antibodies now destroy peripheral nerve tissue causing paralysis (Kuijf *et al.,* 2007; Godschalk *et al.,* 2007). Hospitalisation may occur depending on GBS condition severity typically when it involves respiratory muscles; but if properly managed with good medical care, most patients recover (Brahmer *et al*., 2018; Green *et al*., 2018).

From Patry *et al*. (2019), one needs to understand that not all serotypes from these Campylobacters especially in *C. jejuni* are GBS associated because a number of serotypes have molecular mimicry limited like HS:19. The presence of such serotypes or others alike are able to induce these antibodies that cross-react gangliosides which certainly do not cause GBS to develop (Jiao, 2017), thus both *C. jejuni* and host play significant roles in the process. Other diseases of immune-mediated linked infections from *C. jejuni* are reactive arthritis (Vojdani and Vojdani, 2019) and urethral inflammation (Slingerland *et al*., 2017).

2.6 The Interaction of *C. jejuni* and *C. coli* with Intestinal Surfaces

According to a research by Stahl *et al*. (2016), Campylobacters in the initial stages of the infections in humans colonize the small intestine and then establish themselves in the colon. Especially in poultry or chickens, cecum is the most preferred site even though both *C. jejuni* and *C. coli* have been isolated from other parts like cloaca, large intestine and small intestine which all forms part of the gastrointestinal tract. The colon in humans and cecum in poultry or chicken have similarities in both short chain fatty acids and lactic acid (Stahl *et al*., 2016; Johnson *et al*., 2017), thereby allowing for these species thriving in such environs. Using the corkscrew shaped and darting motility by both *C. jejuni* and *C. coli*, the main barrier for defence in the

gastrointestinal tract (mucus and mucus layer) is overcome in poultry and humans (Hussein, 2018).

2.7 Sources of Campylobacteriosis and *Campylobacter*

2.7.1 Sources of Campylobacteriosis

Humans are not the natural reservoirs or hosts for Campylobacters, even though there have been enormous infections record in humans, they are recognised as accidental pathogens when it occurs in humans and typically considered a zoonotic infection that is transmitted from animal reservoirs or hosts to humans. *C. jejuni* and *C. coli* as primary sources of human cases of Campylobacteriosis are generally linked to poultry as most important reservoirs for *Campylobacter* despite them being commensal in other food-producing animals like cattle, pigs, sheep, goats among others (Anon, 2012; Sheppard and Maiden, 2015; Elmberg *et al*., 2017; Heredia and García, 2018). Pigs and cattle on the other hand are *Campylobacter* carriers despite little information concerning slaughterhouse level of their carcass contamination (Tresse *et al*., 2017).

2.7.2 Common Sources of *Campylobacter*

Poultry especially chickens remains the largest recognised human Campylobacteriosis source of infections (Strachan and Forbes, 2010; de Melo *et al*., 2016; Rosner *et al*., 2017; Thépault *et al*., 2018; Berthenet *et al*., 2019) and incidences are attributed to several factors including poultry from direct handling, contact with poultry faeces, meat preparation and handling, inadequately cooked poultry products consumption and intake of cross-contaminated foods (Gonzalez, 2017).

An evidence supporting a clear link between poultry meats and human *C. coli* or *C. jejuni* infections typically the *C. jejuni* was created by the dioxin crisis in Belgium which occurred during 1999 where foodstuffs of livestock were contaminated with

dioxin. This caused the removal of all Belgian poultry products from sales and in the course, there was an observed *Campylobacter* infections drop in documentation by 40% by the routine surveillance programme (Vellinga and Van Loock, 2002). However, cases of *Campylobacter* infections returned to usual or known "normal level" once shelves were filled back with poultry products (Vellinga and Van Loock, 2002; Newell *et al*., 2017).

2.7.3 *Campylobacter* in Food Producing Birds and Animals

Animals such as pigs, cattle and sheep are food-producing mammals that are known to be *Campylobacter* sources. Besides, establishing the fact of these animals being natural reservoirs for human importance universally remain debatable in literatures for these sources (Wagenaar *et al*., 2015; Seguino *et al*., 2018). Many authors confirm pigs as higher carriers for *C. coli* in terms of proportion to *C. jejuni* and however add that in comparison, frequency of contamination by these species in pigs is not as in poultry (Qin *et al.*, 2011; Quintana-Hayashi and Thakur, 2012; Morales-Partera *et al*., 2018). The carriage level for Campylobacters by cattle is 0-80% (Moore *et al*, 2005; Hasso and Aldraji, 2018), sheep also recorded around 20% (Yang *et al*., 2017; Ocejo *et al*., 2019), and the accepted high incidence is for pigs (ranged from 55-100%), though this may be more attributed to its carcass processing nature than the actual higher rate of carriage in their gut (Chlebicz *et al.*, 2018; Lama and Bachoon, 2018). At a slaughterhouse in Norway, Nesbakken *et al.* (2003) observed 100% *Campylobacter* sp. carriage in pigs` gastrointestinal tract, and Kempf *et al.* (2017) study observed about 70% and 55% *Campylobacter* sp. in both conventional and organic pigs in France and Sweden. Pigs are more carriers of Campylobacters than cattle and sheep, and *C. coli* is very common in pigs than the *C. jejuni* (Matthew-Belmar *et al*., 2015; Pattis *et al*.,

2017; Sahin *et al*., 2017). A study by Wieczorek and Osek (2013a) in Poland showed that 25.6% bovine hides and 2.7% carcasses were positive for *Campylobacter*.

2.8 Sources of *C. jejuni* and *C. coli* on the Poultry Farm

Understanding sources of *C. jejuni* and *C. coli* colonization is a great requirement in the development of an efficient control strategy and there are several studies that have examined such sources on the farm (Oyarzabal and Backert, 2016; Sibanda *et al*., 2018; Upadhyay *et al*., 2019). Though these studies are of great importance, yet most relied on questionnaires and surveys rather than employing the use of genetic tools in the identification of sources as well as clonal relationships among various isolates on the poultry farms.

It is generally considered horizontal transmission when the core source of *Campylobacter* spread is from surroundings to poultry flocks (Hald *et al.*, 2008). According to Andrew *et al.* (2013), the sources of *C. jejuni* and *C. coli* on the poultry farm could be through water, insects, presence of animals other than poultry on farm, and the general farm practices and human movement. A clear illustration of these sources by Andrew *et al.* are seen in figure 2.3.

2.8.1 Water

In the poultry industry, practicing water chlorination has been very common and the usual dose of chlorine used in treating the water is lethal for Campylobacters but detection for contamination is usually observed in water later when flock is shed; thus water might have been contaminated with the organisms excreted from the birds (Jacobs-Reitsma, 1995; Johnson *et al*., 2017). This suggests a minor role for water in introducing *C. jejuni* into the farm, but a potential role for spreading the bacteria within the same poultry house once several birds start shedding.

2.8.2 Insects

Some insects like darkling beetles and houseflies have been idenfied in transmitting *C. jejuni*, hence the consumption of a single infected adult or larva from such insects is said to be sufficient to infect 90% of fowls or chickens in experimental situations (Royden *et al*., 2016; Hazeleger *et al*., 2018). This therefore shows the extent to which pests can serve as vectors for *Campylobacter* and other bacteria; where evidently, similar instances have also been observed in *Salmonella* (Nordentoft *et al*., 2017).

2.8.3 The General Farm Practices and Human Movement

When practices in the poultry farm are poor in the area of hygiene such as used litter retaining and thinning of flock, they are clearly identified to be major potential sources of infections and there has been *Campylobacter* isolation from farmer`s boots, equipment, external clothes, and water used in the farms for purposes of footbath (Rashid *et al*., 2016; Sibanda *et al*., 2018; Wales *et al*., 2019). In ranking study, thinning of flock has been considered as a major source for *Campylobacter* infections in the UK (Higham *et al*., 2018).

2.8.4 The Presence of Animals Other Than Poultry or Insects on the Farm

With respect to flock interactions, the presence of other animal and immediate surroundings of the poultry house or farm has proven in many studies as one major risk factor in flock contamination (Schets *et al*., 2017; Upadhyay *et al*., 2019). Newell *et al*. (2017) and Connerton *et al*. (2018) added that comparing among domestic animals, cattle hold a greater impact than others when it comes to contaminating broiler flocks with *Campylobacter*. To prove this, genotyping was carried out for *flaA* gene of *Campylobacter* isolates when chickens and cattle on the same farm were shed, and same genotype was found in both (An *et al*., 2018; Sibanda *et al*., 2018).

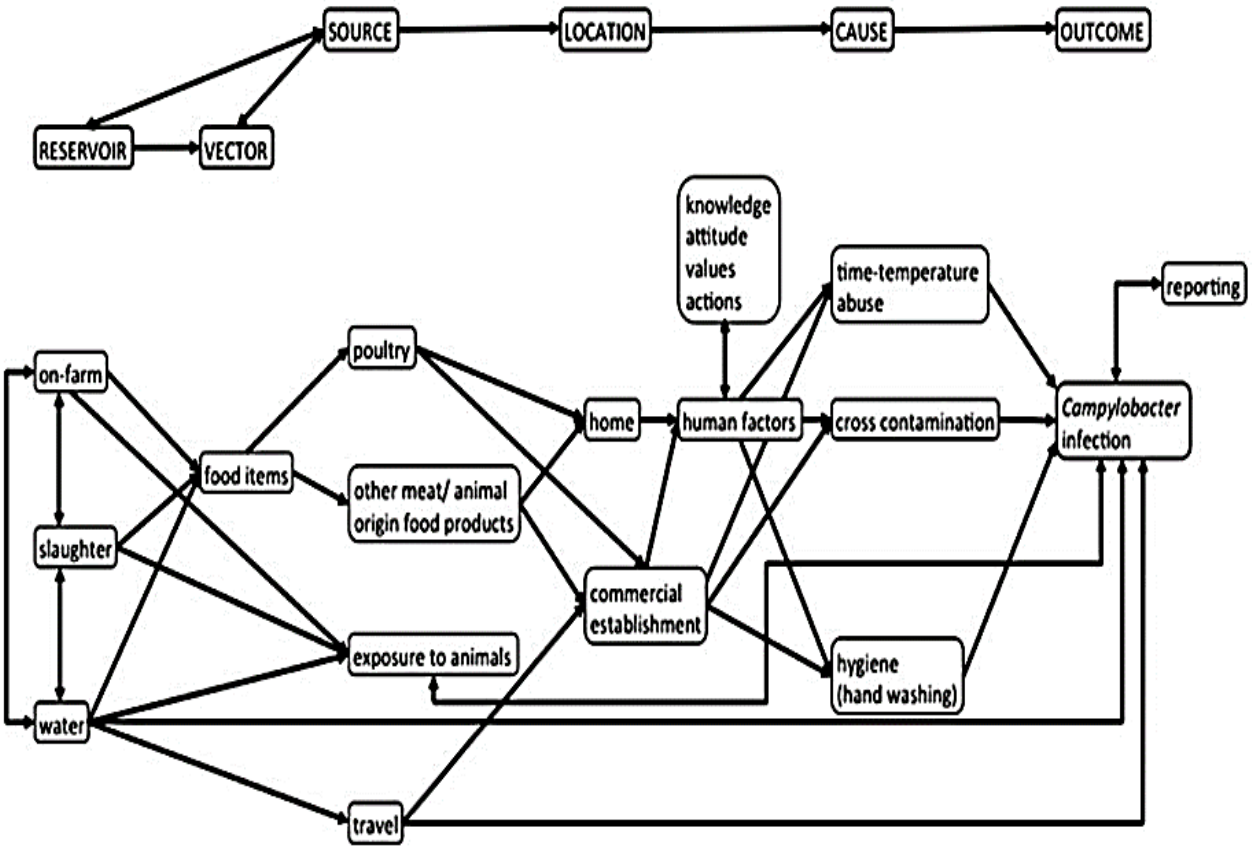


Figure 2. 3: *Campylobacter* Risk Factor Illustration Showing the Source of *Campylobacter* Organisms and Locations Where People are Exposed (Andrew *et al.*, 2013)

2.9 The Need for *Campylobacter* Control in Commercial Poultry

A study by Huang *et al*. (2017) recorded an approximation of 100% of broilers at a time of slaughter being *C. jejuni* colonized. Through several possibilities including feacal contamination of water and food, flies and other farm animals, horizontal transmission of *Campylobacter* can occur (Johnson *et al*., 2017; Hazeleger *et al*., 2018; Upadhyay *et al*., 2019) and once a single chicken is contaminated, the rest within the flock in few days are also colonized (Crotta *et al*., 2017). They remain colonized until slaughter for human consumption passing through and contaminating processing plants, and poultry meat (Jacobs-Reitsma, 1995; Reich *et al*., 2018).

2.10 *Campylobacter* Colonization Factors

Several studies have confirmed that stress can alter the gut microbiota composition (Galley *et al.,* 2014; De Palma *et al.,* 2015). During ingestion in the intestine at the post stage, according to Murphy *et al.* (2006), *Campylobacter* may come across several stressors that potentially can inhibit their optimal growth. Hence, *Campylobacter* high levels in poultry especially chicken, as well as their presence in other animals and even humans in most studies prove their ability to have evolved strategies that are very effective to grow and survive in such harsh intestinal environs.

Hermans *et al.* (2011), mentioned some possible factors contributing to *Campylobacter* colonization in caeca of such sources since those factors are needed for purposes like adhesion, chemotaxis, motility, regulating temperature, responses to oxidative stress, regulating iron, bile salts resistance and resistance to antibiotics or antibiotic use (Persaud *et al.,* 2014). Thus, from these multi-factorial processes, Campylobacters are able to improve their existence or colonization at high rate in the environment of the intestine.

2.11 Conditions for *Campylobacter* Species Growth and Survival

Campylobacters are often described as microaerophilic because they grow best in an atmosphere made of approximately 5% of O_2, 10% of CO_2 and 85% of N_2 (Handley *et al*., 2015; Khaleque and Bari, 2015; Connerton and Connerton, 2017; Hu and Kopecko, 2018). From Ovesen *et al*. (2019) and, Benoit and Maier (2018), there are some species of *Campylobacter* that could also grow under anaerobic or aerobic conditions (e.g. *C. fetus, C. Concisus* etc.). *Campylobacter jejuni* and *C. coli* generally do not grow below temperatures of 30 °C but achieve optimal growth at 42 ºC (Silva *et al.,* 2011; Sharma *et al*., 2016; Natsos *et al*., 2019). Due to these features, the multiplication capability of

Campylobacter outside its animal host, in meat or carcasses when processing or storing is reduced (Sharma *et al*., 2016; Tilmanne *et al*., 2019).

In terms of drying or freezing, *Campylobacter* are known to be sensitive (Sampers *et al.*, 2010; Oh *et al*., 2018). In most cases, temperature determines Campylobacters death rate, and their death record is more rapid during room temperature on dry surfaces as compared to conditions of refrigeration (Silva *et al*., 2011; Coorey *et al*., 2018). In refrigeration, Campylobacters thrive at the normal temperature of 4 °C and at -18 °C to -22 °C in stored frozen meat for several weeks (Sampers *et al.,* 2010; Oyarzabal *et al.*, 2010; Borrusso and Quinlan, 2017).

Also, in water activity terms which determines Campylobacter`s sensitivity to sodium chloride, *Campylobacter* survive best in environments with optimal water activity of 0.997 and do not grow under water activity lower than 0.987. They as well do not grow in sodium chloride concentrations greater than 2% (w/v) but optimum pH of 6.5-7.5 support their growth (Silva *et al*., 2011; Sung and Khan, 2015; Dunlop *et al*., 2016; Biswas *et al*., 2018).

2.12 Different Factors that Enhance *Campylobacter* Survival in the Environment

Campylobacter sp. is known to be a very fragile bacteria and needs to survive once they are debarred from their host into various different hostile conditions of which some may be exposure to oxygen and temperatures under their minimum temperature requirement for growth, desiccation and several other stress elements before it finally colonises a newly found host. Their ability to overcome and survive in this environment with such changing conditions is aided by what is referred to as the two-component regulatory systems (TCS) that helps it through a set of gene regulation, and biofilm

formation which also enhances their survival in conditions that are extreme (Gunther and Chen, 2009). Even on glasses or stainless steels and in aquatic environs, *Campylobacter* can form biofilms (Gunther and Chen, 2009; Kim *et al*., 2017) and in doing so, they enter into viable but non culturable state because they lack adaptive mechanisms to resist stress, and are in microaerobic conditions where very few nutrients are required (Magajna *et al*., 2015; Bronowski *et al*., 2017; Otigbu *et al*., 2018). They also undergo fatty acid composition changes in cell membrane that leads to pressure resistance and integrity changes when they are in stationary phase (Liang *et al*., 2017). In *C. jejuni* for instance, the production of polyphosphate by the enzyme polyphosphate kinase 1 increases in abundance during such conditions to help in survival when nutrient is low (Pina-Mimbela *et al*., 2015). Below are some of the explained ways by which Campylobacters adapt to certain factors;

2.12.1 Heat Stress

Campylobacter sp. are further subdivided into two groups; thermophilic and non-thermophilic groups. These two groups are interchangeably used for the alternative term thermotolerant Campylobacters and more appropriately referred to as thermophily due to the growth and survival characteristics at higher temperatures with range between approximately 20-70 °C and usual excess optimum 50 °C temperature for growth (Kumar *et al*., 2016; Iannino *et al*., 2017; Bhunia, 2018).

The optimal range for non-thermotolerant groups to grow is from 25-37 °C as little to no growth will be observed at 42 °C where the thermotolerant groups display optimum growth, and also even strong growth at 37 °C but with little to no growth at 25 °C also occurring (Kumar *et al*., 2016; Goni *et al*., 2017).

Campylobacter response to thermal stress is mainly mediated by what is referred to as the heat shock proteins (HSPs) that are found to be one of the most preserved coding

sequences (Vinaiphat and Thongboonkerd, 2017). Examples of these genes that encode for these purposes includes *groESIL, dnaJ, hrcA, htrNdegP* and *clpB* (Svensson *et al.*, 2008) which are more articulated in periods of aerobic stresses and heat (Takata *et al.*, 1995) as well in conditions of alkalinity (Wu *et al.*, 1994).

2.12.2 Cold Stress

Despite the lack of cold shock genes in *Campylobacter* like *cspA*, at low temperatures of 4 °C they still uphold their metabolic activity and studies confirm better survival at this temperature in several biological conditions than at 25 °C (Koolman *et al.*, 2016; Oh *et al.*, 2019; Shen *et al.*, 2019). A typical instance is found in a study by Al-Qadiri *et al.* (2015) which showed up regulation of genes to aid *Campylobacter* grow at 5 °C compared to 25 °C that demonstrated that at low temperatures, there is a greater need for energy. According to Alter (2017), *Campylobacter* survival to freeze thawing is attributed to *sodS* and *kalA* genes.

2.12.3 UV Stress

Studies by Prieto-Calvo *et al.* (2016) and Dai *et al.* (2019) showed that some Campylobacters such as the *C. jejuni* has UV stress sensitivity even more than that of *E. coli* and dwells best in a lesser amount of temperature and sunlight climates for few hours in a day. From Strathmann *et al.* (2016), Campylobacters have higher tolerance for UV in river waters which is ascribed to modifications in their physiological properties, and also the high expressions for *recA* genes in strains more resistant to UV.

2.12.4 Acid Stress

Several studies have concluded in their reports that some Campylobacters such as the *C. jejuni* is acid sensitive and a drastic population reduction occurs at pH lower than

5.5 and above 8.0 (Askoura *et al*., 2016; Hafez *et al*., 2018; Frirdich *et al*., 2019). During colonization of the human host, *C. jejuni* is exposed to low pH environments. At first, the bacteria are exposed to inorganic acid (H^+) in the gastric fluid of the stomach and later to organic acids in the small intestine (Audia *et al.,* 2001; Rao *et al.,* 2004). The capacity to counteract environmental stresses is fundamental for survival. Bacteria respond to decreases in pH by inducing different systems to maintain pH homeostasis. These systems may prevent entry of H^+, extrusion of H^+ from the cell, consumption of H^+ in chemical reactions or the repair of damaged cellular material (Baik *et al.,* 1996; Cotter *et al.,* 2000).

Compared to other bacteria, *C. jejuni* is more sensitive to stress and has a limited number of stress regulators. *C. jejuni* lacks the global stationary-phase regulator, sigma factor RpoS, which induces expression of numerous proteins involved in different forms of stress responses (Magnusson *et al.,* 2005). In addition, *C. jejuni* also lacks the oxidative stress response regulatory elements SoxRS and OxyR, and osmotic shock protectants such as BetAB (Parkhill *et al.,* 2000; Svensson *et al.,* 2008).

2.12.5 Aerobic Stress

Campylobacters according to Hu and Kopecko (2018), are able to acclimatize to aerobic growth under 10% CO_2 in humid air (Fraser *et al.,* 1992). There is a branched electron transport chain based on oxygen usage in respiratory metabolism of some Campylobacters especially the *C. jejuni* as a terminal electron acceptor whereas their alternate terminal electron acceptors can also be used (van der Stel *et al*., 2015; Guccione *et al*., 2017; Taylor and Kelly, 2019).

2.12.6 Desiccation Survival

Campylobacter sp. are extremely sensitive to desiccation and studies by Doyle and Roman (1982) proposed some responsible factors for species of *Campylobacter* to

tolerate desiccation to be the strain type, humidity, temperature and the type of medium used in cell suspension.

According to Kwon *et al*. (2016), drying of carcasses helps eliminate *Campylobacter* from pork when cooling rooms are ventilated but does not work for that of poultry meat since its processing requires shorter time of cooling and different skin texture (Sibanda *et al*., 2018; Hansson *et al*., 2018).

2.12.7 Body Temperature

With respect to rate of Campylobacter survival and growth, one key difference in poultry or chicken and humans is the body central temperature; where humans maintain normal temperature of 37 °C, poultry generally maintain 41-45 °C range of temperature. Due to temperature sensitivity, a two-component regulatory system named RacR-RacS has been identified as very vital for wild-type colonization in chickens and *C. jejuni* growth at 42 °C (Bras *et al.*, 1999).

A study by Stintzi (2003) showed approximately 20% genes either up-regulated or down-regulated during the transfer of cultures of *C. jejuni* from 37 °C to 42 °C with similar observations in other genes especially those responsible for protein transportation and modification in membrane structure.

2.13 Health Implications of *Campylobacter*

2.13.1 Human Infections with *Campylobacter*

Surprisingly, both the non-thermotolerant (e.g. *C. fetus venerealis*; *C. fetus fetus*) and thermotolerant (e.g. *Campylobacter jejuni*; *C. coli*; *C. upsaliensis*) Campylobacters are potential pathogens in humans (Penner and Hennessy, 1980).

Findings from Moore *et al.* (2005) showed *C. fetus* as a potential cause of gastroenteritis in humans but rare, and stated that it is associated mostly with systemic infections as a

complication in situations where patients are already unwell. In human infections, *Campylobacter fetus* is known to cause an estimation of 15% mortality with infection signs of fever and metastatic localisation as well as causing diarrhoea in humans that is similar to *C. jejuni* infection as described by Lastovica and Allos (2008). *Campylobacter jejuni*, *C. lari* and *C. coli* are also the major thermotolerant *Campylobacter* sp. commonly identified to cause gastroenteritis infections in humans. Butzler in his reviewed article in 2004 listed Campylobacters that cause infective diarrhoea in humans as *C. coli*, *C. fetus fetus*, *C. jejuni jejuni*, *C. concisus*, *C. hyointestinalis*, *C. jejuni doylei*, *C. upsaliensis* and *C. lari*, though when it comes to human gastroenteritis, the most commonly associated species is the *C. jejuni jejuni*.

Studies concerning epidemiology usually do not differentiate between *C. coli* and *C. jejuni* due to their similarities in infection and characteristics, they either include only the *C. jejuni* or consider both as one group, and labs routinely do not carry out speciation between these two species (Siemer *et al.,* 2005).

2.13.2 *Campylobacter* sp. Occurrence in Foods

There have been *Campylobacter* isolations in many food types which include lamb, raw milk, pork, poultry, seafood, salads and beef in many researches (Wilson and Moore, 1996; Eberhart-Phillips *et al*., 1997; Studahl and Andersson, 2000; Jacobs-Reitsma, 2000; Humphrey *et al.,* 2007; Heuvelink *et al.,* 2009; Suzuki and Yamamoto, 2009; Wijnands *et al.*, 2014). From Humphrey *et al.* (2007), foods can often be contaminated if not handled carefully in the cause of processing when they contain raw materials from animal sources because many food producing animal and poultry species carry *Campylobacter* in their intestines. Nonetheless, most foodborne Campylobacteriosis cases are linked to handling raw poultry, consuming undercooked or raw meat from poultry, or the cross-contamination of raw to prepared foods (

Eberhart-Phillips *et al*., 1997; Studahl and Andersson, 2000; Kramer *et al*., 2000; Nadeau *et al*., 2002; Neimann *et al*., 2003; Humphrey *et al.*, 2007; Suzuki and Yamamoto, 2009; EFSA, 2014). The consumption of unpasteurized milk has been linked to numerous milk-borne Campylobacteriosis occurrences (Heuvelink *et al.*, 2009).

Findings from the EFSA (2015) report indicated that prevalence of *Campylobacter* in fresh broiler meat samples in the year 2013 varied widely across the EU block with rates ranging from 0% records in two countries (Czech Republic and Italy) to 74% in Luxembourg respectively. Besides an European Union wide baseline research showing the average *Campylobacter* incidence for broiler carcass to be around 76% (EFSA, 2010), a study by Suzuki and Yamamoto (2009) also summarized in a global literature survey that 58% of poultry from retail are contaminated with *Campylobacter* on the average.

2.14 Pathogenic Mechanisms of *Campylobacter*

Preferentially, Campylobacters are known to colonise humans, poultry and other animals through their gastrointestinal tract`s mucous layer where the infections hinder the normal absorptive and secretory ability of the intestine thus leading to gastroenteritis. They are further reported to demonstrate four key virulence properties which include invasion, motility, toxin secretion and adherence (Walker *et al.*,1988). In addition, Wassenaar *et al.* (1994) stated that the motility ability and presence of flagella in Campylobacters are purposely for colonization and pathogenesis in their hosts. The presence of flagella assists them to cross to the epithelium through the mucous layer covering in their hosts.

Campylobacter achieves invasion success through series of potential adhesins that mediate attachment to host cells; examples of reported adhesins are outer membrane proteins (such as P95 and PorA), flagella and moieties of surface polysaccharides (Hu *et al.*, 2006).

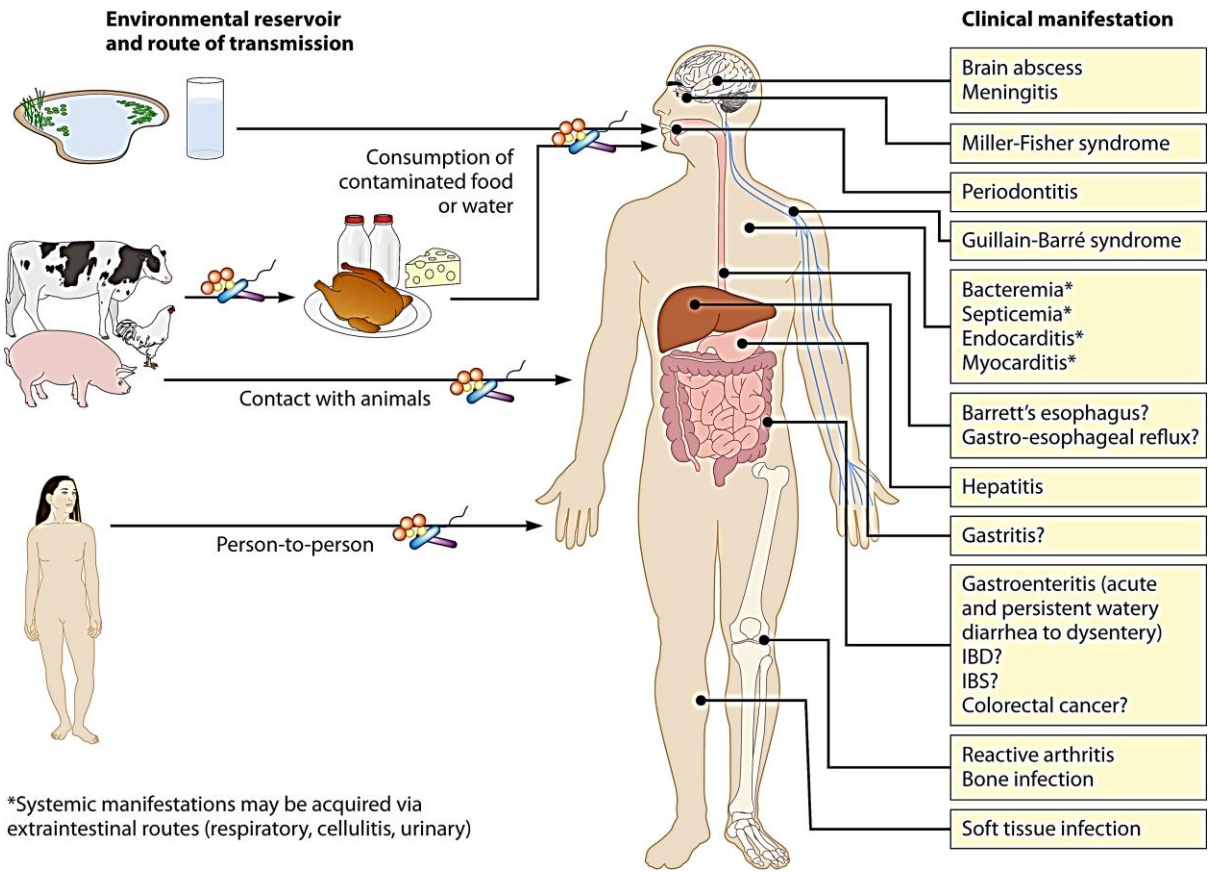
2.15 *Campylobacter* Risk Factors

There remain several risk factors when it comes to *Campylobacter* infections in humans and some of which have been stated in various studies that includes contact with domestic animals, poultry meat consumption or handling, drinking unpasteurized milk, eating undercooked or raw meat, travelling and swimming in natural waters, and drinking untreated water (Rodrigues *et al*., 2001; Kapperud *et al.*, 2003; Neimann *et al*., 2003; Friedman *et al*., 2004; Heuvelink *et al.*, 2009; Wingstrand *et al*., 2006; Ricotta *et al.*, 2014).

In addition, an European Food Safety Authority report also establishedthat preparation, handling and intake of poultry meat accounts for 20%-30% human Campylobacteriosis cases, whereas 50-80% *Campylobacter* infections in humans was ascribed wholly to chicken reservoir (EFSA, 2011). Hence the major key thing to note when it comes to the prevention of Campylobacteriosis in humans as a strategy of public health is especially the control of *Campylobacter* sp. in poultry and its` meat. Therefore, there is a shared view that global agencies that have food safety responsibilities have controlling of *Campylobacter* in the food chain becoming their key target (Office International des Epizooties-OIE, 2008).

Additionally, this then concludes that there are several identified routes for *Campylobacter* transmission which on infection could lead to clinical manifestations,

hence knowing and understanding them as shown in the diagram by Kaakoush *et al.* (2015) may be of preventive importance.



Environmental reservoirs, routes of transmission, and clinical manifestations associated with *Campylobacter* species can be transmitted to humans through consumption of undercooked or contaminated food or via contact with animals. Tap, bore, and pond waters are also sources of *Campylobacter* species. Person-to-person transmission (faecal-oral or via fomites) can occur. Ingestion of a sufficient dose of organisms via the oralgastric route may lead to one or more gastrointestinal and/or extragastrointestinal manifestations; the outcome is dependent on the species or strains of *Campylobacter* involved in the infection. Abbreviations: IBD, inflammatory bowel diseases; IBS, irritable bowel syndrome. Question marks indicate conditions for which a role for *Campylobacter* is implicated but not certain.

Figure 2. 4: An Adopted *Campylobacter* Route of Transmission Diagram Showing the Sources of *Campylobacter* Organisms and the Clinical Manifestations After Human Exposure (Kaakoush *et al.*, 2015)

2.16 *Campylobacter* Isolation and Identification

In the processes of *Campylobacter* isolation and further identification, media that are very selective containing oxygen scavengers like charcoal, defibrinated blood, pyruvate, ferrous iron and highly selective mediators specifically antimicrobials are employed. Following its isolation on the described medium, Gram staining is

performed to observe their typical morphology to aid their characterisation and finally performing biochemical confirmations like the hippurate hydrolysis test or test of indoxyl acetate hydrolysis for the thermophilic *Campylobacter* sp. classification (Humphrey *et al.*, 2007; Silva *et al*., 2011; On, 2013).

The existing conservative procedures intended for thermophilic *Campylobacter* species detection in foods mostly encompasses a liquid medium enrichment in either Bolton or Preston or *Campylobacter* enrichment broths prior to plating it on a selective media such as the modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA), Preston agar or Butzler agar (Silva *et al*., 2011). In the course, any of the following antibiotics; cycloheximide, cefoperazone, rifampicin, trimethoprim, polymyxin B and vancomycin is added to either the selective agar or enrichment media to help inhibit organisms that may be competing (Corry *et al.*, 1995).

Standard procedures from International Organization for Standardization (ISO, 2006a; 2006b) are usually followed or used in *Campylobacter* enumeration, isolation and detection, where broth for enrichment step and the suspension step is incubated for 4-6 hours under microaerophilic condition at 37 °C, following 40-48 hours incubation on the selective mCCDA and extra used medium at 41.5 °C (Silva *et al.*, 2011).

Polymerase chain reaction (PCR) methods have been employed nowadays which aim at identifying *Campylobacter* at the species level where it targets several genes such as 23S rRNA, 16S rRNA, *mapA, hipO, ceuE, bipA* and *glyA* during *Campylobacter* enumeration and identification (García-Gil, 2013). A very typical instance is the identification as well as differentiation of some Campylobacters like *C. jejuni*, *C. lari*, *C. fetus* subsp. *Fetus*, *C. upsaliensis* and *C. coli* using the colony multiplex PCR assay

by Wang *et al.* (2002) targeting the following genes; *glyA* from each of *C. coli*, *C. lari*, and *C. upsaliensis*, and *sapB2* from *C. fetus* subsp. *Fetus*; and finally, *hipO* and 23S rRNA from *C. jejuni*.

Siemer *et al.* (2005) indicated the favourable test is Polymerase Chain Reaction (PCR) when it comes to differentiation or identification of species due to varied results given by other biochemical tests during the differentiation of different strains in same species.

2.17 *Campylobacter* Isolation and Prevalence in Ghana

In 2015, Krumkamp and his associates reported the cases of gastrointestinal infections from patients that visited Agogo Prebyterian Hospital (APH) in the Asante Akim North Municipality in Ghana. They reported that, out of 1,234 stool samples that were collected, 548 children were reported to have diarrhea and 656 children were without gastrointestinal symptoms. *Campylobacter jejuni* which recorded 19.6 % was part of the pathogenic organism that were isolated.

In addition, *Campylobacter* species (17.3 %) were isolated from patients from Komfo Anokye Teaching Hospital in Kumasi-Ghana were having enteritis (128) and urinary tract infection (74). *Campylobacter* species that were recorded from 128 enteritis were to be 20.3 % whiles that of 74 urinary tract infection were 12.2 %. This 17.3 % prevalence of *Campylobacter* species were further screen of which *C. jejuni, C. jejuni sub sp. Doylei, C. coli* and *C. lari* were isolated with a percentage of 40 %, 2.8 %, 37 % and 20 % respectively (Karikari *et al.*, 2017a).

The prevalence of *Campylobacter* species from faecal and carcasses of commercially produced poultry in Kejetia central market in Kumasi-Ghana have also been reported. Campylobacter species were isolated from faecal content of poultry were 22.5 % and

that of poultry carcasses were to be 21.9 %. These species that were found to be in faecal content of poultry were *C. jejuni, C. coli, C. lari* and *C. hyointestinalis* whiles in their carcasses, *C. jejuni, C. coli, C. lari* and *C. jejuni* sub sp. *doylei* were also recorded (Karikari *et al.*, 2017b).

Karikari *et al.* (2016) reported about 22.3 % of *Campylobacter* species from environmental water samples from Kumasi-Ghana. Among this environmental samples, river samples recorded the highest (35.7 %) followed by streams samples (26.2 %), wells samples (21.4 %), ponds samples (9.5 %) and boreholes samples (7.1%).

2.18 Antibiotic Sensitivity Pattern of *Campylobacter*

One of the emerging problems of global concern currently is antimicrobial resistance (AMR), and the major factor that is contributing to this key problem is the usage of antimicrobials (AMU), majorly in humans and animal production (Marshall and Levy, 2011). Antimicrobial usage in the production of animals contributes to the spread, selection, and maintenance of antimicrobial resistant Campylobacters along with other bacteria on farms. The spread of the antimicrobials themselves, resistant bacteria and antimicrobial resistance determinants into the environment may occur through farm waste, and finally get to humans from direct contact with animals, consuming contaminated water, vegetables and foods of animal origin (Da Costa *et al.*, 2013).

Antimicrobials used in producing animals are very similar to the ones in human medicine (FAO/WHO/OIE, 2007), and hence for human medicine, resistance to antimicrobials are of greatest concern (WHO/CIA, 2011).

Due to increasing demand particularly in economies that are emerging, the currently higher antimicrobial quantities used in animal production are expected to increase

further (Van Boeckel *et al.,* 2015). A typical instance is the Southeast Asia (SEA) bloc which is developing rapidly are related economies of such when it comes to high use of antimicrobials in animal production (Richter *et al.,* 2015; Walther *et al.,* 2016), and from studies the area is considered an antimicrobial resistance hotspot (Coker *et al.,* 2011; Antimicrobial Resistance: Global Report on Surveillance, 2014; von Wintersdorff *et al.,* 2014).

The therapeutic use of antibiotics in human medicines as well as the use for therapy, growth promotion and prophylaxis in livestock are identified as major influences on the intensification and spread of antibiotic-resistant bacteria. However, the use of antibiotics for veterinary purposes has importance to livestock industries such as ensuring animal health and welfare, but great selective pressure is exerted under sub-therapeutic levels which influences resistant bacteria emergence (Oosterom *et al.*, 1985; Wittwer *et al.,* 2005).

The increasing resistance to antibiotic in bacteria in most studies especially for intensively housed animals like feedlot cattle, pigs and cattle has been connected to the antibiotic usages in agriculture (Barton, 2000).

Resistant development among various zoonotic bacteria and microorganisms that are non-pathogenic resulting from veterinary use of antibiotics might lead to the fear of treatment failure when humans acquire infections from pigs, poultry, cattle and other animal sources where these antibiotics have been used for treatment. In addition, resistance transfer may leak from non-pathogenic bacteria to human pathogens (Alban *et al.,* 2008).

When it comes to the use of macrolides in veterinary, the World Health Organization (WHO) has great reservations due to the risk of macrolide-resistant (Mres) *Campylobacter* development. This is because those drugs have specifically been chosen

for treatment of children with intestinal disorders hence making that issue particularly to be of concern (Scharff, 2010).

In prolonged or severe cases, antimicrobial treatment such as macrolides and fluoroquinolones are of great need despite most infections from *Campylobacter* being self-resolved.

During epidemiological monitoring and therapeutic guidance for resistance, antibiotic or antimicrobial susceptibility testing continues to play a very critical role. Also the recommended methods of choice for *Campylobacter* by the Clinical and Laboratory Standards Institute (CLSI) are broth microdilution and agar dilution, with the added standardized method by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) called disk diffusion method (Ge *et al.*, 2013).

Susceptibility to several antimicrobial agents including the aminoglycosides, fluoroquinolones, tetracyclines, macrolides, nitrofurans and clindamycin are naturally observed in Campylobacters, and with reported moderate susceptibility to chloramphenicol, cefotaxime, ceftazidime and cefpirome.

Studies by Fliegelman *et al.* (1985) and McNulty (1987) reported intrinsic resistance against most cephalosporins, as well as penicillins with vancomycin, trimethoprim, rifampicin and sulfamethoxazole in *C. coli* and *C. jejuni*. Several other studies during the 20th centuries have also showed numerous and interesting findings on antibiotic susceptibility testing for both *C. jejuni* and *C. coli*, of which some are highlighted below.

Engberg *et al.* (2001) detected *C. coli* and *C. jejuni* resistance for macrolides and quinolones along with resistance trends and mechanisms in isolates from humans. However, in their studies, they advised that erythromycin with other macrolides ought

to remain in most regions as the drugs of choice, but should do so with maintained control measures and systematic surveillance, and further added in many other regions with empiric treatment of infections from *Campylobacter* sp., use should be restricted.

In the context of poultry especially broilers, Avrain *et al.* (2003) conducted a study on *Campylobacter* from broilers considering antimicrobial use and association with production type, performing the test using the following antibiotics; gentamicin, erythromycin, ampicillin, nalidixic acid, ciprofloxacin and tetracycline by employing the method of dilution. They had the following respective percentages of resistance 0, 0.3, 17, 23, 25 and 57% for *C. jejuni* and 0, 31, 40, 29, 43 and 70% for *C. coli* for the used antibiotics.

In 2003, Ge and associates also studied antimicrobial resistance pattern of *Campylobacter* species of 378 isolated *Campylobacter* in retailed raw meats where higher records of resistance to some antibiotics were observed. The highest resistance was recorded in tetracycline with 82%, followed by 77% in doxycycline, 54% in erythromycin, 41% in nalidixic acid and finally 35% in ciprofloxacin. From their findings, *C. coli* displayed higher significant rates of resistance than *C. jejuni* to both erythromycin and ciprofloxacin (Ge *et al.*, 2003).

Pezzotti and the colleagues in 2003 reported resistance to antibiotics by *C. jejuni* and *C. coli* in meat and some animals within North-Eastern Italy when they were investigating the occurrence of *Campylobacter* in the area during 2000 and 2001. Results in general had more resistance for *C. coli* than *C. jejuni*. Results on quinolone resistance was commonly detected in *C. coli* isolates from chicken meat with 78.6%; a slightly lower rate was recorded for *C. jejuni* isolates with 42.2% from broilers, 52.8% from chicken meat and 38.2% from humans. In all the considered sources, *C. coli* isolates also were found frequently resistant to tetracycline and sensitivity to

streptomycin was also most frequently detected with 89.4% in pig isolates (Pezzotti *et al.*, 2003).

Gupta *et al.* (2004) studied *Campylobacter* sp. antimicrobial resistance in United States in 1997–2001. Their study showed prevalence of ciprofloxacin-resistance by *Campylobacter* to be 13% (thus 28 out of 217 isolates) in 1997 and 19% (also 75 out of 384 isolates) in 2001, whereas in 1997 and 2001, erythromycin resistances recorded were 2% (4 of 217) and 2% (8 of 384) respectively. Ciprofloxacin resistance was known among Campylobacters to have emerged since 1990 with increasing prevalence since 1997.

There was another study in that same year by Hart *et al.* (2004) in Australia where they detected *Campylobacter* sp. as well as *Enterococci* and *Escherichia coli* antimicrobial resistance associated with pigs. In the study, they found thermophilic *Campylobacter* sp. in pigs of South Australia showing a range of 60 to 100% widespread resistance to lincomycin, tylosin, ampicillin, erythromycin and tetracycline with no observed resistance to ciprofloxacin.

In addition to studies in 2004, Payota and the team studied *C. coli* incidence and antimicrobial resistance in France from fattening pigs and found *C. coli* resistance higher in tetracycline and erythromycin being 79 and 55% respectively, and resistance of 15, 20 and 34% for enrofloxacin, ampicillin and nalidixic acid respectively observed in the isolates (Payota *et al.*, 2004).

Similar study into *C. coli* in swine was done by Thakur and Gebreyes (2005) where they researched into the molecular epidemiology and antimicrobial resistance mechanisms with findings that indicated multidrug resistance in diverse strains of *C. coli* exhibiting erythromycin and ciprofloxacin resistance which are of great concern,

since in human cases of treating invasive Campylobacteriosis, the two drugs are of choice.

In Northern Thailand, Padungtod *et al.* (2006) studied the detection of antimicrobial resistance in *Campylobacter* strains from humans and food-producing animals' sources, where they found 89% for pig farm isolates and 66% pig slaughter isolates being resistant to three or more of the antimicrobial agents. Erythromycin, nalidixic acid, ciprofloxacin, tetracycline and azithromycin were the most multidrug resistance combinations commonly observed by their study.

In Africa, a study by Dadi and Asrat in Ethiopia in 2008 had described profiles of thermotolerant *Campylobacter* isolates antimicrobial susceptibility in retailed raw meat products and found lower resistance rates of 6%, 4%, 2% to amoxicillin, chloramphenicol, erythromycin respectively but a higher than resistance found for streptomycin, gentamicin, kanamycin, ampicillin and tetracycline with 20, 14, 12, 10 and 10% respectively (Dadi and Asrat, 2008).

Gyles (2008) investigated some selected bacteria from poultry and their resistance to antimicrobials. The research findings showed usual low resistance to macrolides, moderate-high resistance frequency to tetracycline and low-high resistance to fluoroquinolones or quinolone for *Campylobacter*.

Also, Little *et al.* (2008) carried an investigative study in the United Kingdom on *Salmonella* and *Campylobacter* in raw red meats thus considering prevalence, characterization and pattern for antimicrobial resistance. The resistance to erythromycin, ciprofloxacin and nalidixic acid were observed frequently in *C. coli* than in *C. jejuni* strains that they got from beef, lamb and pork.

From Usha *et al.* (2010), they found *Campylobacter* demonstrating resistance range of 20-100% towards cephalothin, gentamicin, ampicillin, tobramycin, norfloxacin,

ciprofloxacin, tetracycline and enrofloxacin during their investigative study on *C. jejuni* and *C. coli* occurrence and antibiotic resistance in retail broiler chicken. They further included that all the isolates at least were resistant to two of the antibiotics used.

Wieczorek *et al.* (2012) also in their study to detect prevalence, molecularly characterize and perform antimicrobial resistance of *C. coli* and *C. jejuni* isolates in retail raw meat in Poland, observed all *Campylobacter* strains to be susceptible to gentamicin and one *C. coli* isolate susceptible to erythromycin. However, highest levels of resistance to ciprofloxacin among the tested Campylobacters was found to be 91% for *C. jejuni* and 86.1% for *C. coli*, and resistance to nalidixic acid also recording 89.3% for *C. jejuni* and 85% for *C. coli*. There were 60.9% of *Campylobacter* sp. resistant to two or more classes of the antibiotics used within which a strain from *C. coli* showed resistance to four different classes of antimicrobials.

In *Campylobacter* antimicrobial resistance, a given review by Ge *et al.* (2013) on resistance trends and susceptibility testing methods stated frequent reports by several national surveillance programs on resistance of higher levels to ciprofloxacin and tetracycline where that of gentamicin and erythromycin remains low in *Campylobacter* sp.

In 2014 in Italy, Di Giannatale and associates did some work on virulence genes detection and characterized antimicrobial resistance patterns in isolates of *Campylobacter* and their findings on antimicrobial susceptibility revealed higher resistance levels for ciprofloxacin with 62.76%, 55.86% of tetracycline resistance and 55.17% for nalidixic acid. On the other hand, resistance of strains to erythromycin was 13.10% (19), streptomycin was 4.83% (7) and 0.69% representing only one isolate was to chloramphenicol (Di Giannatale *et al.*, 2014).

Another study on thermophilic *Campylobacter* sp. frequency, antibiogram and risk factors in Nepal was carried out by Ghimire *et al.* (2014) with isolation from dressed porcine carcass of Chitwan. The results from this study revealed the highest resistance of 92.59% each to erythromycin and ampicillin, 72.2% to colistin, 61.1% to tetracycline, 44.4% each to cotrimoxazole and nalidixic acid, 31.5% to ciprofloxacin and 5.56% to gentamicin. Besides, 77.8% was found for isolates that were multidrug resistant thus those resistant to more than two of the antimicrobials used.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Sites

This research was carried out within Tamale metropolis, Nyankpala community and UDS-Nyankpala Campus. Sampling was done in all the aforementioned sites. Laboratory analyses were carried out on Nyankpala Campus of the University for Development Studies, specifically in the Microbiology division of the Spanish Laboratory Complex. The various sampling sites lie in two areas, thus the Tamale metropolis and Tolon district in the Northern region of Ghana.

Nyankpala community and Nyankpala Campus of the University for Development Studies are located in the Tolon district of the Northern region. Nyankpala is about 20 km South-West of Tamale the capital of Northern region (figure 3.1) and lies on the coordinates of 09º24′N and 0º59′W (Savannah Agricultural Research Institute-SARI, 2001).

Tamale officially called Tamale Metropolitan Area is the capital town of the Northern Region which is one of the sixteen regions of Ghana. Tamale is Ghana`s third-largest city. It has a 2013 projected population of 950,124 according to the 2010 census and is the fastest-growing city in West Africa (Ghana Statistical Service-GSS, 2012). The town is located 600 km North of Accra (coordinates of 09º24′27″N and 00º51′12″W) (SARI, 2001; GSS, 2012).

The area experiences one rainy season starting from April/May to September/October with a peak season in July/August. The mean annual rainfall is 1100 mm within 95 days of intense rainfall. The dry season is usually from November to March. The mean daily temperature ranges from 33 ºC to 39 ºC while mean night temperature range from 20

°C to 22 °C. The mean annual day sunshine is approximately 7.5 hours (SARI, 2001; GSS, 2012). The area is characterized by natural vegetation dominated by grasses with very few shrubs.

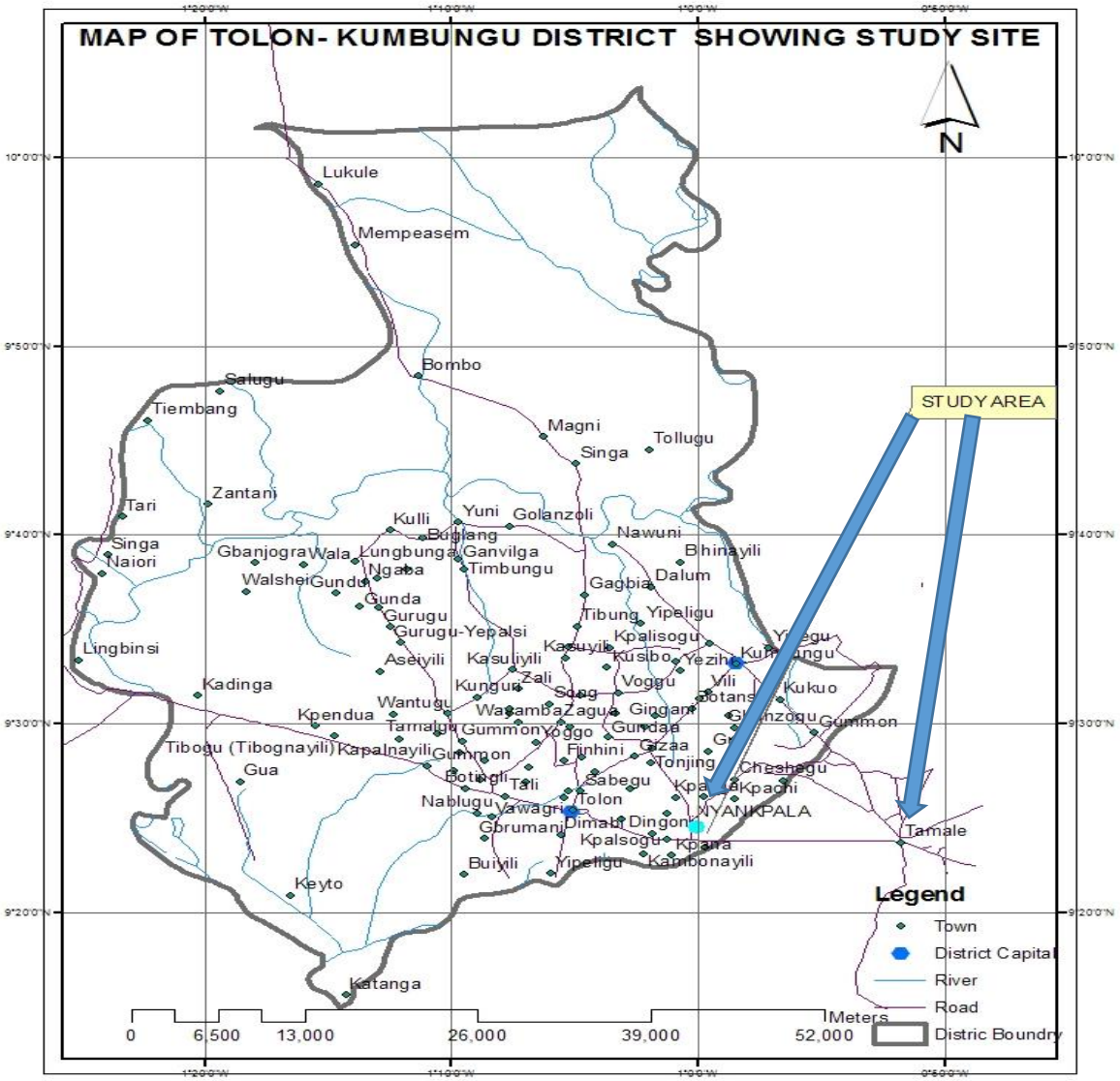


Figure 3. 1: A Map of Tolon-Kumbungu District Showing the Study Areas (Savannah Agricultural Research Institute-SARI, 2001)

3.2 Study Subjects

A total sample of 1087 covering poultry and humans (from hospitals, commercial and households) were collected. The study spanned from 25th October, 2017 to 7th May, 2019.

A total of 192 samples of poultry (chicken) coming from commercial sources (6 farms) in the Tamale Metropolis and 154 poultry from households of locals in the Nyankpala community were randomly collected for the study. In addition to that, human samples (faeces) were sourced from 462 patients in public hospitals in Tamale and 279 healthy individuals (comprising healthy household individuals in Nyankpala, students on Nyankpala campus of the University for Development Studies and finally, human faecal samples from the environment). Patients feacal samples were collected based on convenience sampling, healthy household individual samples were purposively sampled (i.e. dependent on households that have chicken), and human samples from both students and the environment were randomly collected.

3.2.1 Sample Collection

Faecal samples were collected using the following methods respectively;

i. Poultry samples were collected by means of cloacal swabbing with sterile cotton swab in a transport medium container (Copan sterile cotton swab).
ii. Human faecal samples from hospitals and households were also collected with the provision of sterile stool containers to both patients and healthy individuals, and human faeces from the environment were sampled with the use of sterile swabs with transport medium (Copan sterile cotton swab).

All collected faecal samples were then kept in an ice chest containing ice packs and transported within an hour to the Spanish Laboratory Complex on UDS-Nyankpala Campus for laboratory analysis.

All collected samples were labelled and basic information such as date and location were recorded at the point of collection.

3.2.2 Sample Size Determination

The study employed the Fisher`s formula as indicated below for the determination of sample size as used by Charan and Biswas (2013);

$$\text{Sample size} = \frac{Z_{1-\alpha}^2 \, P \, (1-P)}{d^2}$$

Where;

$Z_{1-\alpha}^2$= standard normal variate (at 5% type 1 error ($p<0.05$) it is 1.96 and at 1% type 1 error ($p<0.01$) it is 2.58 **(**1.96 was used for the determination of my sample size**)**

P= is the expected percentage in population based on earlier researches or pilot studies (50%=0.50, 25%=0.25 both were employed for humans and poultry respectively**)**

d= is the absolute precision or error which has to be decided by researcher (5%=0.05)

From the above formula, considering the indicators for its parameters came to a conclusion of 385 and 289 as sample sizes for humans and poultry, respectively.

Assumptions;

Expected proportion in population based on previous studies or pilot studies in Ghana were around 13-17% and since studies on *Campylobacter* in the Northern part of Ghana is very limited, the study then aimed at increasing its sample size by using 50% for humans and 25% for poultry instead. Despites the assumed percentages which would have given about 289 and 385 samples size respectively to poultry and humans; the study covered 346 samples for poultry and 741 to humans.

3.3 Isolation of *Campylobacter* sp.

Samples collected were streaked with the aid of sterile inoculating loop on Charcoal Cefoperazone Deoxycholate Agar (CCDA, Oxoid, UK) and incubated under microaerophilic condition generated by a gas generating pack (CampyGen™ 2.5L, Oxoid) in canisters at 42 °C for 24-48 hours.

Samples that were taken with the sterile cotton swabs upon arriving at the laboratory, were first spread on the CCDA agar plate following streaking with a sterile inoculating loop.

Fresh faecal samples that were collected in the sterile stool containers were also streaked on the CCDA agar plates using loopful each and finally following incubation. However, the study employed the isolation approach used by Schets *et al.* (2017).

3.3.1 Purification of Identified *Campylobacter* sp.

The incubated plates were then examined for typical *Campylobacter* colonies, characterized by greyish, creamy/white, flat, moistened colonies with tendencies to spread. Colonies exhibiting typical *Campylobacter* morphology on the CCDA were selected and sub-cultured on prepared Columbia Blood Agar (CBA) (Oxoid, Basingstoke, UK) supplemented with 5% defibrinated sheep blood and also incubated under microaerophilic condition generated by a gas generating pack (CampyGen™ 2.5L, Oxoid) in a canister at 42 °C for 24-48 hours.

3.3.2 Confirmation of Presumptively Identified *Campylobacter* Isolates

The following detailed approaches and/or tests below were employed for the confirmation of all 245 presumptive *Campylobacter* isolates identified from the study.

A. Microscopy

Gram staining was performed on colonies morphologically identified as *Campylobacter*. Smear-slides were first prepared and heat-fixed before gram staining reagents which includes the crystal violet, iodine (a mordant), acetone or decolouriser and safranin (a counter stain) were used to flood the smeared slides (at time periods of 3 minutes, 2 minutes, 10 seconds and 30 seconds respectively for the aforementioned reagents) with washing after each added reagent (appendix 1.1). Prepared microscopic slides were examined using phase contrast microscopy with oil immersion.

Slides demonstrating characteristic morphology of *Campylobacter* corkscrew-like shape were regarded as presumptively positive.

B. Oxidase Test

Tests were performed on presumptive positive isolates using oxidase test strips. The oxidase test was performed using Microbact™ Oxidase Detection Strips (Oxoid Ltd., Basingstoke, UK) and following the procedure below;

i. Colonies of Campylobacters to be tested were transferred onto the oxidase detection test strips using sterile loop and spread on the strips.

ii. Upon waiting for 5 seconds, observation was made and results recorded (a deep blue/violet colour indicates a positive reaction and no blue colour change for negative reaction as shown in plate 3.1: 2A).

C. Catalase Test

Hydrogen peroxide was used in performing catalase test on presumptive positive isolates. The test was performed following the slide (drop) method for freshly prepared cultures. The test was simply done following the procedures below;

i. A small amount of organism from a well-isolated colony was collected carefully while preventing collection of agars with a plastic disposable loop and placed onto a surface of clean, dried glass microscope slide.

ii. Using a dropper, one (1) drop of 3% H_2O_2 was placed onto the organism on the slide and mixed.

iii. A dark background that would enhance readability was used in observation for immediate bubble formation and results recorded as;

- *Catalase positive reaction*: Evident by immediate **effervescence or rapid evolution of oxygen** (bubble formation within 5-10 seconds) was considered; thus, as shown in plate 3.1: 2B.
- *Catalase negative reaction*: thus, considering those with no bubble formation.

iv. After which, used slides were disposed-off in a biohazard glass disposal container.

2A. Oxidase test using test strip 2B. Catalase test using H_2O_2

Plate 3. 1: An Oxidase Test Strip (2A) and Slide of Catalase Test (2B) Showing Positive Reaction for *Campylobacter*

D. Latex Agglutination Immunoassay

A rapid confirmatory latex agglutination test for *Campylobacter* was done using the Thermo Scientific *Campylobacter* Test Kit (Oxoid Ltd., Basingstoke, UK). Each presumptively positive colony was confirmed by testing an additional portion of the colony using the *Campylobacter* Test Kit following the manufacturer`s procedure. Thus, 50 µL of sample diluent was first dispensed onto each of two ovals of the agglutination slide, and colonies of *Campylobacter* sp. scooped into each and mixed uniformly. One drop (50 µL) of the control latex reagent was added to one of the suspension and one drop test latex reagent dispensed to the other bacterial suspension. Slides were gently rocked while keeping the fluid suspensions in constant movement for 2 minutes and observed for agglutination and test results were read with interpretation based on the indication by visible aggregation of the latex particles. Detail of the steps followed is presented in appendix 1.2.

From the Thermo Scientific *Campylobacter* test, results were interpreted as in table 3.1 and a typical example of positive *Campylobacter* sp. from the study is shown in plate 3.2.

Table 3. 1: *Campylobacter* Agglutination Test Results Interpretation

Reaction with test latex	Reaction with control latex	Interpretation
+	-	Campylobacters present.
-	-	Campylobacters not present in sufficient numbers detected by the test.
+	+	Non-specific agglutination.

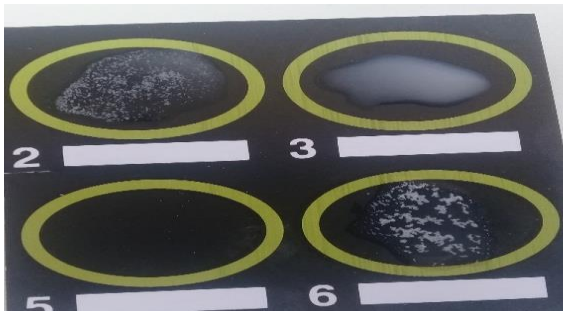


Plate 3. 2: A Picture Showing Positive *Campylobacter* Agglutination in Oval Window Labelled 2 and 6 After the Addition of Test Latex

3.4 Biotyping of the *Campylobacter* Isolates

The total 245 confirmed isolates of *Campylobacter* were re-streaked onto 5% sheep blood agar (Oxoid, UK) and incubated microaerophilically before biotyping.

In performing the biotyping, the isolates were subjected to Lior's scheme (Lior, 1984) and according to Lior's scheme of biotyping, *C. jejuni*, *C. coli*, *C. lari* were divided into seven biotypes based on the three phenotypic tests viz., hippurate hydrolysis test, rapid H_2S production and deoxyribonuclease enzyme production (DNase) test as shown in the table 3.2.

Table 3. 2: Lior`s Scheme of Biotyping

Test	*C. jejuni*				*C. coli*		*C. lari*	
	Biotype-I	Biotype-II	Biotype-III	Biotype-IV	Biotype-I	Biotype-II	Biotype-I	Biotype-II
Hippurate hydrolysis	+	+	+	+	-	-	-	-
Rapid H_2S	-	-	+	+	-	-	+	+
DNase	-	+	-	+	-	+	-	+

3.4.1 Hippurate Hydrolysis Test

In performing this test, hippurate strips kit (01869 Hippurate Strips Kit, Sigma-Aldrich) was employed. The hydrolysis of hippurate is indicated by colour change due to the release of glycine. A loopful of 24-hour test culture from blood agar was picked up and then followed the procedure below;

- A bacterial suspension from the pure test culture of Campylobacter was prepared in a narrow tube containing 0.5 ml of physiological saline solution (0.89%, w/v).
- The density of the suspension was then determined by adjusting the turbidity to approximately 2° on the McFarland turbidity scale using DEN-1B McFarland densitometer.
- A substrate strip (saturated with sodium Hippurate and 350 mg chromogen) was kept into the tube with the suspension with the whole paper zone dipped and mixed gently following incubation at 37 °C for 24 hours.
- After incubation, approximately 200 µl (approx. 4 drops) of a diluent (reagent) added alongside the tube wall gently and not mixed.
- It was then incubated at a temperature of 18-24 °C for 5-10 minutes and results of the test read and evaluated (negative reaction for no colour change while

positive reaction for a "ringed" blue-purple colour development mainly in the place of contact between the reagent and inoculum.

3.4.2 Hydrogen Sulfide Production (H_2S) Test

For H_2S production test, prepared slant of Triple Sugar Iron (TSI, Oxoid) agar was used. Colonies of inoculums of a 24-hour test culture of *Campylobacter* sp. from non-selective blood agar plate was picked up and inoculated by stabbing into the butt and streaking on slant in test medium (TSI) just below the surface. The tube was then kept in an incubator at 37 ^{o}C for 24 hours and observed for colour change. The positive reaction was indicated by blackening of the test medium around the inoculums of test culture in tube.

3.4.3 DNase Test

The production of nucleases by various bacteria has been demonstrated by growing the organism on DNA-containing media with methyl green (MG) or toluidine blue O as an indicator.

However, in this study, prepared plates of DNase agar (Oxoid, UK) medium was used and does not contain any indicator. The procedures followed for the test were;

i. A loopful of 24-hour test culture was used to inoculate heavily on an area about 5 mm in diameter on the DNase agar plate and incubated at 37 ^{o}C under microaerophilic condition.
ii. After incubation, the plates were further flooded with diluted 1M HCl (Hydrochloric acid).
iii. Flooded plate was allowed to stand on a laboratory bench (lids uppermost) for few minutes with result read and evaluated.

iv. Result was based on looking for positive reactions indicated by appearance of large, clear zones of hydrolysis on DNase agar around the colony.

3.5 Antibiotic Susceptibility Test

All the 245 *Campylobacter* isolates were subjected to antibiotic susceptibility test by Kirby-Bauer disc diffusion method according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) on plates of Mueller-Hinton agar (Oxoid, UK) supplemented with 5% defibrinated sheep blood (EUCAST, 2019). Incubation of plates was done at 42 °C for 24 hours under microaerophilic condition generated by a gas generating pack (CampyGen^{TM} 2.5L, Oxoid) in canisters. The plates before incubation, discs of the following antimicrobials were kept on them: ampicillin (10 µg), sulphamethoxazole/trimethoprim (25 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), nalidixic acid (30 µg), chloramphenicol (30 µg), ceftriaxone (30 µg), norfloxacin (10 µg), amikacin (30 µg), imipenem (10 µg) and tetracycline (30 µg) as suggested by External Quality Assurance System (EQAS, 2013) with some additions from local research works (Ahiabu *et al.*, 2016; Karikari *et al.*, 2017a,b). The antibiotics used in this study are also commonly used in Ghana. After 24 hours, the diameter of the zone of inhibition for each antibiotic disc were measured and the sensitivity of the bacteria to each antibiotic then determined by employing the general guidelines of EUCAST (EUCAST, 2019) for interpretation of the results. Detail of the procedures followed in performing the antibiotic test is shown in appendix 1.3. All the antibiotics used were sourced from Oxoid, Basingstoke, UK. There were no recommended antimicrobial breakpoints for *Campylobacter* sp. for nine of the antibiotics (gentamicin, ceftriaxone, chloramphenicol, norfloxacin, ampicillin, amikacin, imipenem, nalidixic acid and sulphamethoxazole/trimethoprim) used, breakpoints for Enterobacteriaceae were then employed (EUCAST, 2019) (Table 3.3).

Table 3. 3: EUCAST 2019 Breakpoints for the Various Antibiotics Used

ANTIBIOTICS	DISK CONTENT (µg)	BREAKPOINTS		
		S≥	I	R<
Ciprofloxacin-CIP	10	26	-	26
Gentamicin-CN	10	17	16-14	14
Erythromycin-E	15	20	-	20
Ceftriaxone-CRO	30	25	24-22	22
Chloramphenicol-C	30	17	-	17
Norfloxacin-NOR	10	22	21-19	19
Tetracycline-TE	30	30	-	30
Ampicillin-AMP	10	14	-	14
Amikacin-AK	30	18	17-15	15
Imipenem-IPM	10	22	21-16	16
Nalidixic Acid-NA	30	19	18-14	14
Sulphamethoxazole/ Trimethoprim-SXT	25	14	13-11	11

3.6 Storage of Confirmed *Campylobacter* Isolates

Prior to storage, confirmed *Campylobacter* isolates were streaked on prepared 5% sheep blood Columbia Blood Agar (CBA) (Oxoid, Basingstoke, UK) and incubated under microaerophilic condition generated by a gas generating pack (CampyGen™ 2.5L, Oxoid) in a canister at 42 °C for 24-48 hours.

A storage medium containing 15% glycerol Brain Heart Infusion was also prepared and 1 ml each pipetted into sterile cryo vials.

The freshly incubated bacteria were then scooped into the cryo vials containing the storage medium and labelled respectively. The isolates were stored in a freezer at -21 °C.

3.7 Data Analysis

Laboratory results and data collected on poultry, patients and healthy individuals were entered into Microsoft Excel and analysed in IBM SPSS (v 20; Statistical Package for

the Social Sciences). Frequencies and percentages were calculated for study variables using Fisher's exact test. *P* values of less than 0.05 were considered significant. The results obtained are presented in tables.

3.8 Ethical Approval and Verbal Consent

Prior to this study, ethical clearance was obtained from the Research Ethical Committee of the Tamale Teaching Hospital (appendix 1.4).

Verbal consent was sought from Commercial poultry farmers, various households in Nyankpala as well as students prior to sample collection.

CHAPTER FOUR

RESULTS

4.1 Prevalence of *Campylobacter* species

4.1.1 Prevalence of *Campylobacter* species in Poultry and Humans in the Northern Region of Ghana

A total of 1087 samples comprising both poultry and humans were investigated for *Campylobacter* species. From which, 346 (31.8%) were poultry and 741 (68.2%) were human samples. Out of the total samples, 245 (22.5%) were positive for *Campylobacter* species whereas 842 (77.5%) were negative (Table 4.1). There was significant difference (P< 0.05) in the prevalence of *Campylobacter* species from poultry and human sources (Table 4.1).

The overall prevalence of *Campylobacter* species was 22.5%, 43.1% in poultry, and 13% in humans (Table 4.1).

Table 4. 1: Prevalence of *Campylobacter* species in Poultry and Humans in Northern Region of Ghana

	FREQUENCY (%)			
SOURCES	**No. of Sample**	**No. of Positive**	**No. of Negative**	**P value**
POULTRY	346	149(43.1)	197(56.9)	
HUMANS	741	96(13)	645(87)	
TOTAL	1087	245(22.5)	842(77.5)	0.001

Values in bracket indicate percentage.

4.1.2 Prevalence of *Campylobacter* species Among the Various Sources in Poultry and Humans

Examination of 346 poultry for intestinal carriage by rectal swab revealed that 149 (43.1%) were harbouring *Campylobacter* sp. (Table 4.2). Out of the total 149 *Campylobacter* sp. isolated from poultry, 97 (65.1%) were from commercial sources and 52 (34.9%) were from domestic/household sources. The difference in prevalence of *Campylobacter* sp. between these two sources was significant ($P < 0.05$).

Similarly, within sources, the prevalence of *Campylobacter* sp. in commercial and domestic/households were 50.5% and 33.8% respectively, with a significant difference in rates ($P < 0.05$). (Table 4.2).

Table 4. 2: Prevalence of *Campylobacter* species in the Various Sources of Poultry

POULTRY SOURCES	FREQUENCY (%)			
	No. of Sample	No. of Positive	No. of Negative	P value
Commercial	192	97(50.5)	95(49.5)	
Domestic/Households	154	52(33.8)	102(66.2)	
TOTAL	346	149(43.1)	197(56.9)	0.001

Values in bracket indicate percentage.

Also, 741 faeces from human sources examined revealed that 96 (13%) were positive for *Campylobacter* sp. (Table 4.3). From the 96 *Campylobacter* sp. isolated, 71 (73.96%) were from hospital while 25 (26.04%) were from domestic/household sources.

The prevalence rate in hospital was higher than domestic/household and there was a significant difference between the two ($P < 0.05$) (Table 4.3).

Table 4. 3: Occurrence of *Campylobacter* species in the Sources of Human

HUMAN SOURCES	FREQUENCY (%)			
	No. of Sample	No. of Positive	No. of Negative	P value
Hospital	462	71(15.4)	391(84.6)	
Domestic/Households	279	25(9)	254(91)	
TOTAL	741	96(13)	645(87)	0.001

Values in bracket indicate percentage.

4.1.3 Prevalence of *Campylobacter jejuni* and Non- *jejuni* sp. in Humans and Poultry

Of the 245 isolated *Campylobacter* species stored, only 105 were culturable at the time of characterisation. The characterisation showed 72 (68.6%) being *C. jejuni* and 33 (31.4%) Non-*jejuni* species out of the total 105 *Campylobacter* species screened (Table 4.4). From the 72 *C. jejuni*, 58 (80.6%) were from poultry and 14 (19.4%) were from humans. Additionally, out of the 33 Non-*jejuni*, 17 (51.5%) and 16 (48.5%) were from poultry and humans, respectively. There was a significant difference ($P < 0.05$) in the rate of *C. jejuni* and Non-*jejuni* isolated (Table 4.4).

Table 4. 4: Prevalence of *Campylobacter jejuni* and Non-*jejuni* in Poultry and Human Sources

SOURCES	FREQUENCY (%)			
	No. of Isolates	No. of *C. jejuni*	No. of Non-*jejuni*	P value
Poultry	75	58(77.3)	17(22.7)	
Human	30	14(46.7)	16(53.3)	
TOTAL	105	72(68.6)	33(31.4)	0.001

Values in bracket indicate percentage.

4.1.4 Prevalence of *Campylobacter jejuni* and Non- *jejuni* sp. Among Poultry and Human isolates

A. In Poultry

From the 75 *Campylobacter* isolates from poultry, 58 (77.3%) and 17 (22.7%) were *C. jejuni* and Non-*jejuni*, respectively (Table 4.5).

Within each source, the prevalence of *C. jejuni* in commercial source (84%) was higher than in domestic/household source (64%). For the Non-*jejuni*, commercial source recorded lower prevalence of 16% than that of domestic/household sources (36%) (Table 4.5).

Generally, there was a significant difference ($P< 0.05$) both between sub-sources and within sub-sources.

B. In Humans

The 30 *Campylobacter* isolates from humans included 14 (46.7%) and 16 (53.3%) *C. jejuni* and Non-*jejuni* respectively (Table 4.5).

Within sources, hospital had the least prevalence for *C. jejuni* (45%) while domestic/household had the highest of 50%; whereas in Non-*jejuni*, the highest prevalence was recorded in hospital (55%) with domestic/household recording least prevalence of 50% (Table 4.5).

Table 4. 5: Prevalence of *Campylobacter jejuni* and Non-*jejuni* in Poultry and Humans Sub-Sources

SOURCES	FREQUENCY (%)			
	No. of Isolates	No. of *C. jejuni*	No. of Non-*jejuni*	P value
Poultry				
Commercial	50	42(84)	8(16)	
Domestic/Household	25	16(64)	9(36)	
TOTAL	75	58(77.3)	17(22.7)	0.001
Humans				
Hospital	20	9(45)	11(55)	
Domestic/Household	10	5(50)	5(50)	
TOTAL	30	14(46.7)	16(53.3)	0.001

Values in bracket indicate percentage.

4.1.5 Prevalence of the Biotypes of *Campylobacter jejuni* in Poultry and Humans

The total 72 *Campylobacter jejuni* were characterized into the four known categories of biotypes under *jejuni* sp. while employing the Lior`s scheme of biotyping.

In all, the study recorded the highest prevalence of 31 (43.1%) for biotype-II and the least of 2 (2.8%) for biotype-IV (Table 4.6). Among all the four biotypes, there was a significant difference (P< 0.05) observed in their prevalence rates.

Table 4. 6: Prevalence of the Various Biotypes in *Campylobacter jejuni*

Campylobacter jejuni **Biotypes**	**No. of Isolates (%)**	**P value**
Biotype-I	29(40.3)	
Biotype-II	31(43.1)	
Biotype-III	10(13.9)	
Biotype-IV	2(2.8)	
TOTAL	72 (100)	0.018

Values in bracket indicate percentage.

Out of the total 72 *Campylobacter jejuni* isolates, 58 (80.6%) were from poultry sources and 14 (19.4%) were from human sources.

In poultry (58), the biotype with the highest frequency was the biotype-II 30 (51.7%) and the least of 2 (3.4%) observed in biotype-IV. However, in humans (14), biotype-I recorded the highest of 10 (71.43%) and biotype-II recording the least of 1 (7.14%). There was no record for biotype-IV in humans (Table 4.7).

Table 4. 7: Biotypes of Isolates from Poultry and Humans

Sources	**No. of Isolates**	**No. of *C. jejuni* (%)**				**P value**
		Biotype-I	**Biotype-II**	**Biotype-III**	**Biotype-IV**	
Poultry	58	19 (32.8)	30 (51.7)	7 (12.1)	2 (3.4)	
Humans	14	10 (71.43)	1 (7.14)	3 (21.43)	0	
TOTAL	72	29 (40.3)	31 (43.1)	10 (13.9)	2 (2.8)	0.014

Values in bracket indicate percentage.

4.1.6 Prevalence of the Biotypes of *Campylobacter jejuni* in the Various Sources of Poultry and Humans

The two sources of the poultry were commercial and domestic/household. The total biotypes under the commercial sub-source was 42 (72.4%) and 16 (27.6%) prevalence found under domestic/household sub-source; all of which were from the general 58 biotype isolates in poultry (Table 4.8).

From the total 42 biotype isolates from commercial source, biotype-II and biotype-IV were the highest and least prevalent with 25 (59.5%) and 1 (2.4%), respectively. Also, in the 16 isolates from domestic source, the biotype-I recorded the highest of 9 (app. 56.3%) and two other biotypes (III and IV) recorded the least of 1 (app. 6.3%) each. Despite the different biotype prevalent levels, there was no significant difference recorded between and within the sub-sources (P> 0.05) (Table 4.8).

A total of 14 isolates were recorded from the various biotypes under the human source. Of these total isolates, 9 representing 64.3% were from the hospital sub-source and 5 (35.7%) were from the domestic/household sub-source (Table 4.8).

The biotype with highest prevalence in the hospital source was biotype-I with 6 (%) isolates and least prevalence was biotype-III with 3(%) isolates. There was no record of isolates for biotype II and IV. In prevalence from domestic/household source, biotype I and II recorded the highest and least respectively with 4 (%) isolates and 1 (%) isolate. Despite the different biotype prevalent levels, there was no significant difference recorded between and within the sub-sources (P> 0.05) (Table 4.8).

Table 4. 8: Biotyping of Isolates from Various Poultry and Human Sources

SOURCES	No. of Isolates	No. of *C. jejuni* (%)				P value
		Biotype-I	Biotype-II	Biotype-III	Biotype-IV	
Poultry						
Commercial	42	10 (23.8)	25 (59.5)	6 (14.3)	1 (2.4)	
Domestic	16	9 (56.25)	5 (31.25)	1 (6.25)	1(6.25)	
TOTAL	58	19 (32.8)	30 (51.7)	7 (12.1)	2 (3.4)	0.170
Humans						
Hospital	9	6 (66.7)	0	3 (33.3)	0	
Domestic	5	4 (80)	1 (20)	0	0	
TOTAL	14	10 (71.43)	1 (7.14)	3 (21.43)	0	0.084

Values in bracket indicate percentage.

4.2 Antimicrobial Susceptibility Testing

4.2.1 Antimicrobial Susceptibility Profile of *Campylobacter* Isolates

Of the 105 isolates that were subjected to antimicrobial susceptibility testing, the highest level of resistance of the *Campylobacter* isolates was recorded against tetracycline (100%) while the least resistance was to imipenem (6.7%). Resistance to the remaining drugs were 93.3% to Ceftriaxone, 78.1% to ampicillin 74.3% to erythromycin, 56.2% to ciprofloxacin, 38% to Sulphamethoxazole/Trimethoprim, 33.3% to chloramphenicol, 28.6% to nalidixic acid, 21% to norfloxacin, 11.4% to amikacin and 10.5% to gentamicin (Table 4.9). Differences in susceptibility pattern among the *Campylobacter* species to the various antibiotics was statistically significant ($p< 0.05$) (Table 4.9).

Table 4. 9: Antibiotic Resistance and Susceptibility Patterns of *Campylobacter* species

Antibiotics	Susceptibility Pattern (Freq/%)			P value
	S	I	R	
Amikacin-AK	69 (65.7)	24 (22.9)	12 (11.4)	
Ampicillin-AMP	23 (21.9)	N	82 (78.1)	
Chloramphenicol-C	70 (66.7)	N	35 (33.3)	
Ciprofloxacin-CIP	46 (43.8)	N	59 (56.2)	
Gentamicin-CN	78 (74.3)	16 (15.2)	11 (10.5)	
Ceftriaxone-CRO	6 (5.7)	1 (1)	98 (93.3)	
Erythromycin-E	27 (25.7)	N	78 (74.3)	
Imipenem-IPM	83 (79)	15 (14.3)	7 (6.7)	
Nalidixic Acid-NA	69 (65.7)	6 (5.7)	30 (28.6)	
Norfloxacin-NOR	60 (57.1)	23 (21.9)	22 (21)	
SXT	64 (61)	1(1)	40 (38)	
Tetracycline-TE	0	N	105 (100)	0.001

Values in bracket indicate percentage.

Keys: S=Sensitive; I=Intermediate; R=Resistant; N=Intermediate not available; SXT= Sulphamethoxazole/Trimethoprim

4.2.2 Susceptibility Profile of *Campylobacter* species in Poultry and Humans

Poultry isolates showed highest resistance to tetracycline 75 (100%) and least resistance to imipenem (5.3%) (Table 4.10). Similarly human isolates showed most resistance to tetracycline 30 (100%) and least to amikacin and imipenem (each 10%) (Table 4.10).

The next antibiotics with highest and least isolate resistance was ceftriaxone (93.34% and 93.4%) and gentamicin (8% and 16.7%) respectively in poultry and humans with other remaining varying as shown in the table 4.10;

Table 4. 10: The Antimicrobial Susceptibility Pattern for *Campylobacter* sp. from Poultry and Human Sources

Antibiotics	Susceptibility Pattern (Freq/%)						P value
	Poultry (n=75)			Human (n=30)			
	S	I	R	S	I	R	
AK	43(57.3)	23(30.7)	9(12)	26(86.7)	1(3.3)	3(10)	
AMP	19(25.3)	N	56(74.7)	4(13.3)	N	26(86.7)	
C	56(74.7)	N	19(25.3)	14(46.7)	N	16(53.3)	
CIP	35(46.7)	N	40(53.3)	11(36.7)	N	19(63.3)	
CN	56(74.7)	13(17.3)	6(8)	22(73.3)	3(10)	5(16.7)	
CRO	5(6.7)	0	70(93.3)	1(3.3)	1(3.3)	28(93.4)	
E	25(33.3)	N	50(66.7)	2(6.6)	N	28(93.4)	
IPM	58(77.4)	13(17.3)	4(5.3)	25(83.4)	2(6.6)	3(10)	
NA	51(68)	5(6.7)	19(25.3)	18(60)	1(3.3)	11(36.7)	
NOR	41(54.7)	19(25.3)	15(20)	19(63.4)	4(13.3)	7(23.3)	
SXT	53(70.7)	1(1.3)	21(28)	11(36.7)	0	19(63.3)	
TE	0	N	75(100)	0	N	30(100)	0.001

Values in bracket indicate percentage.

Keys: S=Sensitive; I=Intermediate; R=Resistant; N=Intermediate not available; AK=Amikacin; AMP=Ampicillin; C= Chloramphenicol; CIP= Ciprofloxacin; CN= Gentamicin; CRO= Ceftriaxone; E= Erythromycin; IPM= Imipenem; NA= Nalidixic Acid; NOR= Norfloxacin; SXT= Sulphamethoxazole/Trimethoprim; TE=Tetracycline

4.2.3 Susceptibility Profile of *Campylobacter* species in the Various Sources of Poultry and Humans

In Poultry Sources

Seventy-five (75) isolates of *Campylobacter* were recorded for poultry, out of which 50 were from commercial source and 25 from domestic/household source. There was a significant difference ($p < 0.05$) among the antibiotic susceptibility rate and between the two sub-sources (Table 4.11). Additionally, tetracycline recorded the highest resistance with total resistance (100%) in both sources. However, gentamicin recorded the least resistance with 4% in commercial source while 0% was recorded for both amikacin and imipenem in the domestic/household source (Table 4.11).

Isolates showed the next highest resistance to ceftriaxone (94% and 92%) for both commercial and household sources respectively. Also, imipenem (8%) and norfloxacin (12%) were the next least resisted antibiotics in commercial and domestic/household sources respectively; with others having their resistance trend as presented in table 4.11.

Table 4. 11: The Antimicrobial Susceptibility Pattern for *Campylobacter* sp. from Poultry Sources

Antibiotics	Susceptibility Pattern (Freq/%)						P value
	Commercial (n=50)			Domestic (n=25)			
	S	I	R	S	I	R	
AK	23(46)	18(36)	9(18)	20(80)	5(20)	0	
AMP	11(22)	N	39(78)	8(32)	N	17(68)	
C	41(82)	N	9(18)	15(60)	N	10(40)	
CIP	22(44)	N	28(56)	13(52)	N	12(48)	
CN	38(76)	10(20)	2(4)	18(72)	3(12)	4(16)	
CRO	3(6)	0	47(94)	2(8)	0	23(92)	
E	20(40)	N	30(60)	5(20)	N	20(80)	
IPM	35(70)	11(22)	4(8)	23(92)	2(8)	0	
NA	32(64)	5(10)	13(26)	19(76)	0	6(24)	
NOR	27(54)	11(22)	12(24)	14(56)	8(32)	3(12)	
SXT	37(74)	1(2)	12(24)	16(64)	0	9(36)	
TE	0	N	50(100)	0	N	25(100)	0.001

Values in bracket indicate percentage.

Keys: S=Sensitive; I=Intermediate; R=Resistant; N=Intermediate not available; AK=Amikacin; AMP=Ampicillin; C= Chloramphenicol; CIP= Ciprofloxacin; CN= Gentamicin; CRO= Ceftriaxone; E= Erythromycin; IPM= Imipenem; NA= Nalidixic Acid; NOR= Norfloxacin; SXT= Sulphamethoxazole/Trimethoprim; TE=Tetracycline

In Human Sources

Thirty (30) isolates of *Campylobacter* were recorded for human sources, out of which 20 were from hospital sub-source and 10 from domestic/household sub-source. There was a significant difference ($p < 0.05$) among the antibiotic susceptibility rate between the two sub-sources (Table 4.12). Additionally, tetracycline recorded the highest resistance with total resistance (100%) in both sub-sources. However, amikacin and imipenem recorded the least resistance with 10% (each) in hospital sub-source while four of the antibiotics (amikacin, gentamicin, imipenem and norfloxacin) recorded least of 10% each in the domestic/household source (Table 4.12). The remaining antibiotics had their resistance pattern as in table 4.12.

Table 4. 12: The Antimicrobial Susceptibility Pattern for *Campylobacter* sp. from Human Sources

Antibiotics	Susceptibility Pattern (Freq/%)						P value
	Hospital (n=20)			Domestic (n=10)			
	S	I	R	S	I	R	
AK	17(85)	1(5)	2(10)	9(90)	0	1(10)	
AMP	2(10)	N	18(90)	2(20)	N	8(80)	
C	9(45)	N	11(55)	5(50)	N	5(50)	
CIP	6(30)	N	14(70)	5(50)	N	5(50)	
CN	15(75)	1(5)	4(20)	7(70)	2(20)	1(10)	
CRO	1(5)	0	19(95)	0	1(10)	9(90)	
E	1(5)	N	19(95)	1(10)	N	9(90)	
IPM	17(85)	1(5)	2(10)	8(80)	1(10)	1(10)	
NA	10(50)	1(5)	9(45)	8(80)	0	2(20)	
NOR	12(60)	2(10)	6(30)	7(70)	2(20)	1(10)	
SXT	7(35)	0	13(65)	4(40)	0	6(60)	
TE	0	N	20(100)	0	N	10(100)	0.001

Values in bracket indicate percentage.

Keys: S=Sensitive; I=Intermediate; R=Resistant; N=Intermediate not available; AK=Amikacin; AMP=Ampicillin; C= Chloramphenicol; CIP= Ciprofloxacin; CN= Gentamicin; CRO= Ceftriaxone; E= Erythromycin; IPM= Imipenem; NA= Nalidixic Acid; NOR= Norfloxacin; SXT= Sulphamethoxazole/Trimethoprim; TE=Tetracycline

4.2.4 Species Specific Resistance Profile of the *Campylobacter* sp. in Poultry and Humans in the Northern Region of Ghana

Out of the 105 *Campylobacter* isolates, 72 (68.6%) were *C. jejuni* and 33 (31.4%) Non-*jejuni* sp. In the general susceptibility profile, resistance rate between *C. jejuni* and Non-*jejuni* sp., were all statistically significant ($p < 0.05$) (Table 14). Tetracycline showed the highest of total resistance in both the *C. jejuni* and Non-*jejuni* sp. (each 100%), while imipenem showed the least resistance of 6 (8.3%) and 1 (3%) in *C. jejuni* and Non-*jejuni* sp., respectively. Besides, there was no record of resistance to erythromycin

in the Non-*jejuni* sp. (Table 4.13). Isolates demonstrated varying resistance trend to the other antibiotics and this is shown for *C. jejuni* and Non-*jejuni* sp. respectively in the table below (Table 4.13).

Table 4. 13: Resistance Profile of *C. jejuni* and Non-*jejuni* sp. Isolates

Antibiotics	*C. jejuni* (72)	Non-*jejuni* sp. (33)	P value
	(%) Resistance	(%) Resistance	
Amikacin-AK	10(13.9)	2(6.1)	
Ampicillin-AMP	55(76.4)	27(81.8)	
Chloramphenicol-C	23(31.9)	12(36.4)	
Ciprofloxacin-CIP	42(58.3)	17(51.5)	
Gentamicin-CN	7(9.7)	4(12.1)	
Ceftriaxone-CRO	67(93.1)	31(93.9)	
Erythromycin-E	50(69.4)	0	
Imipenem-IPM	6(8.3)	1(3)	
Nalidixic Acid-NA	20(27.8)	10(30.3)	
Norfloxacin-NOR	16(22.2)	6(18.2)	
SXT	25(34.7)	15(45.5)	
Tetracycline-TE	72(100)	33(100)	0.001

Values in bracket indicate percentage. Keys: SXT= Sulphamethoxazole/Trimethoprim

4.2.5 Resistance Profile of the *Campylobacter jejuni* and Non-*jejuni* sp. in Poultry and Humans

From the two main sources (poultry and humans), there was an observed total resistance (100%) for tetracycline in both *C. jejuni* and Non-*jejuni* sp. However, least resisted antibiotics in poultry were gentamicin and imipenem (6.9% each), and in humans; amikacin and imipenem (14.3% each) for *C. jejuni* strains. For the Non-*jejuni* sp.,

though there was no recorded resistance for imipenem in poultry, amikacin had the lower of 1 (5.9%) while in humans, amikacin and imipenem were the least with 1 (6.3%) each. There were significant differences ($p < 0.05$) observed between sources, specific species and within the antibiotics (Table 4.14).

Table 4. 14: Resistance Profile of *C. jejuni* and Non-*jejuni* sp. from Poultry and Human Sources

Antibiotics	*C. jejuni* (72)		Non-*jejuni* (33)		P value
	Poultry (58)	Humans (14)	Poultry (17)	Humans (16)	
	Resistance (%)	Resistance (%)	Resistance (%)	Resistance (%)	
AK	8(13.8)	2(14.3)	1(5.9)	1(6.3)	
AMP	44(75.9)	11(78.6)	12(70.6)	15(93.8)	
C	13(22.4)	10(71.4)	6(35.3)	6(37.5)	
CIP	32(55.2)	10(71.4)	8(47.1)	9(56.3)	
CN	4(6.9)	3(21.4)	2(11.8)	2(12.5)	
CRO	54(93.1)	13(92.9)	16(94.1)	15(93.8)	
E	38(65.5)	12(85.7)	12(70.6)	16(100)	
IPM	4(6.9)	2(14.3)	0	1(6.3)	
NA	15(25.9)	5(35.7)	4(23.5)	6(37.5)	
NOR	13(22.4)	3(21.4)	2(11.8)	4(25)	
SXT	17(29.3)	8(57.1)	4(23.5)	11(68.8)	
TE	58(100)	14(100)	17(100)	16(100)	0.001

Values in bracket indicate percentage.

Keys: AK=Amikacin; AMP=Ampicillin; C= Chloramphenicol; CIP= Ciprofloxacin; CN= Gentamicin; CRO= Ceftriaxone; E= Erythromycin; IPM= Imipenem; NA= Nalidixic Acid; NOR= Norfloxacin; SXT= Sulphamethoxazole/Trimethoprim; TE=Tetracycline

4.2.6 Species Specific Resistance Profile of the *Campylobacter* sp. in Poultry and Humans in the Northern Region of Ghana

In the two sources of poultry, the resistance rates of *C. jejuni* and Non-*jejuni* sp. between the two sub-sources were significantly different ($p < 0.05$). There was an observed total resistance (100%) to tetracycline in all the sources for both *C. jejuni* and Non-*jejuni* sp. However, the lowest resistance in commercial source was noted in gentamicin 1 (2.9%) for *C. jejuni* while in domestic/household, norfloxacin had the least with 1 (6.3%). Amikacin and imipenem had no resistance recorded for the domestic source (Table 4.15). Also, Non-*jejuni* sp. showed least resistance rate of 12.5% to four of the antibiotics (gentamicin, amikacin, nalidixic acid and sulphamethoxazole/trimethoprim) in commercial source and 11.1% to gentamicin in the domestic/household source. Besides, there were absence of resistance to imipenem and norfloxacin in the commercial sub-source and in the domestic sub-source, no resistance was observed for amikacin and imipenem (Table 4.15).

Table 4. 15: Resistance Profile of *C. jejuni* and Non-*jejuni* sp. from Poultry Sources

Antibiotic	*C. jejuni* (n=58)		Non-*jejuni* (n=17)		P value
	Commercial (n=42)	Domestic (n=16)	Commercial (n=8)	Domestic (n=9)	
	Resistance (%)	Resistance (%)	Resistance (%)	Resistance (%)	
AK	8(19)	0	1(12.5)	0	
AMP	33(78.6)	11(68.8)	6(75)	6(66.7)	
C	7(16.7)	6(37.5)	2(25)	4(44.4)	
CIP	25(59.5)	7(43.8)	3(37.5)	5(55.6)	
CN	1(2.9)	3(18.8)	1(12.5)	1(11.1)	
CRO	39(92.9)	15(93.8)	8(100)	8(88.9)	
E	25(59.5)	13(81.3)	5(62.5)	7(77.8)	
IPM	4(9.5)	0	0	0	
NA	12(28.6)	3(18.8)	1(12.5)	3(33.3)	
NOR	12(28.6)	1(6.3)	0	2(22.2)	
SXT	11(26.2)	6(37.5)	1(12.5)	3(33.3)	
TE	42(100)	16(100)	8(100)	9(100)	0.001

Values in bracket indicate percentage.

Keys: AK=Amikacin; AMP=Ampicillin; C= Chloramphenicol; CIP= Ciprofloxacin; CN= Gentamicin; CRO= Ceftriaxone; E= Erythromycin; IPM= Imipenem; NA= Nalidixic Acid; NOR= Norfloxacin; SXT= Sulphamethoxazole/Trimethoprim; TE=Tetracycline

Similarly in human sources, there was an observed total resistance to tetracycline in all the two sources for both *C. jejuni* and Non-*jejuni* sp. However, the antibiotics with no observed resistance from strains of *C. jejuni* and Non-*jejuni* sp. were seen in five antibiotics (amikacin, gentamicin, imipenem, nalidixic acid and norfloxacin) either under the hospital or domestic/household sources as in the table below (Table 4.16).

Table 4. 16: Resistance Profile of *C. jejuni* and Non-*jejuni* sp. from Human Sources

Antibiotic	*C. jejuni* (n=14)		Non-*jejuni* (n=16)		P value
	Hospital (n=9)	Domestic(n=5)	Hospital (n=11)	Domestic(n=5)	
	Resistance (%)	Resistance (%)	Resistance (%)	Resistance (%)	
AK	2(22.2)	0	0	1(20)	
AMP	8(88.9)	3(60)	10(90.9)	5(100)	
C	6(66.7)	4(80)	5(45.5)	1(20)	
CIP	7(77.8)	3(60)	7(63.6)	2(40)	
CN	2(22.2)	1(20)	2(18.2)	0	
CRO	9(100)	4(80)	10(90.9)	5(100)	
E	8(88.9)	4(80)	11(100)	5(100)	
IPM	2(22.2)	0	0	1(20)	
NA	3(33.3)	2(40)	6(54.5)	0	
NOR	2(22.2)	1(20)	4(36.4)	0	
SXT	5(55.6)	3(60)	8(72.7)	3(60)	
TE	9(100)	5(100)	11(100)	5(100)	0.001

Values in bracket indicate percentage.

Keys: AK=Amikacin; AMP=Ampicillin; C= Chloramphenicol; CIP= Ciprofloxacin; CN= Gentamicin; CRO= Ceftriaxone; E= Erythromycin; IPM= Imipenem; NA= Nalidixic Acid; NOR= Norfloxacin; SXT= Sulphamethoxazole/Trimethoprim; TE=Tetracycline

4.2.7 Biotype Distribution Resistance Profile of the *Campylobacter jejuni* in Poultry and Human Sources

Isolates of *Campylobacter jejuni* under the four biotypes in poultry showed statistical significance ($p < 0.05$) in total. However, within biotypes, except the *C. jejuni* biotype-IV which was statistically insignificant ($p > 0.05$), the remaining first three *C. jejuni* biotypes (I, II, III) were all significant ($p < 0.05$). All biotypes showed total resistance to tetracycline (Table 4.17).

In *C. jejuni* biotype-I, imipenem (5.3%) was least resisted antibiotic; gentamicin (3.3%) was least resisted in *C. jejuni* biotype-II; in *C. jejuni* biotype-III, ceftriaxone also showed total resistance with zero record for amikacin and least in gentamicin, imipenem, nalidixic acid, norfloxacin with 14.3% each; and finally in *C. jejuni* biotype-IV. While ceftriaxone was totally resisted, an observed share percentage of 50 each for ampicillin, chloramphenicol, ciprofloxacin, sulphamethoxazole/trimethoprim was also recorded (Table 4.17).

In the human isolates, there were absence of *C. jejuni* biotype-VI. Though resistance in *C. jejuni* biotype-I was statistically significant ($p < 0.05$), the biotype-II and III were insignificant ($p > 0.05$). Similarly, as in the poultry source, total resistance to tetracycline was also seen in the three biotypes in humans. There was some total resistance also seen in *C. jejuni* biotype-II for four other antibiotics (ampicillin, ciprofloxacin, ceftriaxone and sulphamethoxazole/ trimethoprim) and three others in *C. jejuni* biotype-II (i.e. ceftriaxone, erythromycin and chloramphenicol). There were some records of zero resistances demonstrated by the strains towards some antibiotics (i.e. amikacin, gentamicin, erythromycin, imipenem, nalidixic acid and norfloxacin) as presented in table 4.17.

Table 4. 17: Resistance Distribution for the Biotype Groups Under Poultry and Human Sources

Anti	Poultry (n=58)				Humans (n=14)			P value
	Biotyp-I (19)	Biotyp-II (30)	Biotyp-III (7)	Biotyp-IV (2)	Biotyp-I (10)	Biotyp-II (1)	Biotyp-III (3)	
	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	
AK	4(21.1)	4(13.3)	0	0	2(20)	0	0	0.001
AMP	14(73.7)	24(80)	5(71.4)	1(50)	8(80)	1(100)	2(66.7)	
C	8(42.1)	2(6.7)	2(28.6)	1(50)	7(70)	0	3(100)	
CIP	10(52.6)	19(63.3)	2(28.6)	1(50)	7(70)	1(100)	2(66.7)	
CN	2(10.5)	1(3.3)	1(14.3)	0	2(20)	0	1(33.3)	
CRO	19(100)	26(86.7)	7(100)	2(100)	9(90)	1(100)	3(100)	
E	15(78.9)	19(63.3)	4(57.1)	0	9(90)	0	3(100)	
IPM	1(5.3)	2(6.7)	1(14.3)	0	2(20)	0	0	
NA	7(36.8)	7(23.3)	1(14.3)	0	4(40)	0	1(33.3)	
NOR	3(15.8)	9(30)	1(14.3)	0	2(20)	0	1(33.3)	
SXT	5(26.3)	9(30)	2(28.6)	1(50)	5(50)	1(100)	2(66.7)	
TE	19(100)	30(100)	7(100)	2(100)	10(100)	1(100)	3(100)	
P value	0.001	0.001	0.001	0.252	0.001	0.347	0.099	

Values in bracket indicate percentage.

Keys: Anti=Antibiotics; Biotyp=Biotype; R=Resistance; AK=Amikacin; AMP=Ampicillin; C= Chloramphenicol; CIP= Ciprofloxacin; CN= Gentamicin; CRO= Ceftriaxone; E= Erythromycin; IPM= Imipenem; NA= Nalidixic Acid; NOR= Norfloxacin; SXT= Sulphamethoxazole/Trimethoprim; TE=Tetracycline

4.2.8 Biotype Resistance Profile of the *Campylobacter jejuni* in the Various Sources of Poultry and Humans

In Poultry

From the commercial poultry source, three of the biotypes (I, II, III) were statistically significant ($p < 0.05$) within biotypes while the difference in resistance in biotype-IV was insignificant ($p > 0.05$) (Table 4.18). Total resistance to tetracycline (100%) was revealed for all the four biotypes. From the biotype-I, ceftriaxone had 100% resistance with no resistance shown in gentamicin. In biotype-III, amikacin and gentamicin had no resistance. Only one isolate was found under the biotype-IV and that one isolate was resistant to ampicillin, ciprofloxacin, ceftriaxone in addition to the tetracycline. However, there were no resistance seen in the remaining antibiotics (Table 4.18).

Moreover, resistance rate in two of the biotypes (I, II) in the domestic source were significant ($p < 0.05$) and two (III, IV) others remaining were not significant ($p > 0.05$) within biotypes, though significant difference ($p<0.05$) was observed in total (Table 4.18). Amikacin and imipenem had no resistance from the biotype-I strains. Erythromycin was totally resisted as tetracycline by biotype-II strains and in addition to this, five other antibiotics (amikacin, chloramphenicol, gentamicin, norfloxacin and nalidixic acid) showed no resistance in the biotype-II. Also, in the biotype-III and IV, there was only one isolate each and the resistance demonstrated vary among the antibiotics used with 50% record (Table 4.18).

Table 4. 18: Resistance Distribution for the Biotype Groups in Poultry Sources

Anti	Poultry (n=58)							
	Commercial (n=42)				Domestic/Household (n=16)			
	Biotyp-I (10)	Biotyp-II (25)	Biotyp-III (6)	Biotyp-IV (1)	Biotyp-I (9)	Biotyp-II (5)	Biotyp-III (1)	Biotyp-IV (1)
	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)
AK	4(40)	4(16)	0	0	0	0	0	0
AMP	8(80)	20(80)	4(66.7)	1(100)	6(66.7)	4(80)	1(100)	0
C	4(40)	2(8)	1(16.7)	0	4(44.4)	0	1(100)	1(100)
CIP	6(60)	16(64)	2(33.3)	1(100)	4(44.4)	3(60)	0	0
CN	0	1(4)	0	0	2(22.2)	0	1(100)	0
CRO	10(100)	22(88)	6(100)	1(100)	9(100)	4(80)	1(100)	1(100)
E	8(80)	14(56)	3(50)	0	7(77.8)	5(100)	1(100)	0
IPM	1(10)	2(8)	1(16.7)	0	0	2(40)	0	0
NA	4(40)	7(28)	1(16.7)	0	3(33.3)	0	0	0
NOR	2(20)	9(36)	1(16.7)	0	1(11.1)	0	0	0
SXT	2(20)	8(32)	1(16.7)	0	3(33.3)	1(20)	1(100)	1(100)
TE	10(100)	25(100)	6(100)	1(100)	9(100)	5(100)	1(100)	1(100)
P value	0.001	0.001	0.002	0.364	0.001	0.001	0.347	0.347

Values in bracket indicate percentage.

Keys: Anti=Antibiotics; Biotyp=Biotype; R=Resistance; AK=Amikacin; AMP=Ampicillin; C= Chloramphenicol; CIP= Ciprofloxacin; CN= Gentamicin; CRO= Ceftriaxone; E= Erythromycin; IPM= Imipenem; NA= Nalidixic Acid; NOR= Norfloxacin; SXT= Sulphamethoxazole/Trimethoprim; TE=Tetracycline

A total of fourteen (14) isolates of *C. jejuni* from human sources were examined, out of which 9 were from hospital sub-source and 5 from domestic/household source. Biotypes I and III were observed in the hospital source showing resistance rates of significant difference (P=0.001) while biotypes I and II were seen in the domestic/household source, also with no significant difference (P=0.050).

In the hospital source, 100% resistance to tetracycline across the two biotypes was revealed. From the biotype-I, strains also showed additional total resistance (100% each) to ampicillin and ceftriaxone. In biotype-III there were 3 isolates recorded, and they showed 100% resistance each to chloramphenicol, ceftriaxone, erythromycin and tetracycline (Table 4.19).

Moreover, the biotype-I strains in the domestic/household source were sensitive to amikacin and imipenem, while 100% each resistance rate was shown to erythromycin and chloramphenicol. Also, in the biotype-II, there was only one isolate and it was resistant to five of the antibiotics used (i.e. ampicillin, ciprofloxacin, ceftriaxone, sulphamethoxazole/ trimethoprim and tetracycline). No resistance was seen in the other remaining antibiotics for the biotype-II (Table 4.19).

Table 4. 19: Resistance Distribution for the Biotype Groups in Human Sources

Antibiotic	Hospital (n=9)		Domestic/Household (n=5)		P value
	Biotype-I (n=6)	Biotype-III (n=3)	Biotype-I (n=4)	Biotype-II (n=1)	
	Resistance (%)	Resistance (%)	Resistance (%)	Resistance (%)	
AK	2(33.3)	0	0	0	0.001
AMP	6(100)	2(66.7)	2(50)	1(100)	
C	3(50)	3(100)	4(100)	0	
CIP	5(83.3)	2(66.7)	2(50)	1(100)	
CN	1(16.7)	1(33.3)	1(25)	0	
CRO	6(100)	3(100)	3(75)	1(100)	
E	5(83.3)	3(100)	4(100)	0	
IPM	2(33.3)	0	0	0	
NA	2(33.3)	1(33.3)	2(50)	0	
NOR	1(16.7)	1(33.3)	1(25)	0	
SXT	3(50)	2(66.7)	2(50)	1(100)	
TE	6(100)	3(100)	4(100)	1(100)	
P value	0.013	0.099	0.040	0.347	

Values in bracket indicate percentage.

Keys: Antibiotic=Antibiotics; AK=Amikacin; AMP=Ampicillin; C= Chloramphenicol; CIP= Ciprofloxacin; CN= Gentamicin; CRO= Ceftriaxone; E= Erythromycin; IPM= Imipenem; NA= Nalidixic Acid; NOR= Norfloxacin; SXT= Sulphamethoxazole/Trimethoprim; TE=Tetracycline

4.3 Multidrug Resistant Distribution

4.3.1 Multidrug Resistant Distribution of *Campylobacter* species from Poultry and Humans

The study defines multidrug resistance as resistance to 3 or more classes of the antibiotics used in the antimicrobial susceptibility testing for the isolates *Campylobacter* strains. Multidrug resistance in the defined context was seen in 96.2% (101/105) of the *Campylobacter* strains. However, 3.8% (4/105) were not multidrug resistant though 3 of that four isolates were also resistant to two of the antibiotics used and 1 strain resistant to only one antibiotic. Resistance to 11 of the 12 antibiotics used was observed, the highest frequencies of 21 strains each were resistant to 4 and/or 5 of the antibiotics were also revealed (as presented in table B of appendix 1.5).

Considering the multidrug resistance proportion within each source, 94.7% (71/75) and 100% (30/30) of multidrug resistance were seen in poultry and human isolates, respectively. The rate of multidrug resistance was more in humans than in poultry. However, there was no significant difference ($p > 0.05$) of multidrug resistance observed between the two sources (Table 4.20).

Table 4. 20: Profile of Multidrug Resistance in Poultry and Humans

	FREQUENCY (%)			
SOURCES	**No. of Isolates**	**No. of Multidrug**	**No. of Non-Multidrug**	**P value**
Poultry	75	71(94.7)	4(5.3)	
Human	30	30(100)	0	
TOTAL	105	101(96.2)	4(3.8)	0.254

Values in bracket indicate percentage.

4.3.2 Multidrug Resistant Distribution of *Campylobacter* species from the Various Poultry and Human Sources

In the poultry sub-sources, domestic/household source had a relative higher percentage of multidrug resistance than the commercial source. There was no significant difference ($p > 0.05$) in the multidrug resistance rate in both sub-sources (Table 4.21). The study showed one strain of commercial source that was resistant to 9 of the antibiotics used while 2 strains were resistant to the same maximum number of antibiotics in domestic/household source. However, most strains (11 strains each) were resistant to 5 and/or 6 antibiotics in commercial while a maximum of 7 strains were resistant to 4 of the antibiotics used (as presented in table B of appendix 1.5).

Also, in the human sub-sources, there was no significant difference ($p > 0.05$) in the multidrug resistance rate in both sub-sources. Multidrug resistance was on equal rate (100%) in both hospital and domestic/household sub-source (Table 4.21). One strain from the hospital sub-source resisted 11 of the antibiotics used while a maximum of 9 antibiotics were also resisted by one strain in domestic/household sub-source (as presented in table B of appendix 1.5).

Table 4. 21: Profile of Multidrug Resistance in Various Poultry and Human Sources

MAIN SOURCE	SUB-SOURCES	FREQUENCY (%)			P value
		No. of Isolates	No. of Multidrug	No. of Non-Multidrug	
Poultry	Commercial	50	47(94)	3(6)	
	Domestic/Household	25	24(96)	1 (4)	
	TOTAL	75	71(94.7)	4(5.3)	0.605
Humans	Hospital	20	20(100)	0	
	Domestic/Household	10	10(100)	0	
	TOTAL	30	30(100)	0	0.605

Values in bracket indicate percentage.

4.3.3 Multidrug Resistant Distribution of *Campylobacter jejuni* and Non-*jejuni* in Poultry and Human Sources

Out of the 101 isolates of multidrug resistance, 68 (67.3%) were *C. jejuni* and 33 (32.7%) Non-*jejuni*. No significant difference (p=0.215) was observed between *C. jejuni* and Non-*jejuni*.

It was observed that all the 33 (100%) Non-*jejuni* strains were multidrug resistant while 68 (94.4%) of the 72 strains of *C. jejuni* were also multidrug resistant. All human isolates in both *C. jejuni* and Non-*jejuni* were multidrug resistant. Also, 93.1% of the *C. jejuni* of poultry were multidrug resistant while 100% was observed in humans (Table 4.22). No significant difference (p > 0.05) was observed between the two sources in *C. jejuni* and Non-*jejuni* (Table 4.22).

Table 4. 22: Profile of Multidrug Resistance of *Campylobacter jejuni* and Non-*jejuni* in Poultry and Human Sources

SOURCES	*C. jejuni*		Non-*jejuni*		P value
	No. of Isolates	MDR (%)	No. of Isolates	MDR (%)	
Poultry	58	54 (93.1%)	17	17(100%)	0.412
Humans	14	14(100%)	16	16(100%)	
TOTAL	72	68(94.4%)	33	33(100%)	

Values in bracket indicate percentage.

Keys: MDR=Multidrug Resistance

4.3.4 Multidrug Resistant Distribution of *Campylobacter jejuni* and Non-*jejuni* in the Various Poultry and Human Sources

In the poultry, domestic/household source had the highest percentage (93.8%) rate of multidrug resistance compared to the commercial source in terms of *C. jejuni* isolates but 100% each was seen for both sources under the Non-*jejuni* multidrug resistance rate (Table 4.23).

All *C. jejuni* and Non-*jejuni* isolates of humans in respective sources (hospital and domestic) showed 100% rate of multidrug resistance each. There was no significant difference recorded between *C. jejuni* and Non-*jejuni* among the sources ($p > 0.05$) (Table 4.23).

Table 4. 23: Profile of Multidrug Resistance of *C. jejuni* and Non-*jejuni* in the Various Poultry and Human Sources

SOURCES	SUB-SOURCES	*C. jejuni*		Non-*jejuni*		P value
		No. of Isolates	MDR (%)	No. of Isolates	MDR (%)	
Poultry	Commercial	42	39 (92.9%)	8	8(100%)	0.792
	Domestic/ Hsd	16	15(93.8%)	9	9(100%)	
	TOTAL	58	54(93.1)	17	17(100%)	
Humans	Hospital	9	9 (100%)	11	11(100%)	
	Domestic/Hsd	5	5(100%)	5	5(100%)	
	TOTAL	14	14(100%)	16	16(100%)	

Values in bracket indicate percentage.

Keys: MDR=Multidrug Resistance; Hsd=Household

4.3.5 Multidrug Resistant Distribution Among the Biotypes of *Campylobacter jejuni* from Poultry and Human Sources

It has been initially revealed that 68 (94.4%) of the 72 *C. jejuni* strains were multidrug resistant (as presented in table C of appendix 1.5). With the 68 multidrug resistant isolates in general, *C. jejuni* biotype-I recorded the highest rate of 42.6% (29 strains) followed by 39.7% (27 strains) of biotype-II, 14.7% (10 strains) of biotype-III and least of 2.9% (2 strains) of biotype-IV (as presented in table C of appendix 1.5). Though there was a significant difference ($p < 0.05$) observed across or throughout, the percentage rate of multidrug resistance observed was the same 100% under each biotype in any of the two sources (poultry and humans) (Table 4.24).

Table 4. 24: Multidrug Resistance Profile of the Biotypes of *Campylobacter jejuni* in Poultry and Human Sources

SOURCES	Biotype-I		Biotype-II		Biotype-III		Biotype-IV	
	No. of Isolates	MDR (%)	No. of Isolates	MDR (%)	No. of Isolates	MDR (%)	No. of Isolates	MDR (%)
Poultry (58)	19	19(100)	30	30(100)	7	7(100)	2	2(100)
Human (14)	10	10(100)	1	1(100)	3	3(100)	0	0
TOTAL	29	29(100)	31	31(100)	10	10(100)	2	2(100)
P value								0.014

Values in bracket indicate percentage.

Keys: MDR=Multidrug Resistance

4.3.6 Multidrug Resistance in Biotypes of *Campylobacter jejuni* Among the Various Sources in Poultry and Humans

In general, there was a significant difference ($p < 0.05$) observed across or throughout all biotypes multidrug resistant rates in various sources of both poultry and humans. Though frequencies of strains were not the same under each biotype and each sub-sources, a percentage rate of 100% was recorded in all (Table 4.25).

Table 4. 25: Multidrug Resistance Profile of the Biotypes of *Campylobacter jejuni* in Various Sources of Poultry and Humans

SOURCES	Biotype-I		Biotype-II		Biotype-III		Biotype-IV	
	No. of Isolates	MDR (%)	No. of Isolates	MDR (%)	No. of Isolates	MDR (%)	No. of Isolates	MDR (%)
Poultry								
Commercial	10	10(100)	25	25(100)	6	6(100)	1	1(100)
Domestic/Hsd	9	9(100)	5	5(100)	1	1(100)	1	1(100)
TOTAL	19	19(100)	30	30(100)	7	7(100)	2	2(100)
Humans								
Hospital	6	6(100)	0	0	3	3(100)	0	0
Domestic/Hsd	4	4(100)	1	1(100)	0	0	0	0
TOTAL	10	10(100)	1	1(100)	3	3(100)	0	0
P value								0.018

Values in bracket indicate percentage.

Keys: MDR=Multidrug Resistance; Hsd=Household

CHAPTER FIVE

DISCUSSION

5.1 Prevalence of *Campylobacter* species

This study is the first to document *Campylobacter* sp. prevalence in both poultry and human sources in the Northern region of Ghana, and to further determine their antimicrobial susceptibility levels while biotyping for the *C. jejuni* sp. found. The study revealed 22.5% overall prevalence of *Campylobacter* sp. with significant difference (p=0.001) between the two sources. It also showed 68.6% and 31.4% prevalence level of *C. jejuni* and Non-*jejuni* respectively. In developing countries, Lior scheme of biotyping has been used to subtype *C. jejuni* and Non-*jejuni* strains especially *C. coli* (Taylor, 1992; Oberhelman and Taylor, 2000). Biotyping for the *C. jejuni* in this study demonstrated prevalence of 40.3% in biotype-I, 43.1% in biotype-II, 13.9% in biotype-III and 2.8% in biotype-IV with significant difference (p=0.018). Thus, from both human and non-human sources, the most prevalent biotype was the *C. jejuni* biotype-II. This study biotyping results do not conform to a similar study by Lior in which 57.3%, 36%, 4% and 2.7% were the revealed prevalence demonstrated in *C. jejuni* biotype I, II, III and IV respectively (Lior, 1984).

5.1.1 Prevalence of *Campylobacter* species in Poultry

In this study, 43.1% of poultry cloacal swab from both commercial and domestic sources were contaminated by *Campylobacter* sp. There was a higher prevalence of 50.5% in commercial source than in domestic (33.8%) with significant difference (p=0.001). These observed prevalence in this study was not disquieting since they fall within the global reported range of 10-90% among birds, especially in poultry which have Campylobacters frequently colonising their intestinal tracts (Jacobs-Reitsma *et*

al., 1994; Newell and Fearnley, 2003). The general findings from this study for poultry is higher than an earlier report by Karikari *et al.* (2017b) and Sackey *et al.* (2001) with rates of 22.5% and 14.1% respectively, but lower than that in Abraham *et al.* (1990) which reported a rate of 43.6% in Ghana.

Several higher prevalence of *Campylobacter* sp. in poultry from different countries and sources have been reported in both Africa and outside Africa with instances like 47% (from commercial and industrial broilers) and 94% (from industrial layers) in South Africa, 51.5% reported in Nigeria, 63.8% described in Cote d'Ivoire, 83.1% also in Ireland and 87.2% in Poland (EFSA, 2010; Bester and Essack, 2012; Salihu *et al.*, 2012; Bernadette *et al.*, 2012; Wieczorek *et al.*, 2012). A study conducted in a broiler farm had a lower colonisation rate of 16.83% in Assiut Governorate of Egypt by Abushahba (2018) from commercial source and in Giza Governorate in that same Egypt, Hassanain in 2011 recorded an overall prevalence of 10.96% (10% from broiler chicken and 11.42% from intestines) *Campylobacter* sp. A 2018 study found a varied prevalence of 33-44% in Kenya which is similar to the rate observed in this study (Carron *et al.*, 2018). Other studies in Africa recorded a higher rate of 47-68% (Asuming-Bediako *et al.*, 2019). These variances in rates could be due to differences in sampling size and number of poultry farms sampled (Ewnetu and Mihret, 2010; Komba, 2017). Further, a higher rate of 42.5% *Campylobacter* sp. in poultry has been observed in a Tanzanian study where cloacal swab sampling were done across various breeds of chicken (Chuma *et al.*, 2016). In Kenyan broilers, Turkson *et al.* reported a higher rate of 51.5% (Turkson *et al.*, 1988) while in another recent 2018 study in chickens from Nairobi, higher prevalence rate of 69.5% *Campylobacter* sp. was also recorded (Mageto *et al.*, 2018). A study in Ethiopia reported a rate of 28.9% (Nigatu *et al.*, 2015). Unlike this study where rate of prevalence in commercial sources were more than the domestic

with significant difference, similar studies have shown dominance of Campylobacters in indigenous chickens (76.49% and 75%) in comparison to broilers (26.4% and 50%) from two studies in Tanzania (Kazwala *et al.*, 1993; Chuma *et al.*, 2016). However, a recent 2018 study in Nairobi-Kenya shares same observations with this study with 91.07% in broilers, 70.96% in layers but found lower prevalence of *Campylobacter* in indigenous chickens from the peri-urban areas (Mageto *et al.*, 2018).

The high rates of *Campylobacter* colonization found in some countries according to Johnsen *et al.* (2006) could be due to numerous small-scale poultry farms with low levels of biosecurity ensured in such sub-regions. The frequent colonisation and higher prevalence records for *Campylobacter* sp. especially in the intestinal tracts of poultry needs not to be overlooked since it can easily cross-contaminate poultry carcasses especially during slaughtering and end in consumption. Karikari *et al.* (2017b) supports this idea by stating that poultry carcasses could be contaminated through inadequate rinsing and washing facilities during open air slaughtering, poor environmental hygiene and lack of well-structured facility for processing.

Also, the increased prevalence of *Campylobacter* in the commercial sources have been attributed to poultry farm workers who fail to change clothes and boots used for animal production when moving from one flock to another and end up carrying Campylobacters between flocks. Studies state that applying hygiene barriers reduces *Campylobacter* prevalence in poultry significantly; therefore the practices of changin new clothes and boots before entering another flock, frequent flock water and feed changing, and disinfecting farm floors are all measures that will help break the link for possible cross-contamination on poultry farms (Cardinale *et al.*, 2004; Saleha, 2004).

In poultry, the study found 77.3% *C. jejuni* (84% in commercial and 64% in domestic) and 22.7% Non-*jejuni* (16% in commercial and 36% in domestic). *Campylobacter*

jejuni was the dominant species and this finding is in agreement with similar work done in Ghana by Karikari and associates with other authors that reported domination of *C. jejuni* in poultry and poultry products (Jorgensen *et al.,* 2002; Son *et al.,*2007; Salihu *et al.,* 2012; Karikari *et al.,* 2017b). Thermophilic Campylobacters recovered were more than the non-thermophiles which could be attributed to the use of CampyGen gas generating system used in the isolation of *Campylobacter* sp. that creates a selective nature or environment optimizing the thermophilic Campylobacters growth and thereby inhibiting the growth of non-thermophilic Campylobacters that require an atmosphere enriched in hydrogen (Workman *et al.,* 2005).

The study further revealed different levels of biotypes in the *C. jejuni* sp. in poultry with 32.8% in biotype-I, 51.7% biotype-II, 12.1% biotype-III and 3.4% biotype-IV. Biotype-II was dominant and there was significant difference (p=0.014) among the various biotypes. With regards to the various sources for poultry, the commercial sample had 23.8% biotype-I, 59.5% biotype-II, 14.3% biotype-III and 2.4% biotype-IV while the domestic sample also had 56.25% biotype-I, 31.25% biotype-II, 6.25% each biotype-III and IV. Biotype-II and I were dominant in commercial and domestic sources respectively. There was no significant difference (p=0.170) between the sub-sources though prevalence rates varied between the two.

According to Salihu *et al.* (2009), biotyping results showing a prevalence of biotype-I in *C. jejuni* from poultry and also found in humans is an indication of the zoonotic nature of the pathogen. This study showed higher prevalence of *C. jejuni* biotype-II which along with the remaining, are not comparable to that of Scarcelli *et al.* (2001) which indicated prevalence rates of 76.5%, 17.6% and 5.8%, respectively to *C. jejuni* biotype I, II and III. Additionally, the study contradicts results from other studies which indicate 60.46% *C. jejuni* biotype-I and 39.53% *C. jejuni* biotype-II with no record for

C. jejuni biotype III or IV from poultry in India (Tayde and Brahmbhatt, 2014), 63.9% and 25% occurrence of *C. jejuni* biotype-I and II, respectively in Nigeria (Salihu *et al.*, 2009) and occurrence of 85.2% and 14.8% for *C. jejuni* biotype-I and II, respectively in Trininad (Shelly *et al.*, 2005). While this study isolated the four *C. jejuni* biotypes from poultry sources, a study by Aboaba and Smith had no *C. jejuni* biotype-III and IV recovered; though they reported significant lower prevalence rates of 20% and 6.67% for the *C. jejuni* biotype I and II (Aboaba and Smith, 2005). In another study which contradicts this study, respective isolation rate for *C. jejuni* biotype I and II were 20% and 0% (Baserisalehi *et al.*, 2007).

The preponderance of *C. jejuni* and its associated biotypes from both the commercial and domestic sources indicate that poultry can be a major reservoir in terms of human campylobacteriosis spread.

5.1.2 Prevalence of *Campylobacter* species in Humans

Globally, *Campylobacter* prevalence has been recorded in both rural and urban areas, though it is particularly among children. These prevalence rates vary between countries. The study sampled stools from patients undergoing routine examination in the hospitals and healthy individuals from households where rearing of domestic fowls was key. The presence of *Campylobacter* species was established among patients that presented themselves for different medical attention in the two public hospitals and in homes of individuals in the northern region of Ghana with an overall prevalence rate of 13%. The findings from the study showed 15.4% and 9% from both hospitals and domestic sources, respectively. There was an observed significant difference (p=0.001) between the two sources. Nonetheless, similar work done by Karikari *et al.* (2017a) in the same country recorded a prevalence rate of 17.3% which is a bit higher than in this study

from patients who visited Komfo Anokye Teaching hospital in Kumasi, but higher than a report by Abraham *et al.* who had 6.6% in urban and 12.8% in rural Ghana (Abraham *et al.*, 1990).

Several studies have demonstrated higher and lower prevalence rates in comparison to findings from this study in patients from hospitals both in Africa and other countries in different continents around the globe. Few reports from the East of Africa had higher prevalence rate than in this study. In Ethiopia, Lengerh *et al.* (2013) recorded a prevalence rate of 15.4% *Campylobacter* sp. in a hospital at Gondar similar to results from this study.

Additionally, research from other places that had higher prevalence of *Campylobacter* sp. from humans in hospital sources reported rates of 16.7% in Jimma-Ethiopia, 16.8% (from Abassia) and 27.6% (from Assiut) in Egypt, 20-21% in South Africa, 21% in Malawi, 43-70% in Nigeria and 87% in Spain (Mackenjee *et al.*, 1984; Saenz *et al.*, 2000; Wasfy *et al.*, 2000; Obi and Bessong, 2002; Samie *et al.*, 2007; Mason *et al.*, 2013; Tafa *et al.*, 2014; Nwankwo *et al.*, 2016a; Nwankwo *et al.*, 2016b; Abushahba, 2018).

Besides several studies reporting higher rates of *Campylobacter* prevalence, some other lower recordings than what this study recorded from hospital sources were also noted; 1.7% in Mozambique, 2% in Sudan, 2.3% in Burkina Faso, 5.8% in Kenya, 9.3% in Kampala-Uganda, 2.7% (from Zagazig) and 9.37% (from Nile Delta) all in Egypt, 9.7% each in Madagascar and Mwanza-Tanzania, 15% in Angola (Mshana *et al.*, 2009; Randremanana *et al.*, 2012; Sangaré *et al.*, 2012; Swierczewski *et al.*, 2013; Awadallah *et al.*, 2014; Deogratias *et al.*, 2014; Saeed *et al.*, 2015; Pelkonen *et al.*, 2018; Sainato *et al.*, 2018).

This study had *Campylobacter* sp. prevalence rate of 9% from the domestic source and is not comparable to other research reports which indicate 28-44.9% in Liberia by Molbak *et al.* (1988), 16.66% in Egypt, 11.4% and 9.3% in Tanzania (Mdegela *et al.*, 2006; Hassanain, 2011; Komba *et al.*, 2015).

In humans, the study found rates of 45% and 50% in *C. jejuni* and 55% and 50% in Non-*jejuni*, respectively for isolates from hospital and domestic sources with significant difference.

Several research findings showed higher *C. jejuni* in hospitals than in this study; 80.9% in the Ugandan study by Meryem *et al.* (2016), 93% in the Nigerian report by Ohanu and Offune (2009), 85% by Samie *et al.* (2007) in South Africa, 59% by Wasfy *et al.* (2000) in Egypt and 51.8% by Sangaré *et al.* (2012) in Burkina Faso. Other lower findings for *C. jejuni* prevalence in hospitals were 23.8% by Nwankwo *et al.* (2016a) in Nigeria and 40% by Lastovica (2006) in South Africa.

The study findings on Non-*jejuni* in hospital was higher than other research reports who found lower Non-*jejuni* (38.8% *C. coli* and 37.5% *C. lari*) by Nwankwo *et al.* (2016a) and Non-*jejuni* (24.6% *C. concisus* and 23.6% *C. upsaliensis*) by Lastovica (2006).

Also, from domestic sources, a higher prevalence of 90% *C. jejuni* has been noted by Mdegela *et al.* (2006) while a lower prevalence of 12.3% observed by El-Tras *et al.* (2015) with lower Non-*jejuni* (2.8% *C. coli*).

The disparity observed in the case of higher reports than this study was expected since in those works samples used were from risk groups while the samples used in this study were sourced from the general population. The study speculates that the observed different rates of prevalence in hospitals and homes or domestic sources could be as a result of contaminations from foods and water, and poor environmental conditions

because the media through which Campylobacters are frequently transmitted involve ingesting contaminated water or food and contact with faeces from humans or animals sources (Ugboma *et al.,* 2013). Additionally, poultry is a major source of *Campylobacter* transmission to humans and coops with wet litter and poor sanitation in households increase human risk of exposure (El-Tras *et al.,* 2015).

The dominance of biotype-I in humans in this study is similar to other African studies; thus, a Nigerian study had 52.5% and 28.7%, respectively for *C. jejuni* biotype I and II (Alabi *et al.,* 1986), Georges-Courbot and associates in the Central African Republic also revealed same trend (31.9% of biotype-I, 11% of biotype-II and 2.4% of biotype-III) from enteric *Campylobacter* isolated from children in Bangui (Georges-Courbot *et al.,* 1989). Finally, a South African study though had a higher prevalence in biotype-I than this study, yet the study showed similar trend of 95.4% and 1.5% for biotype I and II, respectively (Lastovica *et al.,* 1986). These biotypes found in humans especially the biotype-I confirms the zoonotic nature of the pathogen (Salihu *et al.,* 2009).

5.2 Antimicrobial Susceptibility Pattern of *Campylobacter* species

In many parts of the world according to the World Health Organisation, significant levels of resistance to erythromycin and fluoroquinolones have been identified in antimicrobial resistance surveillance and it appears to be attributed to the use of these drugs in livestock and poultry production (WHO, 2013). Additionally, there has been an established relationship between antimicrobial resistance and antibiotics used in humans and animals through epidemiological studies (Moore *et al.,* 2005; Jonker and Picard, 2010; de Vries *et al.,* 2018).

5.2.1 Antimicrobial Susceptibility Pattern of *Campylobacter* species in Poultry

Commercial Poultry Source:

With regards to the commercial poultry isolates, resistance that were commonly observed against erythromycin, ampicillin, ciprofloxacin, chloramphenicol, gentamicin, norfloxacin and sulphamethoxazole/trimethoprim in this study is in contrast to the higher resistance rates by Karikari *et al.* (2017b); though in the same country. Both studies were carried out at different locations within the country and different levels of farmer`s exposure to drugs used on poultry farms might have caused the observed difference in rates. This study speculates that chickens sampled might have had long term exposure to these antibiotics from farmers through abuse and misuse. Despite this, the *Campylobacter* isolates from Karikari`s studies were sensitive to imipenem while this study recorded a significant resistant rate of 8% to imipenem but on the same level when it comes to tetracycline (i.e. 97-100%) (Karikari *et al.*, 2017b).

Similar trend for the quinolones which is not comparable to this study has been recorded by Bester and Essack in Kwazulu Natal in South Africa where they found resistance to ciprofloxacin (91% and 76%) and to gentamicin (98% and 81%), respectively for commercial broilers and layers (Bester and Essack, 2012). Other non-comparable higher trends have been reported in works from different countries (Sukhapesna *et al.*, 2005; Tang *et al.*, 2009; Usha *et al.*, 2010; Mansouri-najand *et al.*, 2012; Kovalenko *et al.*, 2014). There are also studies that had low resistance rates for the quinolones which are not comparable to the study findings as in Fallon *et al.* (2003) and the rate of 19.6% to ciprofloxacin (Salihu *et al.*, 2012).

Resistance to tetracycline revealed in this study is however not in agreement to the rates of 24.1% resistance to tetracycline (Salihu *et al.*, 2012), 68% resistance to tetracycline

recorded in chicken from Durban (Reddy and Zishiri, 2017) and in addition from a study in Egypt by Hassanain (2011) who reported *Campylobacter* isolates from poultry showing 58.82% resistance each to tetracycline. Isolates` resistant record to tetracycline in this study affirms the rates reported by Bester and Essack (2012) in commercial poultry sources; where they recorded resistance rates of 100% from commercial free range broilers, 98.9% from industrial raised broilers and 100% from industrial layers to tetracycline, respectively.

The resistance to erythromycin among the isolates from commercial source in this study is lower than the one reported by Bester and colleague where 87.9% resistant rate was recorded from commercial free range broilers. However, the findings from this study for isolates` resistance to erythromycin is also higher than the same study with 47.6% from industrially raised broilers and 43.7% from industrial layers, respectively (Bester and Essack, 2012). Additionally, lower resistant rates of 58.82% and 11.6% to erythromycin were reported by Hassanain (2011) and Salihu *et al.* (2012), respectively.

While Karikari *et al.* (2017b) and Hassanain (2011) were reporting higher resistance rates of 72% and 64.7%, respectively to chloramphenicol than this study recorded, no resistance to chloramphenicol was seen by Salihu *et al.* (2012). However, Salihu *et al.*(2012) indicated that the zero resistance to chloramphenicol in the study was because the antibiotic has been banned for use in both humans and animals due to its effects like high residue in animal products and its documentation in cases of aplastic anaemia in Nigeria. In Ghana, chloramphenicol usage is allowed and hence the significant levels of resistance detected this study may be attributed to misuse and abuse.

Karikari and associates reported 100% resistant rate to ampicillin which was higher than the this study findings (Karikari *et al.*, 2017b), but the rate of 32.1% to ampicillin by Salihu *et al.* (2012) was lower than in this report.

Furthermore, in the commercial poultry source, the study showed varied resistance trend among the *C. jejuni* and Non-*jejuni* strains.

First, a study by Uaboi-Egbenni *et al.* (2012) reported lower resistance rate of 29% to ciprofloxacin except for nalidixic acid where resistance rate was higher than in this study for *C. jejuni*. The study further attributed the found resistance to the ciprofloxacin among chickens to the indirect use of sarafloxacin and enrofloxacin that form compositional part of some broad spectrum antimicrobials used on poultry farms.

This study findings is further in contrast to the lower rates of *C. jejuni* resistance to tetracycline reports of 15-36% in Australia, 43% in USA and 35.4% from four European countries (Barton and Wilkins, 2001; Bywater *et al.*, 2004; Gupta *et al.*, 2004); and the 56.7% record in Uaboi-Egbenni *et al.* (2012) in Limpopo.

Secondly, similar trends of lower resistance rate than what the study had for Non-*jejuni* to ciprofloxacin, tetracycline and erythromycin from commercial poultry sources have been demonstrated in some researches (Tambur *et al.*, 2009; Uaboi-Egbenni *et al.*, 2012).

Additionally, there is literature reporting varied resistance profiles of *C. jejuni* and Non-*jejuni* sp.; whiles some studies indicated higher resistance among Non-*jejuni* sp. like *C. coli* (Jonker and Picard, 2010), other ones also establishes their higher resistance rates among *C. jejuni* (Tambur *et al.*, 2009). There are also demonstration of no differences observed in resistance rates between the two species (*C. jejuni* and Non-*jejuni* sp.) in some studies (Uzunovic-Kamberovic *et al.*, 2009; Ewnetu and Mihret, 2010); though explanations to differences observed between the two species are lacking (Luangtongkum *et al.*, 2009).

This observed high resistance to especially the fluoroquinolones among others has emerged as a significant problem not only affecting the Northern region of Ghana but the entire nation at large. In Ghana, fluoroquinolones have been effective in treating several illnesses and known be common antibiotics (Ahiabu *et al.,* 2016). These resistance genes may end in humans through poultry products if proper handling and processing are overlooked by processors, posing different health threats. Most Ghanaian health institutions lack the ability in performing antimicrobial therapy and hence clinicians rely on empirical use of antimicrobials during prescriptions; this practical shortcomings in rapidly and accurately diagnosing of infectious diseases, its causative pathogen, susceptibility of the pathogen to a particular amicrobial therapy and, additional emerging resistance to some drugs remain key challenges to Ghana (Newman *et al.,* 2011; Michael *et al.*, 2014; Ahiabu *et al.*, 2016).

The multidrug resistant rate in commercial source that the study established ranged from 92.9-100% records. These established multidrug records are in agreement to Karikari *et al.* (2017b) in Ghana and Tang *et al.* (2009) in Malaysia who established 100% each, and also comparable to 97% recorded in Thailand by Sukhapesna *et al.* (2005). Low and lower multidrug resistance rates like 82.1%, 75% and 35% have also been established with the first two in Nigeria and last in Malaysia respectively (Akwuobu *et al.,* 2010; Mansouri-najand *et al.,* 2012; Salihu *et al.,* 2012). In South Africa, multidrug resistance was lower and found in 23% and 43% of the isolates from commercial broilers and layers respectively; and additionally, from 25-37.5% in Uaboi-Egbenni *et al.* (2012).

Domestic Poultry Source:

Meanwhile, in the domestic poultry sub-source resistances of 100%, 92%, 80%, 68%, 48%, 40%, 36%, 24%, 16% and 12% was reported for tetracycline, ceftriaxone, erythromycin, ampicillin, ciprofloxacin, chloramphenicol, sulphamethoxazole/trimethoprim, nalidixic acid, gentamicin and norfloxacin, respectively with no resistance recorded for amikacin and imipenem.

This discovery by the study showed higher resistance rates to tetracycline, ciprofloxacin and erythromycin than a similar study in the Kwazulu Natal of South Africa where isolated *Campylobacter* species from rural chickens by Bester and Essack (2012) revealed 21.6%, 7.9% and 0%, respectively to the aforementioned antibiotics.

This study showed higher resistance rates of *C. jejuni* to tetracycline and chloramphenicol than the one from Kenya that isolated *C. jejuni* from backyard chickens in Thika which had 71% and 25.8% respectively, but that same study inversely had higher resistance rates of 77.4% to nalidixic acid and 71% to ciprofloxacin than it showed in this study (Nguyen *et al.*, 2016). Sensitivity to amikacin and imipenem was recorded by both *C. jejuni* and Non-*jejuni* sp. strains from the domestic source. The relative high resistance records from the study could be speculated that it is due to uncontrolled use and ease of access to antibiotics by small-scale household-base poultry farmers had been the reason for the high records (Iovine, 2013).

The multidrug resistant rate from domestic source was 96%. In addition, 93.8% and 100% multidrug resistance rate in domestic source were reported for *C. jejuni* and Non-*jejuni* sp respectively and no significant difference was observed.

The varying results from both commercial and domestic sources observed for high or low resistant rates to the antibiotics in comparison to other studies might be due to the

factors like differences in policies regarding control use of antimicrobials, regional or geographical differences in terms of exposure and in addition, differences in farm managements (Uaboi-Egbenni *et al.,* 2012).

5.2.2 Antimicrobial Susceptibility Pattern of *Campylobacter* species in Humans

Typically, campylobacteriosis is a self-limiting disease which does not need any treatment with antibiotics but antibiotics are administered in some cases in humans. The recommended antibiotics for treatment of *Campylobacter* infections are usually ciprofloxacin and erythromycin which are fluoroquinolones and macrolides, respectively (Coker *et al.,* 2002; Ghunaim *et al.,* 2015). However, resistance to these antibiotics has emerged due to misuse and abuse.

Resistance of the *Campylobacter* species in humans from this study to erythromycin was very high (90-95%) but not as the reported range of 96-100% by Karikari *et al.* (2017a) who also carried out the study in the southern part of Ghana, besides Campylobacters resistance to macrolide in a long time period been reported to be low and stable (Sackey *et al.,* 2001; Lehtopolku, 2011). On the other hand, resistance rates of 79% from Nigeria, 51% from Singapore and 31% from Bulgaria have been reported (Gibreel and Taylor, 2006). Similarly in Egypt, report has indicated *Campylobacter* isolates from humans showed 50% resistance to erythromycin (Hassanain, 2011). Lower report at resistant rate of 10.3% to erythromycin in Burkina Faso (Sangaré *et al.,* 2012) and another study in Ethiopia reported erythromycin effectiveness for more than 80% in *Campylobacter* isolates from humans (Tafa *et al.,* 2014) have been recorded. While this study demonstrated significantly higher resistance along with other studies, *Campylobacter* isolates showed zero resistance to erythromycin from few studies in Nigeria (Adekunle *et al.,* 2009; Samuel *et al.,* 2006). *Campylobacter* resistance to

erythromycin is known to be a gradual process and hence strains demand prolonged exposure to this antibiotic before becoming resistant (Wieczorek and Osek, 2013b). According to some Ghanaian studies, antibiotics like erythromycin, ampicillin, tetracycline and others for a relatively long time period, have commonly been found in the Ghanaian market (Newman *et al.*, 2011; Karikari *et al.* (2017a). And thus, this study speculates that long term exposure through abuse and misuse by individuals in both homes and hospitals to some of these common antibiotics according to Ahiabu *et al.* (2016) might have caused the significant high resistance levels recorded.

The *Campylobacter* strains in this study showed total resistance (100%) to tetracycline across and this was different from Karikari *et al.* (2017a) in Ghana who had within a range of 92.3-100%, but 75%, 72% and 64% have been described in a report from Egypt in North Africa, Spain and Durban in South Africa, respectively (Prats *et al.*, 2000; Hassanain, 2011; Reddy and Zishiri, 2017). In addition, low and lower rates have also been documented in human isolates with 10.3% in Burkina Faso (Sangaré *et al.*, 2012), 12% in Ilorin-Nigeria (Samuel *et al.*, 2006), 22% in Mozambique (Mandomando *et al.*, 2007), and other 22% and 39.5% of tetracycline-resistant *Campylobacter* isolates all from Ethiopia by Ewnetu and Mihret (2010) and Tafa *et al.* (2014), respectively. However, a report from Adekunle *et al.* (2009) revealed 0% resistance to tetracycline. According to Iovine (2013), tetracycline class of antibiotics is heavily used globally in both veterinary and human medicines. In Ghana, tetracycline is heavily applied in both humans and animal husbandry which could lead to the widespread resistance observed in this study (Turkson, 2008; Newman *et al.*, 2011).

Furthermore, the study revealed varied ranges of resistance to the quinolones (thus, 50-70% for ciprofloxacin, 20-45% for nalidixic acid, 10-30% for norfloxacin). Karikari *et al.* (2017a) which is also a Ghanaian study had a resistance range of 23-78% to

quinolones which was higher than the found range of 10-70% in this study. However, Tafa *et al.* (2014) reported the quinolone effectiveness for more than 80% towards isolated *Campylobacter* strains. Additionally, lower resistance to some quinolones have been reported. Mandomando *et al.* (2007) had 11% each resistance to two antibiotics (ciprofloxacin and nalidixic acid), Sangaré *et al.* (2012) had 13.8% and 34.5% respective resistance to ciprofloxacin and nalidixic acid, Samuel *et al.* (2006) reported 24% resistance rate to nalidixic acid with 0% resistance recorded for ciprofloxacin, and another 0% resistance to ciprofloxacin also reported by Adekunle *et al.* (2009). In Kampala-Uganda, Mshana *et al.* (2009) reported 5% resistance to ciprofloxacin. Though Mandomando *et al.* (2007) indicated that the low rates of resistance to the quinolones were expected because its use is not recommended in young children as the study sampled children and however admits as all the other reports also stated unrestricted use of antibiotics for treatment contributed to the resistance of which this study shares that possibility.

Resistance to chloramphenicol in this research was comparable to 54-56% in Karikari`s study (Karikari *et al.*, 2017a) but higher than the ones in the study by Tafa *et al.* (2014) who reported 31.6% resistance to the phenicols and 11% resistance to chloramphenicol by Mandomando *et al.* (2007).

A study in Egypt had a total resistance of 100% to ß-lactams (Girgis *et al.*, 2014) and these higher comparable rates according to reports could be attributed to intrinsic resistance demonstrated by some *Campylobacter* strains like *C. coli* and *C. jejuni* (Fitzgerald *et al.*, 2008), and the study stands to state that some of its isolated *C. jejuni* and Non-*jejuni* could be ß-lactamase producing strains causing that high resistance to the ß-lactams.

This current study though showed 10% resistance to imipenem is in contrast to 0% reported by Karikari *et al.* (2017a). Studies confirm carbapenems being exception to the ß-lactam resistance generally and also known to be effective in treating campylobacteriosis; however, this needs to be reconsidered with time since there are upcoming report of resistance (Luangtongkum *et al.*, 2009; Wieczorek and Osek, 2013b). Also, Karikari *et al.* (2017a) indicated that imipenem has proven to very effective and this is also known by users in Ghana, hence the recorded resistance to imipenem in this study could be attributed to abuse and misuse since it has been known to be very effective by users and thus users no longer consider its high cost. In this study, the isolated *C. jejuni* and Non-*jejuni* sp. from overall humans and sub-sources had higher resistance rates to erythromycin (80-88.9 and 100) and tetracycline (100 and 100), respectively. The study still attributes these higher resistance rates to abuse and misuse along with heavy applications and abundance in the Ghanaian market.

Similarly from a previous Ghanaian study, a higher rate of 100% to erythromycin was reported for *C. jejuni* (Karikari *et al.*, 2017a) in contrast to reports by this study. As Ghanaian studies were reporting higher rates, other studies were also reporting low and lower rates such as 9% to erythromycin for *C. jejuni* in humans (Wasfy *et al.*, 2000); also in Durban, Shobo *et al.* (2016) reported 31.5% of *C. jejuni* isolates from humans were resistant to erythromycin.

Additionally, Karikari *et al.* (2017a) reported resistance among *C. jejuni* to quinolones to be below 50% but the finding from this study is dissimilar to their report since *C. jejuni* resistance to quinolones ranged from 21.4-77.8%. This recorded rates by this study is however comparable to the documented 72% and 80% in Spain, Thailand and Hong Kong, respectively; but in contrast to the findings of rates of 41-46% in Germany, and 19-47% in USA and Canada (Luber *et al.*, 2003; Luangtongkum *et al.*, 2009). Also

in Cairo, Wasfy *et al.* (2000) reported 40% resistance to nalidixic acid by *C. jejuni*; where the rate exhibited was quite similar to this findings from this study.

Also, resistance of *C. jejuni* isolates to tetracycline in humans from this study was higher than the rates of 93.3% found by Karikari *et al.* (2017a), 6% by Wasfy *et al.* (2000) and the 25.9% in the study report by Shobo *et al.* (2016).

In the Non-*jejuni* sp., isolates from humans in the research showed 100% resistance to both erythromycin and tetracycline and this finding is not comparable to lower rates that have been reported by some studies for *C. coli* (Wasfy *et al.*, 2000; Shobo *et al.*, 2016; Karikari *et al.*, 2017a).

A study by Adekunle *et al.* (2009) reported 66% resistance to nalidixic acid by *C. coli* and is quite higher than the findings from this study which had 0-54.5% resistance to nalidixic acid for Non-*jejuni* sp.

Multidrug resistance among the study isolates from humans was 100%, and this then agrees with the high report from Karikari *et al.* (2017a) who also had 97.1% in Ghana. The finding further agrees to the report from China (90%) but disagrees to those established in France and Korea with lower rates of 37% and 56%, respectively (Payot *et al.*, 2004; Chen *et al.*, 2010; Shin and Lee, 2010). However, 78.9% rate of multidrug resistant has been recorded in Jimma-Ethiopia (Tafa *et al.*, 2014).

The high resistance levels revealed by the study in human isolates from both sources may be attributed to abuse, misuse, self-medication, wrong and unapproved prescription by physicians or overuse by individuals without prescription which are key indicators that have contributed greatly to spread and growth of the antibiotic resistance. In Ghana, antibiotics are readily available to patients and individuals from hawkers, in market stalls and by roadsides, across the counters of pharmaceutical stores, and even

leftovers from folks. All these practices possibly lead to underutilisation or overutilization among humans that increases antibiotic resistant rates and also creation of selective pressure. The study shares all the aforementioned key factors of attributes with Belongia and Schwartz (1998) and Karikari *et al.* (2017a) who further added that fluoroquinolones and macrolides may receive failure in terms of use in the treatments of *Campylobacter* infections among patients with such presented cases in Ghanaian hospitals.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study provides information on the prevalence of *Campylobacter* in poultry from commercial and domestic sources and, in humans.

Overall, some poultry and humans from this study were found to be *Campylobacter* carriers, with the found rate being more in poultry than humans. Likewise, the prevalence of isolated *C. jejuni* were dominant in poultry sources than in humans. Inversely, Non-*jejuni* sp. identified in poultry were lower than in human sources. From the *C. jejuni* strains isolated, all the four known biotypes (I, II, III and IV) were found in poultry sources but the *C. jejuni* biotype-IV was absent in the isolates from human sources.

Campylobacter strains showed remarkable resistance to the commonly used antibiotics in poultry industry and human medicine. Additionally, *Campylobacter* species from this study showed highest rate of total resistance to tetracycline across all sources. In poultry, imipenem was the antibiotic with least isolates resistance while human strains showed same least resistance to amikacin and imipenem, respectively. The observed multidrug resistance rates ranged from 94.7-100% in both poultry and humans, respectively.

Though some of the antibiotics (imipenem, gentamicin and amikacin) proved to be effective with little to no resistance rates, most of these cheap and common antibiotics may not be reliable in the treatment of Campylobacteriosis. Therefore, laboratory confirmations to determine effective antibiotics is very essential.

6.2 Recommendations

- More extensive studies at multi-regional and local levels would help create a clearer picture of *Campylobacter* prevalence and their antimicrobial resistance pattern in Ghana.
- The antibiotic resistance profiles observed in the *C. jejuni* and Non-*jejuni* sp. isolates across sources to the fluoroquinolones, macrolides and tetracycline which are often used as first line treatment is of concern. Therefore, it is necessary to undertake continuous monitoring of antibiotic resistance to inform effective treatment regimens for *Campylobacter* infections.
- The study further recommends constant education on reduction or avoidance of animal contact by individuals and judicious use of antimicrobial agents which could safeguard antimicrobial efficacy and substantially reduce *Campylobacter* prevalence resulting from cross-contamination with contacts.

REFERENCES

Abbasi, E., Belkum, A. V. and Ghaznavi-Rad, E. (2019). Quinolone and macrolide-resistant *Campylobacter jejuni* in pediatric gastroenteritis patients from Central Iran. *Microbial and Drug Resistance, 25*(7), 1080-1086.

Aboaba, O. O. and Smith, S. I. (2005). Occurrence of *Campylobacter* species in poultry forms in Lagos area of Nigeria. *Jounal of Environmental Biology*, *26*(2 Suppl), 403–408.

Abraham, C. A., Agbodaze, D., Nakanot, A., Fari, A. and Longmatey, H. E. K. (1990). Prevalence and antibiogram of *Campylobacter jejuni* in domestic animals in rural Ghana. *Archives of Environmental Health*, (45), 59–62.

Abubakar, M. K., Muigai, A. W., Ndung'u, P. and Kariuki, S. (2019). Investigating carriage, contamination, antimicrobial resistance and assessment of colonization risk factors of *Campylobacter* sp. in broilers from selected farms in Thika, Kenya. *Microbiology Research Journal International, 27*(6), 1-16.

Abushahba, M. F. N. (2018). Prevalence of zoonotic species of *Campylobacter* in broiler chicken and humans in Assiut Governorate, Egypt. *Approaches in Poultry, Dairy and Veterinary Sciences*, (3), 1–9.

Acke, E., Carroll, C., O'Leary, A., McGill, K., Kelly, L., Lawlor, A., Madden, R. H., Moran, L., Scates, P., McNamara, E., Moore, J. E., Jones, B. R., Fanning, S. and Whyte, P. (2011). Genotypic characterisation and cluster analysis of *Campylobacter jejuni* isolates from domestic pets, human clinical cases and retail food. *Irish Veterinary Journal, 64*(1), 6.

Acuff, G. R. and Dickson, J. S. (2017). Major zoonotic pathogens related to cattle: *Escherichia coli*, *Salmonella* and *Campylobacter*: *Escherichia coli.* In Ensuring safety and quality in the production of beef. Burleigh Dodds Science Publishing; Vol 1 (pp. 29-33).

Addis, M. and Sisay, D. (2015). A review on major food borne bacterial illnesses. *Journal of Tropical Diseases, 3*(4), 176-183.

Adekunle, O. C., Coker, A. O. and Kolawole, D. O. (2009). Antibiotic susceptibility pattern of strains of *Campylobacter coli* isolated in Osogbo, Nigeria.

Biology and Medicine, (1), 20–23.

Adley, C. C. and Ryan, M. P. (2016). The nature and extent of foodborne disease. In Antimicrobial Food Packaging (pp. 1-10). Academic Press.

Agunos, A., Waddell, L., L´eger, D. and Taboada, E. (2014). A systematic review characterizing on-farm sources of *Campylobacter* sp. for broiler chickens. *PLoS ONE*. (9), e104905.

Ahiabu, M. A., Tersbøl, B. P., Biritwum, R., Bygbjerg, I. C. and Magnussen, P. (2016). A retrospective audit of antibiotic prescriptions in primary health-care facilities in Eastern Region, Ghana. *Health Policy and Planning*, *31*(2), 250–258.

Aidley, J., Wanford, J. J., Green, L. R., Sheppard, S. K. and Bayliss, C. D. (2018). PhasomeIt: an 'omics' approach to cataloguing the potential breadth of phase variation in the genus *Campylobacter*. *Microbial Genomics*, *4*(11), e000228.

Akwuobu, C. A., Oboegbulem, S. I. and Ofukwu, R. A. (2010). Characterization and antibiogram of local isolates of *Campylobacter* species from chicken in Nsukka area, Southeast Nigeria. *American-Eurasian Journal of Sustainable Agriculture*, *4*(2), 117–121.

Alabi, S. A., Coker, A. O., Dosunmu-Ogunbi, O. and Odugbemi, T. (1986). Biotype and serogroup distribution of *Campylobacter* isolates from children in Nigeria. *Journal of Clinical Microbiology*, (24), 856–858.

Alarjani, K. M. (2019). Investigation of the influence of phase variation on the biological phenotypes and immunity to the flagella in *Campylobacter jejuni* (Doctoral dissertation, Department of Genetics).

Alba, P., Leekitcharoenphon, P., Franco, A., Feltrin, F., Ianzano, A., Caprioli, A., Stravino, F., Hendriksen, R. S., Bortolaia, V. and Battisti, A. (2018). Molecular epidemiology of mcr-encoded *colistin* resistance in Enterobacteriaceae from food-producing animals in Italy revealed through the EU harmonized antimicrobial resistance monitoring. *Frontiers in Microbiology*, (9), 1217.

Alban, L., Nielsen, E. O. and Dahl, J. (2008). A human health risk assessment for macrolide-resistant *Campylobacter* associated with the use of macrolides in Danish pig production. *Preventive Veterinary Medicine, 83*(2), 115–129.

Al-Banna, N. A., Cyprian, F. and Albert, M. J. (2018). Cytokine responses in Campylobacteriosis: Linking pathogenesis to immunity. *Cytokine and Growth Factor Reviews*, (41), 75-87.

Al-neama, R. T. (2017). Exploring the ecologies of *Campylobacter* and eimeria infections in UK sheep (Doctoral dissertation, University of Salford).

Alonso, R., Girbau, C. and Astorga, A. F. (2011). *Campylobacter*. In Molecular Detection of Human Bacterial Pathogens ed. Liu, D. pp.1125-1139. Boca Raton, Florida: CRC Press.

Al-Qadiri, H., Sablani, S. S., Ovissipour, M., Al-Alami, N., Govindan, B. and Rasco, B. (2015). Effect of oxygen stress on growth and survival of *Clostridium perfringens*, *Campylobacter jejuni*, and *Listeria monocytogenes* under different storage conditions. *Journal of Food Protection, 78*(4), 691-697.

Alrubaye, B. A. (2018). Microbiota metabolic product deoxycholic acid prevents *Campylobacter jejuni* chicken colonization through modulating ceca anaerobes. (Masters Dissertation, Theses and Dissertations-3017; University of Arkansas, Fayetteville, Department Biological Sciences)

Alter, T. (2017). Prevention and mitigation strategies for *Campylobacter* with focus on poultry production. In *Campylobacter* (pp. 111-129). Academic Press.

An, J. U., Ho, H., Kim, J., Kim, W. H., Kim, J., Lee, S., Mun, S. H., Guk, J. H., Hong, S. and Cho, S. (2018). Dairy cattle, a potential reservoir of human *Campylobacter*iosis: epidemiological and molecular characterization of *Campylobacter jejuni* from cattle farms. *Frontiers in Microbiology*, (9), 3136.

Andreoletti, O., Budka, H., Buncic, S., Collins, J. D., Griffin, J., Hald, T., Havelaar, A., Hope, J., Klein, G., McLauchlin, J., Müller-Graf, C., Nguyen-The, C., Noerrung, B., Peixe, L, Maradona, M. P., Ricci, A., Sofos, J., Threlfall, J., Vågsholm, I. and Vanopdenbosch, E. (2011). Scientific opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. *EFSA Journal*, *9*(4), 2105 (pp. 141)

Andrew, P., Emily, V., Mai, P., Ian, Y. and Nicole, B. (2013). Using risk factor weighting to target and create effective public health policy for

*Campylobacter*iosis prevention in Ontario, Canada. *American Journal of Public Health Research*, *1*(1), 32–37.

Anon. (2012). European Food Safety Authority, European Centre for Disease Prevention and Control; the Euro- pean Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. *EFSA Journal*, (10), 2597 (pp 442).

Antimicrobial Resistance: Global Report on Surveillance. (2014). Retrieved from http://www.who.int/drugresistance/documents/surveillancereport/en/ (accessed on 2nd August 2018)

Askoura, M., Sarvan, S., Couture, J. F. and Stintzi, A. (2016). The *Campylobacter jejuni* ferric uptake regulator promotes acid survival and cross-protection against oxidative stress. *Infection and Immunity*, *84*(5), 1287-1300.

Asuming-Bediako, N., Kunadu, A.-H., Abraham, S. and Habib, I. (2019). *Campylobacter* at the human–food interface: The African perspective (Review). *Pathogens*, *8*(2), pii E87.

Avrain, L., Humbert, F., L'Hospitalier, R., Sanders, P., Vernozy-Rozand, C. and Kempf, I. (2003). Antimicrobial resistance in *Campylobacter* from broilers: association with production type and antimicrobial use. *Veterinary Microbiology*, *96*(3):267-276.

Audia, J. P., Webb, C. C. and Foster, J. W. (2001). Breaking through the acid barrier: an orchestrated response to proton stress by enteric bacteria. *International Journal of Medical Microbiology*, *291*(2), 97–106.

Awadallah, M. A. I., Ahmed, H. A., El-Gedawy, A. A. and Saad, A. M. (2014). Molecular identification of *C. jejuni* and C. *coli* in chicken and humans, at Zagazig, Egypt, with reference to the survival of *C. jejuni* in chicken meat at refrigeration and freezing temperatures. *International Food Research Journal*, *21*(5), 1801–1812.

Baik, H. S., Bearson, S., Dunbar, S. and Foster, J. W. (1996). The acid tolerance response of *Salmonella* Typhimurium provides protection against organic acids. *Microbiology*, *142*(Pt 11), 3195–3200.

Baker, M. G., Sneyd, E. and Wilson, N. A. (2007). Is the major increase in notified

*Campylobacter*iosis in New Zealand real? *Epidemiology and Infection, 135*(1), 163–170.

Barrett, J. and Fhogartaigh, C. N. (2017). Bacterial gastroenteritis. *Medicine, 45*(11), 683-689.

Barton, M. D. (2000). Antibiotic use in animal feed and its impact on human healt. *Nutrition Research Reviews, 13*(2), 279–299.

Barton, M. D. and Wilkins, J. (2001). *Antibiotic resistance in bacteria isolated from poultry. Rural Industries Research and Development Corporation Publication No 1/105 Report of RIRDC Project No USA9A.*

Basardien, L. (2012). Molecular characterization of *Campylobacter* isolates from free range and commercial chicken in South Africa. (Masters Dissertation, Theses and Dissertaions; University of the Western Cape, Department of Biotechnology).

Baserisalehi, M., Bahador, N. and Kapadnis, B. P. (2007). Isolation and characterization of sp. from domestic animals and poultry in south Iran. *Pakistan Journal of Biological Sciences, 10*(9), 1519–1524.

Belongia, E. and Schwartz, B. (1998). Strategies for promoting judicious use of antibiotics by doctors and patients. *British Medical Journal, 317*(7159), 668–671.

Benoit, S. L. and Maier, R. J. (2018). Site-directed mutagenesis of *Campylobacter* concisus respiratory genes provides insight into the pathogen's growth requirements. *Scientific Reports, 8*(1), 14203.

Bernadette, G., Essoh, A. E., Solange, .K.E., Natalie, G., Souleymane, B., Sebastien, N. L. and Mireille, D. (2012). Prevalence and antimicrobial resistance of thermophilic *Campylobacter* isolated from chicken in Cote d'Ivoire. *International Journal of Microbiology*, (150612), 5p.

Berthenet, E., Thépault, A., Chemaly, M., Rivoal, K., Ducournau, A., Buissonnière, A., Bénéjat, L., Bessède, E., Mégraud, F., Sheppard, S. K. and Lehours, P. (2019). Source attribution of *Campylobacter jejuni* shows variable importance of chicken and ruminants reservoirs in non-invasive and invasive French clinical isolates. *Scientific Reports, 9*(1), 8098.

Bester, L. A. and Essack, S. Y. (2012). Observational study of the prevalence and

antibiotic resistance of *Campylobacter* sp. from different poultry production systems in KwaZulu-Natal, South Africa. *Journal of Food Protection*, (75), 154–159.

Bhunia, A. K. (2018). *Campylobacter* and *Arcobacter*. In Foodborne Microbial Pathogens (pp. 289-299). Springer, New York, NY.

Biswas, D., Comes, C., Sardine, J., Lebovic, A. and Biswas, D. (2018). Safer post-harvest processing, proper storage and transport of broiler products to the retail markets. *Journal of Food Processing and Beverages, 6*(2), 4.

Black, R. E., Levine, M. M., Clements, M. L., Hughes, T. P. and Blaser, M. J. (1988). Experimental *Campylobacter jejuni* infection in humans. *Journal of Infectious Diseases, 157*(3), 472–479.

Blaser, M. and Engberg, J. (2008). Clinical aspects of *Campylobacter jejuni* and *Campylobacter coli* infections. In: I. Nachamkin, C.M. Szymanski, M.J. Blaser (Eds.), *Campylobacter*, 3rd ed. DC, USA, (pp. 99-122), ASM Press.

Borrusso, P. A. and Quinlan, J. J. (2017). Prevalence of pathogens and indicator organisms in home kitchens and correlation with unsafe food handling practices and conditions. *J. of Food Prot.,(* Journal of Food protection*) 80*(4), 590-597.

Boysen L., Rosenquist H., Larsson J. T., Nielsen E. M., Sørensen G., Nordentoft S. and Hald, T. (2014). Source attribution of human *Campylobacter*iosis in Denmark. *Epidemiology and Infection, 142*(8), 1599–1608.

Brahmer, J. R., Lacchetti, C., Schneider, B. J., Atkins, M. B., Brassil, K. J., Caterino, J. M., Chau, I., Ernstoff, M. S., Gardner, J. M., Ginex, P., Hallmeyer, S., Holter Chakrabarty, J., Leighl, N. B., Mammen, J. S., McDermott, D. F., Naing, A., Nastoupil, L. J., Phillips, T., Porter, L. D., Puzanov, I., Reichner, C. A., Santomasso, B. D., Seigel, C., Spira, A., Suarez-Almazor, M. E., Wang, Y., Weber, J. S., Wolchok, J. D. and Thompson, J, A. (2018). Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American Society of Clinical Oncology Clinical Practice Guideline. *Journal of Clinical Oncology, 36*(17), 1714-1768.

Bras, A. M., Chatterjee, S., Wren, B. W., Newell, D. G. and Ketley, J. M. (1999). "A novel *Campylobacter jejuni* two component regulatory system important for temperature dependent growth and colonization." *Journal of Bacteriology*, *181*(10), 3298–3302.

Bronowski, C., Mustafa, K., Goodhead, I., James, C. E., Nelson, C., Lucaci, A., Wigley, P., Humphrey, T. J., Williams, N. J. and Winstanley, C. (2017). *Campylobacter jejuni* transcriptome changes during loss of culturability in water. *PLoS ONE, 12*(11), e0188936.

Buchanan, C. J. (2018). Development of a molecular-based risk assessment assay for human pathogenic *Campylobacter jejuni* (Doctoral dissertation, Lethbridge, Alta.: Universtiy of Lethbridge, Department of Biological Sciences).

Butzler, J. P. (2004). *Campylobacter*, from obscurity to celebrity. *Clinical Microbiology and Infection*, *10*(10), 868–876.

Butzler, J. P. (2018). *Campylobacter* Infection in Man and Animals: 1st edition. CRC Press, (pp. 256)

Butzler, J. P., Dekeyser, P., Detrain, M. and Dehaen, F. (1973). "Related Vibrio in stools". *Journal of Pediatrics*, *82*(3), 493–495.

Bywater, R., Deluyker, H., Deroover, E., Deroover, E., de Jong, A., Marion, H., McConville, M., Rowan, T., Shryock, T, Shuster, D., Thomas, V., Vallé, M. and Walters, J. (2004). European survey of antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals. *Journal of Antimicrobial Chemotherapy*, *54*(4), 744–754.

Cardinale, E., Tall, F., Guèye, E. F., Cisse, M. and Salvat, G. (2004). Risk factors for *Campylobacter* sp. infection in Senegalese broiler-chicken flocks. *Preventive Veterinary Medicine*, *64*(1), 15–25.

Carron, M., Chang, Y.-M., Momanyi, K., Akoko, J., Kiiru, J., Bettridge, J., Chaloner, G., Rushton, J., O'Brien, S., Williams, N., Fèvre, E. M. and Hasler, B. (2018). *Campylobacter*, a zoonotic pathogen of global importance: Prevalence and risk factors in the fastevolving chicken meat system of Nairobi, Kenya. *PLoS Neglected Tropical Diseases*, *12*(8), e0006658.

Centre for Disease Control and Prevention (CDC). (2011). *CDC estimates of foodborne illness in the United States*. Retrieved from http://www.cdc.gov/foodborneburden/2011-%0Afoodborne-estimates.html.

Chaban, B., Ngeleka, M. and Hill, J. (2010). Detection and quantification of 14 *Campylobacter* species in pet dogs reveals an increase in species richness in faeces of diarrheic animals. *BMC Microbiology,* (10), 73–73.

Charan, J., and Biswas, T. (2013). How to calculate sample size for different study designs in medical research? *Indian Journal of Psychological Medicine*, *35*(2), 121–126.

Chen, X., Naren, G. W., Wu, C. M., Wang, Y., Dai, L., Xia, L. N., Luo, P. J., Zhang, Q. and Shen, J. Z. (2010). Prevalence and antimicrobial resistance of *Campylobacter* isolates in broilers from China. *Veterinary Microbiology*, *144*(1-2), 133–139.

Chlebicz, A. and Śliżewska, K. (2018). Campylobacteriosis, salmonellosis, yersiniosis, and listeriosis as zoonotic foodborne diseases: a review. *International Journal of Environmental Research and Public Health*, *15*(5), pii: 863.

Chuma, I. S., Nonga, H. E., Mdegela, R. H. and Kazwala, R. R. (2016). Epidemiology and RAPD-PCR typing of thermophilic *Campylobacter*s from children under five years and chickens in Morogoro Municipality, Tanzania. *BMC Infectious Diseases*, (16), 692.

Cody, A. J., Bray, J. E., Jolley, K. A., McCarthy, N. D. and Maiden, M. C. (2017). Core genome multilocus sequence typing scheme for stable, comparative analyses of *Campylobacter jejuni* and *C. coli* human disease isolates. *Journal of Clinical Microbiology*, *55*(7), 2086-2097.

Coker, A. O., Isokpehi, R. D., Thomas, B. N., Amisu, K. O. and Obi, C. L. (2002). Human *Campylobacter*iosis in developing countries. *Emerging Infectious Diseases*, *8*(3), 237-44.

Coker, R., Hunter, B., Rudge, J., Liverani, M. and Hanvoravongchai, P. (2011). Emerging infectious diseases in Southeast Asia: Regional challenges to control. *Lancet*, *377*(9765), 599–609.

Connerton, I. F. and Connerton, P. L. (2017). *Campylobacter* foodborne disease. In Foodborne Diseases (pp. 209-221). Academic Press.

Connerton, P. L., Richards, P. J., Lafontaine, G. M., O'Kane, P. M., Ghaffar, N., Cummings, N. J., Smith, D. L., Fish, N. M. and Connerton, I. F. (2018). The effect of the timing of exposure to *Campylobacter jejuni* on the gut microbiome and inflammatory responses of broiler chickens. *Microbiome, 6*(1), 88.

Coorey, R., Ng, D. S. H., Jayamanne, V. S., Buys, E. M., Munyard, S., Mousley, C. J., Njage, P. M. K. and Dykes, G. A. (2018). The impact of cooling rate on the safety of food products as affected by food containers. *Comprehensive Reviews in Food Science and Food Safety*, *17*(4), 827-840.

Corry, J. E. L., Post, D. E., Colin, P. and Laisney, M. J. (1995). Culture media for the isolation of Campylobacters. *International Journal of Food Microbiology*, *26*(1), 43–76.

Cotter, P. D., Gahan, C. G. and Hill, C. (2000). Analysis of the role of the *Listeria monocytogenes* F0F1 -AtPase operon in the acid tolerance response. *International Journal of Food Microbiology, 60*(2–3):137–146.

Crotta, M., Georgiev, M. and Guitian, J. (2017). Quantitative risk assessment of *Campylobacter* in broiler chickens–Assessing interventions to reduce the level of contamination at the end of the rearing period. *Food Control,* (75), 29-39.

Da Costa, P., Loureiro, L. and Matos, A. J. (2013). Transfer of multidrug-resistant bacteria between intermingled ecological niches: The interface between humans, animals and the environment. *International Journal of Environmental Research and Public Health, 10*(1), 278–294.

Dadi, L. and Asrat, D. (2008). Prevalence and antimicrobial susceptibility profiles of thermotolerant *Campylobacter* strains in retail raw meat products in Ethiopia. *Ethiopian Journal of Health Development*, *22*(2), 195–200.

Dai, L., Sahin, O. and Zhang, Q. (2019). Identification of a nth-like gene encoding an endonuclease III in *Campylobacter jejuni*. *Frontiers in Microbiology*, (10), 698.

De Boer, R. F., Ott, A., Gu¨ ren, P., van Zanten, E., van Belkum, A. and Kooistra-Smid, A. M. (2013). Detection of *Campylobacter* species and *Arcobacter butzleri*

in stool samples by use of real-time multiplex PCR. *Journal of Clinical Microbiology*, *51*(1), 253–259.

De Melo, R. T., Monteiro, G. P., Mendonça, E. P. and Rossi, D. A. (2016). *Campylobacter* sp.: Capacity of biofilm formation and other strategies of survival and adaption to remain in the poultry industry. In *Campylobacter* sp. and related organisms in poultry (pp. 151-164). Springer, Cham.

De Palma, G., Blennerhassett, P., Lu, J., Deng, Y., Park, A. J., Green, W., Denou, E., Silva, M. A., Santacruz, A., Sanz, Y., Surette, M. G., Verdu, E. F., Collins, S. M. and Bercik, P. (2015). Microbiota and host determinants of behavioural phenotype in maternally separated mice. *Nature Communications*, (6), 7735.

De Vries, S. P. W., Vurayai, M., Holmes, M., Gupta, S., Bateman, M., Goldfarb, D., Maskell, D. J., Matsheka, M. I. and Grant, A. J. (2018). Phylogenetic analyses and antimicrobial resistance profiles of *Campylobacter* sp. from diarrhoeal patients and chickens in Botswana. *PLoS ONE*, *13*(3), e0194481.

Debruyne, L., Gevers, D. and Vandamme, P. (2008). Taxonomy of the family *Campylobacter*aceae. In I. Nachamkin, C. . Szymanski, & M. . Blaser (Eds.), *Campylobacter* (3rd editio, pp. 3–26). Washington DC, USA: ASM Press.

Dennehy, P. H. (2019). Infectious gastroenteritis. In introduction to clinical infectious diseases. (pp. 157-168). Springer, Cham.

Deogratias, A.-P., Mushi, M. F., Paterno, L., Tappe, D., Seni, J., Kabymera, R., Kidenya, B. R. and Mshana, S. E. (2014). Prevalence and determinants of *Campylobacter* infection among under five children with acute watery diarrhea in Mwanza, North Tanzania. *Archives of Public Health*, *72*(1), 17.

Di Giannatale, E., Di Serafino, G., Zilli, K., Alessiani, A., Sacchini, L., Garofolo, G., Aprea, G. and Marotta, F. (2014). Characterization of antimicrobial resistance patterns and detection of virulence genes in *Campylobacter* isolates in Italy. *Sensors*, *14*(2), 3308–3322.

Doyle, M. P. and Roman, O. J. (1982). "Recovery of *Campylobacter jejuni* and *Campylobacter coli* from inoculated foods by selective enrichment." *Applied and Environmental Microbiology*, *43*(6), 1343–1353.

Dunlop, M. W., McAuley, J., Blackall, P. J. and Stuetz, R. M. (2016). Water activity of poultry litter: Relationship to moisture content during a grow-out. *Journal of Environmental Management*, (172), 201-206.

Eberhart-Phillips, J., Walker, N., Garrett, N., Bell, D., Sinclair, D., Rainger, W. and Bates, M. (1997). *Campylobacter*iosis in New Zealand: results of a case-control study. *Journal of Epidemiology and Community Health*, *51*(6), 686-691.

Economou, V. and Gousia, P. (2015). Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infection and Drug Resistance*, (8), 49.

Egger, R., Korczak, B. M., Niederer, L., Overesch, G. and Kuhnert, P. (2012). Genotypes and antibiotic resistance of *Campylobacter coli* in fattening pigs. *Veterinary Microbiology*, *155*(2-4), 272-278.

Elmberg, J., Berg, C., Lerner, H., Waldenström, J. and Hessel, R. (2017). Potential disease transmission from wild geese and swans to livestock, poultry and humans: a review of the scientific literature from a One Health perspective. *Infection Ecology and Epidemiology*, *7*(1), 1300450.

El-Radhi, A. S. (2018). Fever in common infectious diseases. In clinical manual of fever in children (pp. 85-140). Springer, Cham.

El-Tras, W. F., Holt, H. R., Tayel, A. A. and El-Kady, N. N. (2015). *Campylobacter* infections in children exposed to infected backyard poultry in Egypt. *Epidemiology and Infection*, *143*(2), 308–315.

El-Zamkan, M. A. and Hameed, K. G. A. (2016). Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in raw milk and some dairy products. *Veterinary World*, *9*(10), 1147.

Engberg, J., Aarestrup, F. M., Taylor, D. E., Gerner-Smidt, P. and Nacbamkin, I. (2001). "Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: Resistance mechanisms and trends in human isolates." *Emerging Infectious Diseases*, *7*(1), 24–34.

Escherich, T. (1886). "Beitrage sur kenntniss der darmbacterien. III. Uber das vorkommen van vibrionen im darmcanal und den stuhlglingen der slauglinge" *Unch.Med. VVochenschr.*, (33), 815-817-83.

European Committee on Antimicrobial Susceptibility Testing (EUCAST). (2019). Available at: http://www.eucast.org/ (17.04.2019).

European Food Safety Authority (EFSA). (2010). Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008. *EFSA Journal*, *8*(3), 1503.

European Food Safety Authority (EFSA). (2011). Scientif ic opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. *EFSA Journal*, *9*(4), 2105.

European Food Safety Authority (EFSA). (2014). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012. *EFSA Journal*, *12*(2), 3547.

European Food Safety Authority (EFSA). (2015). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. *EFSA Journal*, *13*(1), 3991.

Ewnetu, D. and Mihret, A. (2010). Prevalence and antimicrobial resistance of *Campylobacter* isolates from humans and chickens in Bahir Dar, Ethiopia. *Foodborne Pathogens and Disease*, (7), 667–670.

External Quality Assurance System (EQAS). (2013). Protocol. National Food Institute, Technical University of Denmark (DTU Food), in collaboration with partners and Regional Sites in WHO GFN. Retrieved from; http://www.antimicrobialresistance.dk/data/images/ eqas/who_2013protocol-text.pdf. Accessed on: 10-09-2019.

Fallon, R., O'Sullivan, N. O., Maher, M. and Carroll, C. (2003). Antimicrobial resistance of *Campylobacter jejuni* and *C. coli* isolates from broiler chickens isolated at an Irish poultry-processing plant. *Letters in Applied Microbiology*, *36*(5), 277–281.

FAO/WHO/OIE. (2007). *Food and Agriculture Organization of the United Nations. Joint FAO/WHO/OIE Expert Meeting on Critically Important Antimicrobials*. Rome, Italy.

Ferri, M., Ranucci, E., Romagnoli, P. and Giaccone, V. (2017). Antimicrobial resistance: a global emerging threat to public health systems. *Critical Reviews in Food Science and Nutrition*, *57*(13), 2857-2876.

Fica, C. A., Porte, T. L., Braun, J. S., Veas, P. N., Pavez, A. C., Dabanch, P. J. and Morales, I. R. (2011). Bacteremia and endarteritis cases secondary to *Campylobacter* sp. in a metropolitan hospital: our experience along a quarter of a century. *Revista Chilena de Infectologia*, (28), 211–216.

Fitzgerald, F., Whichard, J. and Nachamkin, I. (2008). Diagnosis and antimicrobial susceptibility of *Campylobacter* species. In I. Nachamkin, C. M. Szymanski, & M. J. Blaser (Eds.), *Campylobacter, American Society for Microbiology* (eds, pp. 227–243). Washington DC.

Fliegelman, R., Petral, R., Goodman, L., Segreti, J., Trenholme, G. and Kaplan, R. . (1985). Comparative in vitro activities of twelve antimicrobial agents against *Campylobacter* species. *Antimicrobial Agents and Chemotherapy*, (27), 429–430.

Florent, A. (1953). Isolement d'un vibrion saprophyte du sperme du Taureau et du vagin de la vache (*Vibrio bubulus*). *Comptes Rendus des Seances de la Societe de Biologie et des ses Filiales*, (147), 2066–2069.

Food Standards Agency (FSA). (2014). A microbiological survey of *Campylobacter* contamination in fresh whole UK-produced chilled chickens at retail sale-interim report to cover quarters 1–3. Food Standards Agency, London, United Kingdom.

Fraser, A. D. E., Chandan, V., Yamazaki, H., Brooks, B. W. and Garcia, M. M. (1992). "Simple and economical culture of *Campylobacter jejuni* and *Campylobacter coli* in $C0_2$ in moist air." *International Journal of Food. Microbiology*, *15*(3–4), 377–382.

Friedman, C. R., Hoekstra, R. M., Samuel, M., Marcus, R., Bender, J., Shiferaw, B., Reddy, S., Ahuja, S. D., Helfrick, D. L., Hardnett, F., Carter, M., Anderson, B. and Tauxe, R. V. (2004). Risk factors for sporadic *Campylobacter* infection in the United States: A case-control study in FoodNet sites. Emerging Infections Program FoodNet Working Group. *Clinical Infectious Diseases*, (38), S285-S296.

Frirdich, E., Biboy, J., Pryjma, M., Lee, J., Huynh, S., Parker, C. T., Girardin, S. E., Vollmer, W. and Gaynor, E. C. (2019). The *Campylobacter jejuni* helical to coccoid transition involves changes to peptidoglycan and the ability to elicit an immune response. *Molecular Microbiology*, *112*(1):280-301.

Galley, J. D., Nelson, M. C., Yu, Z., Dowd, S. E., Walter, J., Kumar, P. S., Lyte, M. and Bailey, M. T. (2014). Exposure to a social stressor disrupts the community struc- ture of the colonic mucosa-associated microbiota. *BMC Microbiology*, (14), 189.

García -Gil, L. J. (2013). Detection of pathogenic thermotolerant *Campylobacter* species by Real-time PCR. In D. Rodrí-Lázaro (Ed.), *Real-time PCR in Food Science* (pp. 117–124). Norfolk, UK: Caister Academic Press.

García-Sánchez, L., Melero, B. and Rovira, J. (2018). *Campylobacter* in the Food Chain. In Advances in food and nutrition research (Vol. 86, pp. 215-252). Academic Press.

Ge, B., David, G., Patrick, F., Webb, G., Zhao, S., Susannan, H. and Ianghong, M. (2003). Antimicrobial-resistant *Campylobacter* species from retail raw meats. *Applied and Environmental Microbiology*, (69), 3005–3007.

Ge, B., Wang, F., Maria, S. and Patrick, F. M. (2013). Antimicrobial resistance in *Campylobacter*: Susceptibility testing methods and resistance trends. *Journal of Microbiological Methods*, (95), 57–67.

Ge, M. C., Kuo, S. F., Chang, S. C., Chien, C. C., You, H. L. and Lu, J. J. (2019). Antimicrobial susceptibility and virulence surveillance of *Campylobacter* sp. isolated from patients in two tertiary medical centers in Taiwan. *Frontiers in Microbiology*, (9), 3186.

Georges-Courbot, M. C., Gouandjika, I., Martin, P. M. and Georges, A. J. (1989). Biotype and Lior serogroup distribution of enteric *Campylobacter* isolated from children in Bangui (Central African Republic), and comparison with Penner serotypes. *Research in Microbiology,* (140), 489–497.

Ghana Statistical Service (GSS) (2012). 2010 Population and Housing Census: Summary Report of Final Results. Retrieved from; www.statsghana.gov.gh Date: 20-10-2018.

Ghimire, L., Dinesh, K., Hom Bahadur, B., Bhattarai, R., Dhakal, S. and Sharma, B. (2014). Prevalence, antibiogram and risk factors of thermophilic *Campylobacter* sp. in dressed porcine carcass of Chitwan, Nepal. *BMC Microbiology*, (14), 85–88.

Ghunaim, H., Behnke, J. M., Aigha, I., Sharma, A. and Doiphode, S. H. (2015). Analysis of resistance to antimicrobials and presence of virulence/stress response genes in *Campylobacter* isolates from patients with severe diarrhoea. *PLoS ONE*, (10), e0119268.

Gibreel, A. and Taylor, D. E. (2006). Macrolide Resistance in *Campylobacter jejuni* and *Campylobacter coli*. *Journal of Antimicrobial Chemotherapy*, (58), 243–255.

Girgis, S. A., Rashad, S. S., Othman, H. B., Bassim, H. H., Kassem, N. N. and El-Sayed, F. M. (2014). Multiplex PCR for identification and differentiation of *Campylobacter* species and their antimicrobial susceptibility pattern in Egyptian patients. *International Journal of Current Microbiology and Applied Sciences*, (3), 861–875.

Godschalk, P. C., Kuijf, M. L., Li, J., St Michael, F., Ang, C. W., Jacobs, B. C., Karwaski, M. F., Brochu, D., Moterassed, A., Endtz, H. P., van Belkum, A. and Gilbert, M. (2007). Structural characterization of *Campylobacter jejuni* lipooligosaccharide outer cores associated with Guillain-Barre and Miller Fisher syndromes. *Infection and Immunity*, *75*(3), 1245–1254.

Goni, M. D., Muhammad, I. J., Goje, M., Abatcha, M. G., Bitrus, A. A. and Abbas, M. A. (2017). *Campylobacter* in dogs and cats; its detection and public health significance: A review. *Advances in Animal and Veterinary Sciences*, *5*(6), 239-248.

Gonzalez, R. (2017). Risk-based evaluation of the public health impact of food safety interventions for the control of *Salmonella* sp. in the chicken meat production chain. (Doctoral dissertation: University of Minnesota).

Green, C., Baker, T. and Subramaniam, A. (2018). Predictors of respiratory failure in patients with Guillain–Barré syndrome: a systematic review and meta-analysis. *Medical Journal of Australia*, *208*(4), 181-188.

Guccione, E. J., Kendall, J. J., Hitchcock, A., Garg, N., White, M. A., Mulholland, F., Poole, R. K. and Kelly, D. J. (2017). Transcriptome and proteome dynamics in chemostat culture reveal how *Campylobacter jejuni* modulates metabolism, stress responses and virulence factors upon changes in oxygen availability. *Environmental Microbiology*, *19*(10), 4326-4348.

Gunther, N. W. and Chen, C. Y. (2009). "The biofilm forming potential of bacterial species in the genus *Campylobacter*." *Food Microbiology*, *26*(1), 44–51.

Gupta, A., Nelson, J. M., Barrett, T. J., Tauxe, R. V., Rossiter, S. P., Friedman, C. R., Joyce, K. W., Smith, K. E., Jones, T. F., Hawkins, M. A., Shiferaw, B., Beebe, J. L., Vugia, D. J., Rabatsky-Ehr, T., Benson, J. A., Root, T. P., Angulo, F. J. and NARMS Working Group. (2004). Antimicrobial resistance among *Campylobacter* Strains, United States, 1997–2001. *Emerging Infectious Diseases*, *10*(6), 1102–1109.

Gyles, C. (2008). Antimicrobial resistance in selected bacteria from poultry. *Animal Health Research Reviews*, *9*(2), 149–158.

Hafez, A. A., Younis, G., El-Shorbagy, M. M. and Awad, A. (2018). Prevalence, cytotoxicity and antibiotic susceptibility of *Campylobacter* species recovered from retail chicken meat in Mansoura, Egypt. *African Journal of Microbiology Research*, *12*(22), 501-507.

Hald, B., Skov, M. N., Nielsen, E. M., Rahbek, C., Madsen, J. J., Wainø, M., Nordentoft, S., Baggesen, D. L. and Madsen, M. (2016). *Campylobacter jejuni* and *Campylobacter coli* in wild birds on Danish livestock farms. *Acta Veterinaria Scandinavica*, *58*(1), 11.

Hald, B., Skovgard, H., Pedersen, K. and Bunkenborg, H. (2008). Influxed insects as vectors for *Campylobacter jejuni* and *Campylobacter coli* in Danish broiler houses. *Poultry Science*, (87), 1428–1434.

Hanada, Y., Khanna, S., Loftus Jr, E. V., Raffals, L. E. and Pardi, D. S. (2018). Non–*Clostridium difficile* bacterial infections are rare in patients with flares of inflammatory bowel disease. *Clinical Gastroenterology and Hepatology, 16*(4), 528-533.

Handley, R. A., Mulholland, F., Reuter, M., Ramachandran, V. K., Musk, H., Clissold, L., Le Brun, N. E. and van Vliet, A. H. (2015). PerR controls oxidative stress defence and aerotolerance but not motility-associated phenotypes of *Campylobacter jejuni*. *Microbiology, 161*(7), 1524-1536.

Hansson, I., Sandberg, M., Habib, I., Lowman, R. and Engvall, E. O. (2018). Knowledge gaps in control of *Campylobacter* for *prevention of* Campylobacteriosis. *Transboundary and Emerging Diseases,* (65), 30-48.

Hart, W., Heuzenroeder, M. and Barton, M. (2004). Antimicrobial resistance in *Campylobacter* sp. *Escherichia coli* and Enterococci associated with pigs in Australia. *Journal* of *Veterinary Medicine*, (51), 216–221.

Hassanain, N. A. (2011). Antimicrobial resistant *Campylobacter jejuni* isolated from humans and animals in Egypt. *Global Veterinaria*, (6), 195–200.

Hasso, S. A. and Aldraji, A. J. (2018). Epidemiological study of thermophilic *Campylobacter* isolated from diarrheic and non-diarrheic cows in Baghdad Governorate. *The Iraqi Journal of Veterinary Medicine, 42*(1), 105-112.

Hazeleger, W. C., Jacobs-Reitsma, W. F., Lina, P. H., De Boer, A. G., Bosch, T., Van Hoek, A. H. and Beumer, R. R. (2018). Wild, insectivorous bats might be carriers of *Campylobacter* sp. *PLoS ONE, 13*(1), e0190647.

Heredia, N. and García, S. (2018). Animals as sources of food-borne pathogens: A review. *Animal Nutrition, 4*(3), 250-255.

Hermans, D., Pasmans, F., Messens, W., Martel, A., Van Immerseel, F., Rasschaert, G., Heyndrickx, M., Van Deun, K. and Haesebrouck, F. (2012). Poultry as a host for the zoonotic pathogen *Campylobacter jejuni*. *Vector Borne and Zoonotic Diseases,* (12), 89–98.

Hermans, D., Van Deun, K., Martel, A., Van Immerseel, F., Messens, W., Heyndrickx, M., Haesebrouck, F. and Pasmans, F. (2011). Colonization

factors of *Campylobacter jejuni* in the chicken gut. *Veterinary Research*, *42*(1), 82.

Heuvelink, A. E., van Heerwaarden, C., Zwartkruis-Nahuis, A., Tilburg, J. J., Bos, M. H., Heilmann, F. G., Hofhuis, A., Hoekstra, T. and de Boer, E. (2009). Two outbreaks of campylobacteriosis associated with the consumption of raw cows' milk. *International Journal of Food Microbiology*, (134), 70–74.

Higham, L. E., Scott, C., Akehurst, K., Dring, D., Parnham, A., Waterman, M. and Bright, A. (2018). Effects of financial incentives and cessation of thinning on prevalence of *Campylobacter*: a longitudinal monitoring study on commercial broiler farms in the UK. *Veterinary Record*, *183*(19), 595.

Hoelzer, K., Wong, N., Thomas, J., Talkington, K., Jungman, E. and Coukell, A. (2017). Antimicrobial drug use in food-producing animals and associated human health risks: what, and how strong, is the evidence? *BMC Veterinary Research*, *13*(1), 211.

Hu, L. and Kopecko, D. D. (2018). *Campylobacter* species. In Food Safety (pp. 73-110). Apple Academic Press

Hu, L., Bray, M. D., Osorio, M. and Kopecko, D. J. (2006). *Campylobacter jejuni* induces maturation and cytokine production in human dendritic cells. *Infection and Immunity*, (74), 2697–2705.

Huang, J., Zang, X., Zhai, W., Guan, C., Lei, T. and Jiao, X. (2017). Quantitative analysis of *Campylobacter* sp. contamination in chicken slaughtering lines by "label tracking method" in eastern China. *Food Control*, (80), 67-73.

Humphrey, T., O'Brien, S. and Madsen, M. (2007). Campylobacters as zoonotic pathogens: a food production perspective. *International Journal of Food Microbiology*, (117), 237–257.

Hussein, M. (2018). Further investigation of the roles of fibronectin-binding proteins CadF and FlpA during *Campylobacter jejuni* interactions with intestinal epithelial cells (Doctoral dissertation, London School of Hygiene & Tropical Medicine).

Iannino, F., Di Donato, G., Ruggieri, E., Salucci, S., De Massis, F. and Di Giannatale, E. (2017). *Campylobacter* infections, a significant issue of

veterinary urban hygiene: dog-related risk factors. *Veterinaria Italiana, 53*(2), 111-120.

Iovine, N. M. (2013). Resistance Mechanisms in *Campylobacter jejuni*. *Virulence*, (4), 230–240.

ISO 10272-1:2006. (2006a). Microbiology of food and animal feeding stuffs-Horizontal method for detection and enumeration of *Campylobacter* sp. Part 1: Detection Method. International Organization for Standardization.

ISO 10272-2:2006. (2006b). Microbiology of food and animal feeding stuffs-Horizontal method for detection and enumeration of *Campylobacter* sp. Part 2: Colony-count technique. International Organization for Standardization.

Jacobs-Reitsma, W. F. (1995). *Campylobacter* bacteria in breeder flocks. *Avian Diseases*, (39), 355–359.

Jacobs-Reitsma, W. F. (2000). *Campylobacter* in the food supply. In: I. Nachamkin, M.J. Blaser (Eds.), *Campylobacter*, 2nd ed. American Society for Microbiology, DC, Washington, pp. 467-481.

Jacobs-Reitsma, W. F., Koenraad, P., Bolder, N. and Mulder, R. (1994). In vitro susceptibility of *Campylobacter* and *Salmonella* isolates from broilers to quinolones, ampicillin, tetracycline, and erythromycin. *Veterinary Quarterly*, (16), 206–208.

Janssen, R., Krogfelt, K. A., Cawthraw, S. A., van Pelt, W., Wagenaar, J. A. and Owen, R. J. (2008). Host-pathogen interactions in *Campylobacter* infections: the host perspective. *Clinical Microbiology Reviews*, (21), 505–518.

Jiao, Y. (2017). Syntheses of carbohydrate antigens expressed by gastric-intestinal bacteria and conjugates thereof (Doctoral dissertation).

Johnsen, G., Kruse, H. and Hofshagen, M. (2006). Genetic diversity and description transmission routes for *Campylobacter* on broiler farms by amplified-fragment length polymorphism. *Journal of Applied Microbiology*, (101), 1130–1139.

Johnson, T. J., Shank, J. M. and Johnson, J. G. (2017). Current and potential treatments for reducing *Campylobacter* colonization in animal hosts and disease in humans. *Frontiers in Microbiology*, (8), 487.

Jonker, A. and Picard, J. (2010). Antimicrobial susceptibility in thermophilic *Campylobacter* species isolated from pigs and chickens in South Africa. *Journal of the South African Veterinary Association*, (81), 228–236.

Jordan, H., Dunt, D., Hollingsworth, B., Firestone, S. M. and Burgman, M. (2016). Costing the morbidity and mortality consequences of zoonoses using health-adjusted life years. *Transboundary and Emerging Diseases, 63*(5), e301-e312.

Jorgensen, F., Bailey, R., Williams, S., Henderson, P., Wareing, D. R., Bolton, F. J., Frost, J. A., Ward, L. and Humphrey, T. J. (2002). Prevalence and number of *Salmonella* and *Campylobacter* sp. on raw, whole chicken in relation to sampling methods. *International Journal of Food Microbiology*, (76), 151–164.

Kaakoush, N. O., Castaño-Rodríguez, N., Mitchell, H. M. and Man, S. M. (2015). Global epidemiology of *Campylobacter* infection. *Clinical Microbiology Reviews, 28*(3), 688–720.

Kapperud, G., Espeland, G., Wahl, E., Walde, A., Herikstad, H., Gustavsen, S., Tveit, I., Natas, O., Bevanger, L. and Digranes, A. (2003). Factors associated with increased and decreased risk of *Campylobacter* infection: a prospective case-control study in Norway. *American Journal of Epidemiology*, (158), 234–242.

Karikari, A. B., Obiri-Danso, K., Frimpong, E. H. and Krogfelt, K. A. (2017a). Antibiotic resistance in *Campylobacter* isolated from patients with gastroenteritis in a teaching hospital in Ghana. *Open Journal of Medical Microbiology*, (7), 1–11.

Karikari, A. B., Obiri-Danso, K., Frimpong, E. H. and Krogfelt, K. A. (2017b). Multidrug resistant *Campylobacter* in faecal and carcasses of commercially produced poultry. *African Journal of Microbiology Research,* (11), 271–277.

Kay, E. J., Gundogdu, O. and Wren, B. (2011). Post-genome analysis of the foodborne pathogen *Campylobacter jejuni*. In M. Wiedmann & W. Zhang (Eds.), *Genomics of foodborne bacterial pathogens.* (pp. 55–94). LLC, New York, USA: Springer Science+Business Media.

Kazwala, R., Jiwa, S. F. H. and Nkya, A. E. (1993). The role of management systems

in the epidemiology of thermophilic *Campylobacter*s among poultry in Eastern zone of Tanzania. *Epidemiology and Infection*, *120*(2), *273*–278.

Kempf, I., Kerouanton, A., Bougeard, S., Nagard, B., Rose, V., Mourand, G., Osterberg, J., Denis, M. and Bengtsson, B. O. (2017). *Campylobacter coli* in organic and conventional pig production in France and Sweden: prevalence and antimicrobial resistance. *Frontiers in Microbiology*, (8), 955.

Ketley, J. M. (1997). Pathogenesis of enteric infection by *Campylobacter*. *Microbiology*, (143), 5–21.

Khaleque, A. and Bari, M. L. (2015). Facts on foodborne pathogens. In Foodborne Pathogens and Food Safety (pp. 45-91). CRC Press.

Khan, J. A., Rathore, R. S., Abulreesh, H. H., Qais, F. A. and Ahmad, I. (2018). Cultural and immunological methods for the detection of *Campylobacter jejuni*: A review. *Indian Journal of Biotechnology and Pharmaceutical Research, 6*(3), 4-10.

Kim, S. H., Park, C., Lee, E. J., Bang, W. S., Kim, Y. J. and Kim, J. S. (2017). Biofilm formation of *Campylobacter* strains isolated from raw chickens and its reduction with DNase I treatment. *Food Control*, (71), 94-100.

Kittl, S., Heckel, G., Korczak, B. M. and Kuhnert, P. (2013). Source attribution of human *Campylobacter* isolates by MLST and fla-typing and association of genotypes with quinolone resistance. *PLoS ONE, 8*(11), e81796.

Kloska, F., Beyerbach, M. and Klein, G. (2017). Infection dynamics and antimicrobial resistance profile of *Salmonella* Paratyphi B d-tartrate positive (Java) in a persistently infected broiler barn. *International Journal of Environmental Research and Public Health, 14*(1), 101.

Koga, T., Aoki, W., Mizuno, T., Wakazono, K., Ohno, J., Nakai, T., Nomiya, T., Fujii, M., Fusegawa, K., Kinoshita, K., Hamada, T. and Ikeda, Y. (2017). Antimicrobial resistance in *Campylobacter coli* and *Campylobacter jejuni* in cynomolgus monkeys (Macaca fascicularis) and eradication regimens. *Journal of Microbiology, Immunology, and Infection*, *50*(1), 75-82.

Komba, E. V. G. (2017). Human and animal thermophilic *Campylobacter* infections

in East African countries : Epidemiology and antibiogram. *Biomedical Journal of Scientific and Technical Research*, *1*(5), 1–10.

Komba, E. V., Mdegela, R. H., Msoffe, P. L., Nielsen, L. N. and Ingmer, H. (2015). Prevalence, antimicrobial resistance and risk factors for thermophilic *Campylobacter* infections in symptomatic and asymptomatic humans in Tanzania. *Zoonoses Public Health*, (62), 557–568.

Koolman, L., Whyte, P., Burgess, C. and Bolton, D. (2016). Virulence gene expression, adhesion and invasion of *Campylobacter jejuni* exposed to oxidative stress (H_2O_2). *International Journal of Food Microbiology*, (220), 33-38.

Kovalenko, K., Roastob, M., Santarea, S., B"erzin, A. and Hörmand, A. (2014). *Campylobacter* species and their antimicrobial resistance in Latvian broiler chicken production. *Food Control*, (46), 86–90.

Kowalcyk, B. B., Pires, S. M., Scallan, E., Lamichhane, A., Havelaar, A. H. and Devleesschauwer, B. (2018). Improving burden of disease and source attribution estimates. In food safety economics (pp. 143-174). Springer, Cham.

Kramer, J.M., Frost, J.A., Bolton, F.J. and Wareing, D.R. (2000). *Campylobacter* contamination of raw meat and poultry at retail sale: identification of multiple types and comparison with isolates from human infection. *Journal of Food Protection*, (63), 1654-1659.

Krumkamp, R., Sarpong, N., Schwarz, N.G., Adlkofer, J., Loag, W., Eibach, D., Hagen, R. M., Adu-Sarkodie, Y., Tannich, E. and May, J. (2015). Correction: gastrointestinal infections and diarrheal disease in Ghanaian infants and children: An outpatient case-control study. *PLOS Neglected Tropical Diseases*, *9*(4), e0003728.

Kuijf, M. L., Godschalk, P. C., Gilbert, M., Endtz, H. P., Tio-Gillen, A. P., Ang, C. W., van Doorn, P. A. and Jacobs, B. C. (2007). Origin of ganglioside complex antibodies in Guillain-Barre syndrome. *Journal of Neuroimmunology*, *188*(1-2), 69–73.

Kumar, A., Gangaiah, D., Torrelles, J. B. and Rajashekara, G. (2016). Polyphosphate and associated enzymes as global regulators of stress response and

virulence in *Campylobacter jejuni*. *World Journal of Gastroenterology*, *22*(33), 7402.

Kuusi, M., Nuorti, J. P., Hänninen, M. L., Koskela, M., Jussila, V., Kela, E., Miettinen, I. and Ruutu, P. (2005). A large outbreak of *Campylobacter*iosis associated with a municipal water supply in Finland. *Epidemiology and Infection*, *133*(4), 593–601.

Kuwabara, S. (2011). Does *Campylobacter jejuni* infection elicit axonal or demyelinating Guillain-Barré syndrome, or both? *Journal of Neurology, Neurosurgery, and Psychiatry*, (82), 238.

Kwon, K. S., Lee, I. B. and Ha, T. (2016). Identification of key factors for dust generation in a nursery pig house and evaluation of dust reduction efficiency using a CFD technique. *Biosystems Engineering*, (151), 28-52.

Lama, J. K. and Bachoon, D. S. (2018). Detection of *Brucella suis*, *Campylobacter jejuni*, and *Escherichia coli* strains in feral pig (*Sus scrofa*) communities of Georgia. *Vector-Borne and Zoonotic Diseases*, *18*(7), 350-355.

Lastovica, A. J. (2006). Emerging *Campylobacter* sp.: The tip of the iceberg. *Clinical Microbiololgy Newsletters*, (28), 49–56.

Lastovica, A. J. and Allos, M. B. (2008). Clinical signifigance of *Campylobacter* and related species other than *Campylobacter jejuni* and *Campylobacter coli*. In I. Nachamkin, C. M. Szymanski, & M. J. Blaser (Eds.), *Campylobacter* (3rd ed.). Washington DC, USA: American Society for Microbiology.

Lastovica, A. J., le Roux, E., Congi, R. V. and Penner, J. L. (1986). Distribution of sero-biotypes of *Campylobacter jejuni* and *C. coli* isolated from paediatric patients. *Journal of Medical Microbiology*, (21), 1–5.

Lehmann, H. C. and Sheikh, K. A. (2017). Guillain-Barré syndrome and acute neuropathy. In Neuroimmune pharmacology (pp. 373-393). Springer, Cham.

Lehtopolku, M. (2011). Antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter coli*. Department of Internal Medicine and Medical Microbiology and Immunology, University of Turku, Turku, Finland and the Antimicrobial Resistance Unit, National Institute for Health. Finland: Former National Public

Health Institute, KT4, Yurku.

Lengerh, A., Moges, F., Unakal, C. and Anagaw, B. (2013). Prevalence, associated risk factors and antimicrobial susceptibility pattern of *Campylobacter* species among under five diarrheic children at Gondar University Hospital, Northwest Ethiopia. *BMC Pediatrics*, (13), 82.

Lévesque, S., Lemay, F., Bekal, S., Frost, E. H. and Michaud, S. (2016). First reported case of *Campylobacter lanienae* enteritis in a human. *JMM Case Reports, 3*(3), e005045.

Liang, L., Qi, C., Wang, X., Jin, Q. and McClements, D. J. (2017). Influence of homogenization and thermal processing on the gastrointestinal fate of bovine milk fat: in vitro digestion study. *Journal of Agricultural and Food Chemistry*, *65*(50), 11109-11117.

Liao, S. J., Marshall, J., Hazelton, M. L. and French, N. P. (2019). Extending statistical models for source attribution of zoonotic diseases: a study of *Campylobacter*iosis. *Journal of the Royal Society Interface, 16*(150), 20180534.

Lior, H. (1984). New, extended biotyping scheme for *Campylobacter jejuni*, *Campylobacter coli*, and "*Campylobacter laridis*". *Journal of Clinical Microbiology*, *20*(4), 636–640.

Little, C., Richardson, J., Owen, R., de Pinna, E. and Threlfall, E. (2008). *Campylobacter* and *Salmonella* in raw red meats in the United Kingdom: Prevalence, characterization and antimicrobial resistance pattern, 2003–2005. *Food Microbiology*, (25), 538–543.

Liu, J., Platts-Mills, J. A., Juma, J., Kabir, F., Nkeze, J., Okoi, C., Operario, D. J., Uddin, J., Ahmed, S., Alonso, P. L., Antonio, M., Becker, S. M., Blackwelder, W. C., Breiman, R. F., Faruque, A. S., Fields, B., Gratz, J., Haque, R., Hossain, A., Hossain, M. J., Jarju, S., Qamar, F., Iqbal, N. T., Kwambana, B., Mandomando, I., McMurry, T. L., Ochieng, C., Ochieng, J. B., Ochieng, M., Onyango, C., Panchalingam, S., Kalam, A., Aziz, F., Qureshi, S., Ramamurthy, T., Roberts, J. H., Saha, D., Sow, S. O., Stroup, S. E., Sur, D., Tamboura, B., Taniuchi, M., Tennant, S. M., Toema, D., Wu, Y., Zaidi, A., Nataro, J. P., Kotloff, K. L., Levine, M. M. and Houpt, E. R. (2016).

Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study. *Lancet, 388*(10051), 1291-1301.

Lluque, A., Riveros, M., Prada, A., Ochoa, T. J. and Ruiz, J. (2017). Virulence and antimicrobial resistance in *Campylobacter* sp. from a Peruvian pediatric cohort. *Scientifica,* 2017: 7848926.

Luangtongkum, T., Jeon, B., Han, J., Plummer, P., Logue, C. M. and Zhang, Q. (2009). Antibiotic resistance in *Campylobacter*: Emergence, transmission and persistence. *Future Microbiology*, (4),189–200.

Luber, P., Wagner, J., Hahn, H. and Bartelt, E. (2003). Antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter coli* strains isolated in 1991 and 2001-2002 from poultry and humans in Berlin, Germany. *Antimicrobial Agents and Chemotherapy*, (47), 3825–3830.

Lunani, I. (2007). Analysis of the public health risks of the urban water system in Accra by microbial risk assessment. Master of Science. UNESCO-IHE.

Mackenjee, M. K., Coovadia, Y. M., Coovadia, H. M., Hewitt, J. and Robins-Browne, R. M. (1984). Aetiology of diarrhoea in adequately nourished young African children in Durban, South Africa. *Annals of Tropical Medicine and Parasitology*, (4), 183–187.

Magajna, B. A. and Schraft, H. (2015). *Campylobacter jejuni* biofilm cells become viable but non-culturable (VBNC) in low nutrient conditions at 4 ºC more quickly than their planktonic counterparts. *Food Control,* (50), 45-50.

Mageto, L. M., Ombui, J. N. and Mutua, F. K. (2018). Prevalence and risk factors for *Campylobacter* infection of chicken in peri-urban areas of Nairobi, Kenya. *Journal of Dairy, Veterinary and Animal Research,* (7), 22–27.

Magnusson, L. U., Farewell, A. and Nystrom, T. (2005). ppGpp a global regulator in *Escherichia coli. Trends in Microbiology, 13*(5):236–242.

Man, S. M. (2011). The clinical importance of emerging *Campylobacter* species. *Nature Reviews Gastroenterology and Hepatology,* (25), 669–685.

Mandomando, M. I., Macete, V. E., Ruiz, J., Sanz, S., Abacassamo, F., Valles, X.,

Sacarlal, J., Navia, M. M., Vila, J., Alonso, P. L. and Gascon, J. (2007). Etiology of diarrhea in children younger than 5 years of age admitted in a rural hospital of Southern Mozambique. *American Journal of Tropical Medicine and Hygiene, 76*(3), 522–527.

Mansouri-najand, L., Saleha, A. A. and Wai, S. S. (2012). Prevalence of multidrug resistance *Campylobacter jejuni* and *Campylobacter coli* in chickens slaughtered in selected markets, Malaysia. *Tropical Biomedicine, 29*(2), 231–238.

Marshall, B. and Levy, S. (2011). Food animals and antimicrobials: Impacts on human health. *Clinical Microbiology Reviews*, (24), 718–733.

Mason, J., Iturriza-Gomara, M., O'Brien, S. J., Ngwira, B. M., Dove, W., Maiden, M. C. and Cunliffe, N. A. (2013). *Campylobacter* infection in children in Malawi is common and is frequently associated with enteric virus co-infections. *PLoS ONE*, (8), e59663.

Matthew-Belmar, V., Amadi, V. A., Stone, D., Subbarao, C., DeAllie, C., Sharma, R. and Hariharan, H. (2015). Antimicrobial resistance profiles of *Campylobacter jejuni* and *Campylobacter coli* recovered from feces of young healthy domestic pigs in Grenada. *International Journal of Current Microbiology and Applied Sciences, 4*(10), 197-206.

McNulty, C. A. (1987). The treatment of *Campylobacter* infections in man. *Journal of Antimicrobial Chemotherapy,* (19), 281-284.

Mdegela, R. H., Nonga, H. E., Ngowi, H. A. and Kazwala, R. R. (2006). Prevalence of thermophilic *Campylobacter* infections in humans, chickens and crows in Morogoro, Tanzania. *Journal of Veterinary Medicine*, (121), 116–121.

Meryem, G., Zehor, G., Fares, A., Sadjia, M. and Amina, H. (2016). *Campylobacter* in sheep, calves and broiler chickens in the central region of Algeria: Phenotypic and antimicrobial resistance profiles. *African Journal of Microbiology Research*, (10), 1662–1667.

Michael, C. A., Dominey-Howes, D. and Labbate, M. (2014). The antimicrobial resistance crisis: causes, consequences, and management. *Frontiers in Public*

Health, *2*, 145.

Michi, A. N., Favetto, P. H., Kastelic, J. and Cobo, E. R. (2016). A review of sexually transmitted bovine trichomoniasis and *Campylobacter*iosis affecting cattle reproductive health. *Theriogenology*, *85*(5), 781-791.

Minor, T., Lasher, A., Klontz, K., Brown, B., Nardinelli, C. and Zorn, D. (2015). The per case and total annual costs of foodborne illness in the United States. *Risk Analysis*, *35*(6), 1125-1139.

Moffatt, C. R., Moloi, S. B. and Kennedy, K. J. (2017). First case report of myopericarditis linked to *Campylobacter coli* entero *colitis*. *BMC Infectious Diseases*, *17*(1), 8.

Molbak, K., Hojlyng, N. and Gaarslev, K. (1988). High prevalence of *Campylobacter* excretors among Liberian children related to environmental conditions. *Epidemiology and Infection*, (100), 227–237.

Moore, J. E., Corcoran, D., Dooley, J. S., Fanning, S., Lucey, B., Matsuda, M., McDowell, D. A., Mégraud, F., Millar, B. C., O'Mahony, R., O'Riordan, L., O'Rourke, M., Rao, J. R., Rooney, P. J., Sails, A. and Whyte, P. (2005). *Campylobacter*. *Veterinary Research*, *36*(3), 351–382.

Morales-Partera, A. M., Cardoso-Toset, F., Luque, I., Astorga, R. J., Maldonado, A., Herrera-León, S., Hernández, M., Gómez-Laguna, J. and Tarradas, C. (2018). Prevalence and diversity of *Salmonella* sp., *Campylobacter* sp., and Listeria monocytogenes in two free-range pig slaughterhouses. *Food control*, (92), 208-215.

Morley, L. A. U. R. A. (2014). Niche-adaptive evolution in *Campylobacter jejuni* (Doctoral dissertation, Nottingham Trent University).

Mshana, S. E., Joloba, M., Kakooza, A. and Kaddu-Mulindwa, D. (2009). *Campylobacter* spp among children with acute diarrhea attending Mulago hospital in Kampala-Uganda. *African Health Sciences*, (9), 201–205.

Mtileni, B. J., Muchadeyi, F. C., Maiwashe, A., Phitsane, P. M., Heliman, T. E., Chimonyo, M. and Dzama, K. (2009). Characterisation of production systems

for indigenous chicken genetic resources of South Africa. Applied Animal Husbandry & Rural Development 2.

Murphy, C., Carroll, C. and Jordan, K. N. (2006). Environmental survival mechanisms of the foodborne pathogen *Campylobacter jejuni*. *Journal of Applied Microbiology*, (100), 623–632.

Nadeau, E., Messier, S. and Quessy, S. (2002). Prevalence and comparison of genetic profiles of *Campylobacter* strains isolated from poultry and sporadic cases of *Campylobacter*iosis in humans. *Journal of Food Protection*, (65), 73-78.

Naito, M., Frirdich, E., Fields, J. A., Pryjma, M., Li, J., Cameron, A., Gilbert, M., Thompson, S. A. and Gaynor, E. C. (2010). Effects of sequential *Campylobacter jejuni* 81-176 lipooligosaccharide core truncations on biofilm formation, stress survival, and pathogenesis. *Journal of Bacteriology*, (192), 2182-2192.

Natsos, G., Mouttotou, N. K., Ahmad, S., Kamran, Z., Ioannidis, A. and Koutoulis, K. C. (2019). The genus *Campylobacter*: detection and isolation methods, species identification & typing techniques. *Journal of the Hellenic Veterinary Medical Society, 70*(1), 1327-1338.

Neimann, J., Engberg, J., Mølbak, K. and Wegener, H.C. (2003). A case- control study of risk factors for sporadic *Campylobacter* infections in Denmark. *Epidemiology and Infection*, (130), 353-366.

Nesbakken, T., Eckner, K., Høidal, H. K. and Røtterud, O.-J. (2003). Occurrence of *Yersinia* entero *coli*tica and *Campylobacter* sp. in slaughter pigs and consequences for meat inspection, slaughtering, and dressing procedures. *International Journal of Food Microbiology*, (80), 231–240.

Newell, D. G. and Fearnley, C. (2003). Sources of *Campylobacter* colonization in broiler chickens. *Applied and Environmental Microbiology*, *69*(8), 4343–4351.

Newell, D. G., Mughini-Gras, L., Kalupahana, R. S. and Wagenaar, J. A. (2017). *Campylobacter* epidemiology-Sources and routes of transmission for human infection. In *Campylobacter* (pp. 85-110). Academic Press.

Newman, M. J., Frimpong, E., Donkor, E. S., Opintan, J. A. and Asamoah-Adu, A. (2011). Resistance to Antimicrobial Drugs in Ghana. *Infection and Drug Resistance*, (4), 215–220.

Nguyen, N. M. T. (2017). Genotyping and antibiotic resistance of thermophilic *Campylobacter* isolates from Vietnam and Kenya (Doctoral dissertation).

Nguyen, T. N., Hotzel, H., Njeru, J., Mwituria, J., El-Adawy, H., Tomaso, H., Neubauer, H. and Hafez, H. M. (2016). Antimicrobial resistance of *Campylobacter* isolates from small scale and backyard chicken in Kenya. *Gut Pathogens*, (8), 39.

Nigatu, S., Mequanent, A., Tesfaye, R. and Garedew, L. (2015). Prevalence and drug sensitivity pattern of *Campylobacter jejuni* isolated from cattle and poultry in and around Gondar town, Ethiopia. *Global Veterinaria*, *14*(1), 43–47.

Nijhof, E. V. (2017). Developing recommendations for preventive animal health farm visits in the EU (Master's thesis).

Nohra, A. (2017). Molecular epidemiological studies of *Campylobacter* isolated from different sources in New Zealand between 2005 and 2015: a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy at Massey University, Manawatu, New Zealand (Doctoral dissertation, Massey University).

Nordentoft, S., Fischer, C., Bjerrum, L., Heckmann, L. H. and Hald, B. (2017). Reduction of *Escherichia coli*, *Salmonella* Enteritidis and *Campylobacter jejuni* in poultry manure by rearing of Musca domestica fly larvae. *Journal of Insects as Food and Feed*, *3*(2), 145-153.

Nwankwo, I. O., Faleke, O. O., Salihu, M. D., Magaji, A. A. and Shu-Yun, Z. (2016b). Prevalence of *Campylobacter* species in out-patients and pregnant women attending Government clinics in Sokoto State, Nigeria. *Journal of Preventive Medicine and Care*, *1*(2), 8–15.

Nwankwo, I. O., Faleke, O. O., Salihu, M. D., Magaji, A. A., Musa, U. and Garba, J. (2016a). Epidemiology of *Campylobacter* species in poultry and humans in the four agricultural zones of Sokoto State, Nigeria. *Journal of Public Health*

Epidemiology, (8), 184–190.

Oberhelman, R. A. and Taylor, D. N. (2000). *Campylobacter* infections in developing countries. In I. Nachamkin & M. J. Blaser (Eds.), *Campylobacter* (2nd editio, pp. 139–153). Washington: American Society for Microbiology.

Obi, C. L. and Bessong, P. O. (2002). Diarrhoeagenic bacterial pathogens in HIV-positive patients with diarrhoea in rural communities of Limpopo Province, South Africa. *Journal of Health, Population and Nutrition,* (20), 230–234.

Ocejo, M., Oporto, B. and Hurtado, A. (2019). Occurrence of *Campylobacter jejuni* and *Campylobacter coli* in cattle and sheep in Northern Spain and changes in antimicrobial resistance in two studies 10-years apart. *Pathogens, 8*(3), 98.

Oh, E., Andrews, K. J., McMullen, L. M. and Jeon, B. (2019). Tolerance to stress conditions associated with food safety in *Campylobacter jejuni* strains isolated from retail raw chicken. *Scientific Reports, 9*(1), 1-9.

Oh, E., Chui, L., Bae, J., Li, V., Ma, A., Mutschall, S. K., Taboada, E. N., McMullen, L. M. and Jeon, B. (2018). Frequent implication of multistress-tolerant *Campylobacter jejuni* in human infections. *Emerging Infectious Diseases, 24*(6), 1037.

Ohanu, M. E. and Offune, J. (2009). The prevalence of *Campylobacter* in childhood diarrhea in Enugu State of Nigeria. *Journal Communicable Disease*, (41), 117–120.

OIE (World Organisation for Animal Health). (2008). Standard for Management and Technical Requirements for Laboratories Conducting Tests for Infectious Animal Diseases. In OIE (Ed.), *Quality Standard and Guidelines for Veterinary Laboratories: Infectious Diseases* (2nd ed.). 12 rue de Prony, 75017 Paris, France: World Organisation for Animal Health (OIE: Office International des Epizooties). Retrieved from www.oie.int.

On, S. L. (2013). Isolation, identification and subtyping of *Campylobacter*. Where to from here? *Journal of Microbiological Methods,* (95), 3–7.

On, S. L., Miller, W. G., Houf, K., Fox, J. G. and Vandamme, P. (2017). Minimal standards for describing new species belonging to the families

*Campylobacter*aceae and Helicobacteraceae: *Campylobacter*, *Arcobacter*, *Helicobacter* and *Wolinella* sp. *Journal of Systematic and Evolutionary Microbiology*, *67*(12), 5296.

Oosterom, J., Dekker, R., Dewilde, G. J. A., Vankempendetroye, F. and Engels, G. B. (1985). "Prevalence of *Campylobacter jejuni* and *Salmonella* during pig slaughtering." *Veterinary Quarterly*, *7*(1), 31–34.

Otigbu, A., Clarke, A., Fri, J., Akanbi, E. and Njom, H. (2018). Antibiotic sensitivity profiling and virulence potential of *Campylobacter jejuni* isolates from estuarine water in the Eastern Cape Province, South Africa. *International Journal of Environmental Research and Public Health*, *15*(5), 925.

Ovesen, S., Durack, J., Kirk, K. F., Nielsen, H. L., Nielsen, H. and Lynch, S. V. (2019). Motility and biofilm formation of the emerging gastrointestinal pathogen *Campylobacter* concisus differs under microaerophilic and anaerobic environments. *Gut Microbes*, *10*(1), 34-44.

Oyarzabal, O. A. and Backert, S. (2016). Control of *Campylobacter* sp. in Commercial Poultry Production. In *Campylobacter* sp. and Related Organisms in Poultry (pp. 137-149). Springer, Cham.

Oyarzabal, O. A. and Carrillo, C. D. (2017). Isolation, identification, and typing of *Campylobacter* strains from food samples. In *Campylobacter* (pp. 61-83). Academic Press.

Oyarzabal, O. A., Oscar, T. P., Speegle, L. and Nyati, H. (2010). Survival of *Campylobacter jejuni* and *Campylobacter coli* on retail broiler meat stored at -20, 4, or 12 degrees C and development of Weibull models for survival. *Journal of Food Protection*, (73), 1438–1446.

Padungtod, P., Kaneene, B. ., Hanson, R., Morita, Y. and Boonmar, S. (2006). Antimicrobial resistance in *Campylobacter* isolated from food animals and humans in northern Thailand. *FEMS Immunology and Medical Microbiology*, (47), 217–225.

Paravisi, M. (2017). Caracterização da frequência de resistência antimicrobiana de *Campylobacter jejuni* isolados de frangos de corte (Masters Dissertation;

Universidade Federal do Rio Grande do Sul. Faculdade de Veterinária. Programa de Pós-Graduação em Ciências Veterinárias).

Parkhill, J., Wren, B. W., Mungall, K., Ketley, J. M., Churcher, C., Basham, D., Chillingworth, T., Davies, R. M., Feltwell, T., Holroyd, S., Jagels, K., Karlyshev, A. V., Moule, S., Pallen, M. J., Penn, C. W., Quail, M. A., Rajandream, M. A., Rutherford, K. M., van Vliet, A. H., Whitehead, S. and Barrell, B. G. (2000). The genome sequence of the foodborne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature, 403*(6770):665–668.

Patry, R. T., Stahl, M., Perez-Munoz, M. E., Nothaft, H., Wenzel, C. Q., Sacher, J. C., Coros, C., Walter, J., Vallance, B. A. and Szymanski, C. M. (2019). Bacterial AB 5 toxins inhibit the growth of gut bacteria by targeting ganglioside-like glycoconjugates. *Nature Communications, 10*(1), 1390.

Pattis, I., Moriarty, E., Billington, C., Gilpin, B., Hodson, R. and Ward, N. (2017). Concentrations of *Campylobacter* sp., *Escherichia coli*, enterococci, and *Yersinia* sp. in the faeces of farmed red deer in New Zealand. *Journal of Environmental Quality, 46*(4), 819-827.

Payot, S., Avrain, L., Magras, C., Praud, K., Cloeckaert, A. and Chaslus-Dancla, E. (2004). Relative contribution of target gene mutation and efflux to fluoroquinolone and erythromycin resistance, in French poultry and pig isolates of *Campylobacter coli*. *International Journal of Antimicrobial Agents,* (23), 468–472.

Payota, S., Sami, D., Michel, L., Michel, F. and Catherine, M. (2004). Prevalence and antimicrobial resistance of *Campylobacter coli* isolated from fattening pigs in France. *International Society for Animal Hygiene,* (7), 25–28.

Pelkonen, T., Dos Santos, M. D., Roine, I., Dos Anjos, E., Freitas, C., Peltola, H., Laakso, S. and Kirveskari, J. (2018). Potential diarrheal pathogens common also in healthy children in Angola. *Pediatric Infectious Disease Journal*, (37), 424–428.

Penner, J. L. and Hennessy, J. N. (1980). Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable

antigens. *Journal of Clinical Microbiology*, (12), 732–737.

Perez-Perez, G. I. and Blaser, M. J. (1991). *Campylobacter* and *Helicobacter*, In: S. Baron (Editors.) Med. Microbiol. Churchill Livingstone, New York, p.337-349.

Persaud, S. P., Parker, C. R., Lo, W. L., Weber, K. S. and Allen, P. M. (2014). Intrinsic CD4+ T cell sensitivity and response to a pathogen are set and sustained by avidity for thymic and peripheral complexes of self peptide and MHC. *Nature Immunology*, *15*(3), 266–274.

Pezzotti, G., Serafin, A., Luzzi, I., Mioni, R., Milan, M. and Perin, R. (2003). Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in northeastern Italy. *International Journal of Food Microbiology*, (82), 281–287.

Pina-Mimbela, R., Madrid, J. A., Kumar, A., Torrelles, J. B. and Rajashekara, G. (2015). Polyphosphate kinases modulate *Campylobacter jejuni* outer membrane constituents and alter its capacity to invade and survive in intestinal epithelial cells in vitro. *Emerging Microbes and Infections*, *4*(1), 1-9.

Pires, S. M. (2014). *Burden of Disease of Foodborne Pathogens in Denmark.* (1st ed.). Retrieved from http://www.food.dtu.dk/ (17.04.2015)

Poly, F., Noll, A. J., Riddle, M. S. and Porter, C. K. (2019). Update on *Campylobacter* vaccine development. *Human Vaccines and Immunotherapeutics*, *15*(6), 1389-1400.

Poly, F., Serichantalergs, O., Kuroiwa, J., Pootong, P., Mason, C., Guerry, P. and Parker, C. T. (2015). Updated *Campylobacter jejuni* capsule PCR multiplex typing system and its application to clinical isolates from South and Southeast Asia. *PLoS ONE*, *10*(12), e0144349.

Prats, G., Mirelis, B., Llovet, T., Muñoz, C., Miro, A. E. and Navarro, F. (2000). Antibiotic resistance trends in enteropathogenic bacteria isolated in 1985-87 and 1995-98 in Barcelona. *Antimicrobial Agents and Chemotherapy*, (44), 1140–1145.

Prieto-Calvo, M. A., López, M., Prieto, M. and Alvarez-Ordóñez, A. (2016). Variability in resistance to cold atmospheric plasma (CAP) and ultraviolet light

(UV) and multiple stress resistance analysis of pathogenic verocytotoxigenic *Escherichia coli* (VTEC). *Food Research International,* (79), 88-94.

Qin, S. S., Wu, C. M., Wang, Y., Jeon, B., Shen, Z. Q., Wang, Y., Zhang, Q. and Shen, J. Z. (2011). Antimicrobial resistance in *Campylobacter coli* isolated from pigs in two provinces in China. *International Journal of Food Microbiology*, (146), 94–98.

Quintana-Hayashi, M. P. and Thakur, S. (2012). Longitudinal study of the persistence of antimicrobial-resistant *Campylobacter* strains in distinct swine production systems on farms, at slaughter, and in the environment. *Applied and Environmental Microbiology*, (78), 2698–2705.

Rahman, M. M., Ghoshal, U. C., Sultana, S., Kibria, M. G., Sultana, N., Khan, Z. A., Ahmed, F., Hassan, M., Ahmed, T. and Sarker, S. A. (2018). Long-term gastrointestinal consequences are frequent following sporadic acute infectious diarrhea in a tropical country: a prospective cohort study. *American Journal of Gastroenterology*, *113*(9), 1363.

Randremanana, R., Randrianirina, F., Gousse, M., Dubois, N., Razafindratsimandresy, R., Hariniana, E. R., Garin, B., Randriamanantena, A., Rakotonirina, H. C., Ramparany, L., Ramarokoto, C. E., Rakotomanana, F., Ratsitorahina, M., Rajatonirina, S., Talarmin, A. and Richard, V. (2012). Case-control study of the etiology of infant diarrheal disease in 14 districts in Madagascar. *PLoS ONE*, *7*(9), e44533.

Rao, K. A., Yazaki, E., Evans, D. F. and Carbon, R. (2004). Objective evaluation of small bowel and colonic transit time using pH telemetry in athletes with gastrointestinal symptoms. *British Journal of Sports Medicine, 38*(4):482–487.

Rashid, T., VonVille, H. M., Hasan, I. and Garey, K. W. (2016). Shoe soles as a potential vector for pathogen transmission: a systematic review. *Journal of Applied Microbiology*, *121*(5), 1223-1231.

Reddy, S. and Zishiri, O. T. (2017). Detection and prevalence of antimicrobial resistance genes in *Campylobacter* sp. isolated from chickens and humans. *Onderstepoort Journal* of *Veterinary Research,* (84), e1–e6.

Reich, F., Valero, A., Schill, F., Bungenstock, L. and Klein, G. (2018). Characterisation of *Campylobacter* contamination in broilers and assessment of microbiological criteria for the pathogen in broiler slaughterhouses. *Food Control*, (87), 60-69.

Richter, C., Custer, B., Steele, J., Wilcox, B. and Xu, J. (2015). Intensified food production and correlated risks to human health in the greater mekong subregion: A systematic review. *Environmental Health, 26*(14), 43.

Ricotta, E. E., Palmer, A., Wymore, K., Clogher, P., Oosmanally, N., Robinson, T., Lathrop, S., Karr, J., Hatch, J., Dunn, J., Ryan, P. and Blythe, D. (2014). Epidemiology and antimicrobial resistance of international travel-associated *Campylobacter* infections in the United States, 2005–2011. *American Journal of Public Health*, (104), 108–114.

Rodrigues, L. C., Cowden, J. M., Wheeler, J. G., Sethi, D., Wall, P. G., Cumberland, P., Tompkins, D. S., Hudson, M. J., Roberts, J. A. and Roderick, P. J. (2001). The study of infectious intestinal disease in England: risk factors for cases of infectious intestinal disease with *Campylobacter jejuni* infection. *Epidemiology and Infection*, (127), 185-193.

Rosner, B. M., Schielke, A., Didelot, X., Kops, F., Breidenbach, J., Willrich, N., Gölz, G., Alter, T., Stingl, K., Josenhans, C., Suerbaum, S. and Stark, K. (2017). A combined case-control and molecular source attribution study of human *Campylobacter* infections in Germany, 2011–2014. *Scientific Reports*, *7*(1), 5139.

Rouby, S. R., Abdel-Latef, G. K. and Aziz, S. A. A. A. (2019). Bacteriological and molecular identification of thermophilic *Campylobacter*s of animal and human origins in Beni-Suef Governorate, Egypt. *Journal of Advanced Veterinary Research, 9*(3), 102-106.

Royden, A., Wedley, A., Merga, J. Y., Rushton, S., Hald, B., Humphrey, T. and Williams, N. J. (2016). A role for flies (Diptera) in the transmission of *Campylobacter* to broilers? *Epidemiology and Infection, 144*(15), 3326-3334.

Sackey, B. A., Mensah, P., Collison, E. and Dawson, E. S. (2001). *Campylobacter*, *Salmonella*, *Shigella* and *E. coli* in live and dressed poultry from metropolitan

Accra. *International Journal Food Microbiology*, (71), 21–28.

Saeed, A., Abd, H. and Sandstrom, G. (2015). Microbial aetiology of acute diarrhoea in children under five years of age in Khartoum, Sudan. *Journal of Medical Microbiology*, (64 Pt 4), 432–437.

Saenz, Y., Zarazaga, M. and Lantero, M. (2000). Antibiotic resistance in *Campylobacter* strains isolated from Animal, Foods and Humans in Spain, in 1997-1998. *Antimicrobial Agents Chemotherapy*, (44), 267–271.

Sahin, O., Yaeger, M., Wu, Z. and Zhang, Q. (2017). *Campylobacter*-associated diseases in animals. *Annual Review of Animal Biosciences*, (5), 21-42.

Sainato, R., ElGendy, A., Poly, F., Kuroiwa, J., Guerry, P., Riddle, M. S. and Porter, C. K. (2018). Epidemiology of *Campylobacter* Infections among Children in Egypt. *American Journal of Tropical Medicine and Hygiene*, (98), 581–585.

Saleha, A. (2004). Epidemiological study on the colonization of chickens with *Campylobacter* in broiler farms in Malaysia: possible risk and management factors. *International Journal of Poultry Science*, (3), 129–134.

Salihu, M. D., Junaidu, A. U., Magaji, A. A. and Yakubu, Y. (2012). Prevalence and antimicrobial resistance of thermophilic *Campylobacter* isolates from commercial broiler in Sokoto, Nigeria. *Research Journal of Veterinary Sciences*, *5*(2), 51–58.

Salihu, M. D., Junaidu, A. U., Magaji, A. A., Abubakar, M. B., Adamu, A. Y. and Yakubu, A. S. (2009). Prevalence of *Campylobacter* in poultry meat in Sokoto, Northwestern Nigeria. *Journal of Public Health Epidemiology*, *1*(2), 41–45.

Samie, A., Obi, C. L., Barrett, L. J., Powell, S. M. and Guerrant, R. L. (2007). Prevalence of *Campylobacter* species, *Helicobacter pylori* and *Arcobacter* species in stool samples from the Venda region, Limpopo, South Africa: Studies using molecular diagnostic methods. *Journal of Infection*, (54), 558–566.

Sampers, I., Habib, I., De Zutter, L., Dumoulin, A. and Uyttendaele, M. (2010). Survival of *Campylobacter* sp. in poultry meat preparations subjected to freezing, refrigeration, minor salt concentration, and heat treatment. *International Journal*

of Food Microbiology, (137), 147–153.

Samuel, S. O., Aboderin, A. O., Akanbi, A.A., I., Adegboro, B., Smith, S. I. and Coker, A. O. (2006). *Campylobacter* enetritis in Ilorin, Nigeria. *East African Medical Journal*, (83), 478–484.

Sangaré, L., Nikiéma, A. K., Zimmermann, S., Sanou, I., Congo-Ouédraogo, M., Diabaté, A., Diandé, S. and Guissou, P. I. (2012). *Campylobacter* sp. epidemiology and antimicrobial susceptibility in a developing country, Burkina Faso (West Africa). *African Journal of Clinical and Experimental Microbiology,* (13), 110–117.

Savanna Agricultural Research Institute (SARI). (2001). A map showing UDS-Nyankpala campus, Tamale in the Northern region of Ghana.

Scarcelli, E., da Costa, E. O., Genovez, M. É., Cardoso, M. V., Bach, E. E. and Torres, A. P. (2001). Comparison of electrophoretic protein profiles of *Campylobacter jejuni* subsp. *jejuni* isolated from different animal species. *Brazilian Journal of Microbiology*, *32*(4), 286–292.

Scharff, R. L. (2010). *Health-related costs from foodborne illness in the United States.* Washington DC. The Produce Safety Project at Georgetown University, A peer reviewed initiative of the Pew Charitable Trusts. Georgetown.

Schets, F. M., Jacobs-Reitsma, W. F., van der Plaats, R. Q. J., Heer, L. K. D., van Hoek, A. H. A. M., Hamidjaja, R. A., de Roda Husman, A. M. and Blaak, H. (2017). Prevalence and types of *Campylobacter* on poultry farms and in their direct environment. *Journal of Water and Health, 15*(6), 849-862.

Sean, F. A., Norman, J. S., Patricia, I. F. and David, L. S. (1999). *Campylobacter jejuni*—an emerging foodborne pathogen. *Emerging Infectious Diseases*, (5), 28–35.

Sebald, M. and Véron, M. (1963). Teneur en bases de l'ADN et classification des vibrions. *Annales de l'Institut Pasteur*, (105), 897–910.

Seguino, A., Chintoan-Uta, C., Smith, S. H. and Shaw, D. J. (2018). Public health significance of *Campylobacter* sp. colonisation of wild game pheasants (Phasianus colchicus) in Scotland. *Food Microbiology,* (74), 163-170.

Seguino, A., Chintoan-Uta, C., Smith, S. H. and Shaw, D. J. (2018). Public health significance of *Campylobacter* sp. colonisation of wild game pheasants (Phasianus colchicus) in Scotland. *Food Microbiology*, (74), 163-170.

Sethi, S. (2017). Unit-4 Bacterial Food Infections. IGNOU. http://hdl.handle.net/123456789/ 12193

Sharma, K. P., Chattopadhyay, U. K. and Naskar, K. (2016). Prevalence of *Campylobacter* species in raw meat samples sold in open markets of Kolkata city. *International Journal of Agriculture, Environment and Biotechnology*, *9*(4), 535-539.

Shelly, R., Abiodun, A. and Asgarali, Z. (2005). Biotypes and serotypes of *Campylobacter* sp. isolated from broilers in Trininad. *British Food Journal*, *107*(11), 884–890.

Shen, C., Lemonakis, L., Etienne, X., Li, K., Jiang, W. and Adler, J. M. (2019). Evaluation of commercial antimicrobials against stress-adapted *Campylobacter jejuni* on broiler wings by using immersion and electrostatic spray and an economic feasibility analysis. *Food Control*, (103), 161-166.

Sheppard, S. K. and Maiden, M. C. (2015). The evolution of *Campylobacter jejuni* and *Campylobacter coli*. *Cold Spring Harbor Perspectives in Biology*, *7*(8), a018119.

Shin, E. and Lee, Y. (2010). Characterization of erythromycin-resistant porcine isolates of *Campylobacter coli*. *Microbial Drug Resistance*, (16), 231–239.

Shobo, C. O., Bester, L. A., Baijnath, S., Somboro, A. M., Peer, A. K. and Essack, S. Y. (2016). Antibiotic resistance profiles of *Campylobacter* species in the South Africa private health care sector. *Journal of Infection in Developing Countries*, (10), 1214–1221.

Shrestha, S. (2018). Efficacy of natural compounds with novel carrier systems for controlling *Campylobacter jejuni* in post-harvest poultry. (Doctoral dissertation, University of Arkansas).

Sibanda, N., McKenna, A., Richmond, A., Ricke, S. C., Callaway, T., Stratakos, A. C., Gundogdu, O. and Corcionivoschi, N. (2018). A review of the effect of

management practices on *Campylobacter* prevalence in poultry farms. *Frontiers in Microbiology*, (9), 2002.

Siemer, B. L., Nielsen, E. M. and On, S. L. (2005). Identification and molecular epidemiology of *Campylobacter coli* isolates from human gastroenteritis, food, and animal sources by amplified fragment length polymorphism analysis and penner serotyping. *Applied and Environmental Microbiology*, (71), 1953–1958.

Silva, J., Leite, D., Fernandes, M., Mena, C., Gibbs, P. A. and Teixeira, P. (2011). *Campylobacter* sp. as a foodborne pathogen: a review. *Frontiers in Microbiology*, (2), 200.

Skirrow, M. B. (1977). "*Campylobacter enteritis* - new disease." *British Medical Journal*, *2*(6078), 9–11.

Slingerland, A. E., Schwabkey, Z., Wiesnoski, D. H. and Jenq, R. R. (2017). Clinical evidence for the microbiome in inflammatory diseases. *Frontiers in Immunology*, (8), 400.

Smibert, R. (1984). "*Campylobacter*" In: In J. Holt & N. Krieg (Eds.), *Bergey's Manual of Systematic Bacteriology Volume 1*. Baltimore, Maryland, USA: The Williams and Wilkins Co.

Smole-Možina, S., Kurinčič, M., Klančnik, A. and Mavri, A. (2011). *Campylobacter* and its multi-resistance in the food chain. *Trends Food Science and Technology*, (22), 91-98.

Son, I., Englen, M. D., Berrang, M. E., Fedorka-Cray, P. J. and Harrison, M. A. (2007). Prevalence of *Acrobacter* and *Campylobacter* on broiler carcasses during processing. *International Journal Food Microbiology*, (113), 16–22.

Sreeja, M. K., Gowrishankar, N. L., Adisha, S. and Divya, K. C. (2017). Antibiotic resistance-reasons and the most common resistant pathogens: A review. *Research Journal of Pharmacy and Technology*, *10*(6), 1886.

Stahl, M., Frirdich, E., Vermeulen, J., Badayeva, Y., Li, X., Vallance, B. A. and Gaynor, E. C. (2016). The helical shape of *Campylobacter jejuni* promotes in vivo pathogenesis by aiding transit through intestinal mucus and colonization of crypts. *Infection and Immunity*, *84*(12), 3399-3407.

Stern, N. J., Wojton, B. and Kwiatek, K. (1992). A differential–selective medium and dry ice–generated atmosphere for recovery of *Campylobacter jejuni*. *Journal Food Protection*, (55), 514–517.

Stingl, K., Knüver, M. T., Vogt, P., Buhler, C., Krüger, N. J., Alt, K., Tenhagen, B. A., Hartung, M., Schroeter, A., Ellerbroek, L., Appel, B. and Käsbohrer, A. (2012). Quo vadis? Monitoring *Campylobacter* in Germany. *European Journal of Microbiology and Immunology*, (2), 88–96.

Stintzi, A. (2003). "Gene expression profile of *Campylobacter jejuni* in response to growth temperature variation." *Journal of Bacteriology*, *185*(6), 2009–2016.

Strachan, N. J. C. and Forbes, K. J. (2010). The growing UK epidemic of human campylobacteriosis. *Lancet*, (376), 665–667.

Strathmann, M., Horstkott, M., Koch, C., Gayer, U. and Wingender, J. (2016). The river Ruhran urban river under particular interest for recreational use and as a raw water source for drinking water: The collaborative research project "Safe Ruhr"–microbiological aspects. *International Journal of Hygiene and Environmental Health*, *219*(7), 643-661.

Studahl, A. and Andersson, Y. (2000). Risk factors for indigenous *Campylobacter* infection: a Swedish case-control study. *Epidemiology and Infection*, (125), 269-275.

Sukhapesna, J., Amavisit, P., Wajjwalku, W., Thamchaipenet, A. and Sukpuaram, T. (2005). Antimicrobial resistance of *Campylobacter jejuni* isolated from chicken in Nakhon Pathom province Thailand. *Kasetsart Journal of Natural Science*, *39*(2), 240–246.

Sung, K. and Khan, S. (2015). Biofilm Development by *Campylobacter jejuni*. In L. Anthony, Pometto III, & A. Demirci (Eds.), *Biofilms in the Food Environment.* (2nd ed., pp. 29–50). Retrieved from https://onlinelibrary.wiley.com/doi/10.1002/9781118864036.ch2.

Suzuki, H. and Yamamoto, S. (2009). *Campylobacter* contamination in retail poultry meats and by-products in the world: a literature survey. *Journal of Veterinary Medicine and Science*, (71), 255–261.

Svensson, S. L., Frirdich, E. and Gaynor, E. C. (2008). Survival strategies of *Campylobacter jejuni*. Stress responses, the vial but nonculturable state and biofilms pp. In I. Nachamkin, C. M. Szymanski, & M. J. Blaser (Eds.), *Campylobacter* (3rd ed., pp. 571–590). Washington, D.C.: ASM Press.

Swierczewski, B. E., Odundo, E. A., Koech, M. C., Ndonye, J. N., Kirera, R. K., Odhiambo, C. P., Cheruiyot, E. K., Shaffer, D. N., Ombogo, A. N. and Oaks, E. V. (2013). Enteric pathogen surveillance in a case-control study of acute diarrhea in Kisii Town, Kenya. *Journal of Medical Microbiology*, (62 Pt 11), 1774–1776.

Szygalski-Biasi, R., Ernlund Freitas de Macedo, R., Scaranello-Malaquias, M. A. and Franchin, P. R. (2011). Prevalence, strain identification and antimicrobial resistance of *Campylobacter* sp. isolated from slaughtered pig carcasses in Brazil. *Food Control*, (22), 702–707.

Tafa, B., Sewunet, T., Tassew, H. and Asrat, D. (2014). Isolation and antimicrobial susceptibility patterns of *Campylobacter* species among diarrheic children at Jimma, Ethiopia. *International Journal* of *Bacteriology*, (2014), 560617.

Takata, T., Wai, S. N., Takade, A., Sawae, Y., Ono, J. and Amako, K. (1995). "The purification of a groEL-like stress protein from aerobically adapted *Campylobacter jejuni*." *Microbiology and Immunology*, *39*(9), 639–645.

Tambur, Z., Miljkovic-Selimovic, B., Bokonjic, D. and Kulisic, Z. (2009). Susceptibility of *Campylobacter jejuni* and *Campylobacter coli* isolated from animals and humans to ciprofloxacin. *Polish Journal* of *Veterinary Sciences*, (12), 269–273.

Tang, J. Y. H., Farinazleen, M. G., Saleha, A. A., Nishibuchi, M. and Son, R. (2009). Comparison of thermophilic *Campylobacter* sp. occurrence in two types of retail chicken samples, hit. *Food Research International Journal*, (16), 277–288.

Tayde, R. S. and Brahmbhatt, M. N. (2014). Biotyping of thermophilic *Campylobacter* sp. isolated from poultry in and around Anand city, Gujarat, India. *Veterinary World*, *7*(5), 321–324.

Taylor, A. J. and Kelly, D. J. (2019). The function, biogenesis and regulation of the electron transport chains in *Campylobacter jejuni*: New insights into the bioenergetics of a major food-borne pathogen. *Advances in Microbial Physiology*, (74), 239-329.

Taylor, D. N. (1992). *Campylobacter* infections in developing countries. In I. Nachamkin, M. J. Blaser, & L. S. Tompkins (Eds.), *Campylobacter jejuni: Current status and future trends.* (pp. 20–30). Washington: American Society for Microbiology.

Tejan, N., Datta, P. and Gupta, V. (2018). Bacterial diarrhoea: a comprehensive review. *International Journal of Pharmaceutical Sciences and Research, 9*(12), 5015-5031.

Teunis, P. F., Marinović, A. B., Tribble, D. R., Porter, C. K. and Swart, A. (2018). Acute illness from *Campylobacter jejuni* may require high doses while infection occurs at low doses. *Epidemics*, (24), 1-20.

Teunis, P., Van den Brandhof, W., Nauta, M., Wagenaar, J., Van den Kerkhof, H. and Van Pelt, W. (2005). A reconsideration of the *Campylobacter* dose-response relation. *Epidemiology and Infection*, (133), 583–592.

Thakur, S. and Gebreyes, W. (2005). *Campylobacter coli* in swine production: Antimicrobial resistance mechanisms and molecular epidemiology. *Journal of Clinical Microbiology*, (43), 5705–5714.

Thépault, A., Rose, V., Quesne, S., Poezevara, T., Béven, V., Hirchaud, E., Touzain, F., Lucas, P., Méric, G., Mageiros, L., Sheppard, S. K., Chemaly, M. and Rivoal, K. (2018). Ruminant and chicken: important sources of Campylobacteriosis in France despite a variation of source attribution in 2009 and 2015. *Scientific Reports, 8*(1), 9305.

Tilmanne, A., Yattara, H. M. K., Herpol, M., Vlaes, L., Retore, P., Quach, C., Vandenberg, O., Hallin, M. and Martiny, D. (2019). Multi-step optimization of the filtration method for the isolation of *Campylobacter* species from stool samples. *European Journal of Clinical Microbiology and Infectious Diseases, 38*(5), 859-864.

Tresse, O., Alvarez-Ordóñez, A. and Connerton, I. F. (2017). About the foodborne pathogen *Campylobacter*. *Frontiers in Microbiology*, (8), 1908.

Turkson, P. K. (2008). Use of drugs and antibiotics in Ghana. *Ghana Journal of Agricultural Science*, (41), 23–33.

Turkson, P. K., Lindqvist, K. J. and Kapperud, G. (1988). Isolation of *Campylobacter* sp. and *Yersinia enterocolitica* from domestic animals and human patients in Kenya. *APMIS*, 141–6. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3345259

Uaboi-Egbenni, P. O., Bessong, P. O., Samie, A. and Obi, C. L. (2012). Potentially pathogenic *Campylobacter* species among farm animals in rural areas of Limpopo province, South Africa: A case study of chickens and cattles. *African Journal of Microbiology Research*, (6), 2835–2843.

Ugboma, A. M., Salihu, M. D., Magaji, A. A. and Abubakar, M. B. (2013). Prevalence of *Campylobacter* species in ground water in Sokoto, Sokoto State, Nigeria. *Veterinary World*, (6), 285–287.

Upadhyay, A., Arsi, K., Upadhyaya, I., Donoghue, A. M. and Donoghue, D. J. (2019). Natural and environmentally friendly strategies for controlling *Campylobacter jejuni* colonization in poultry, survival in poultry products and infection in humans. In food safety in poultry meat production (pp. 67-93). Springer, Cham.

Urdaneta-Vargas, S. H. (2016). Epidemiología de *Campylobacter* sp. en granjas de pollos de engorde: prevalencia, factores de riesgo y dinámica de infección. (Doctoral Dissertation; Universidad Autonoma de Barcelona, Departamento de Sanidad y Anatomia Animales).

Usha, M. R., Fauziah, M., Tunung, R., Chai, L. C., Cheah, Y. K., Farinazleen, M. G. and Son, R. (2010). Occurrence and antibiotic resistance of *Campylobacter jejuni* and *Campylobacter coli* in retail broiler chicken. *International Food Research Journal*, (17), 247–255.

Uzunovic-Kamberovic, S., Zorman, T., Berce, I., Herman, L. and Smole-Mozina, S. (2009). Comparison of the frequency and the occurrence of antimicrobial

resistance among *C. jejuni* and *C. coli* isolated from human infections, retail poultry meat and poultry in Zenica-Doboj Canton, Bosnia and Herzegovina. *Medicinski Glasnik Ljekarske Komore Zenicko-Dobojskog Kantona*, (6), 173–180.

Van Boeckel, T., Brower, C., Gilbert, M., Grenfell, B., Levin, S., Robinson, T., Teillant, A. and Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences of the United States of America*, (112), 5649–5654.

Van der Stel, A. X., van Mourik, A., Heijmen-van Dijk, L., Parker, C. T., Kelly, D. J., van de Lest, C. H., van Putten, J. P. and Wösten, M. M. (2015). The *Campylobacter jejuni* RacRS system regulates fumarate utilization in a low oxygen environment. *Environmental Microbiology*, *17*(4), 1049-1064.

Vandamme, P. (2000). Taxonomy of the family *Campylobactericeae*. In I. Nachamkin & M. Blaser (Eds.), *Campylobacter* (2nd ed., pp. 3–26). Washington DC, USA: ASM Press.

Vandamme, P., Debruyne, L., De Brandt, E. and Falsen, E. (2010). Reclassification of bacteroidesureolyticus as *Campylobacter ureolyticus* comb. nov., and emended description of the genus *Campylobacter*. *International Journal of Systematic and Evolutionary Microbiology*, (60), 2016–2022.

Vandamme, P., Dewhirst, F. E., Paster, B. J. and On, S. L. W. (2015**).** *Campylobacter*. In *Bergey's Manual of Systematics of Archaea and Bacteria* eds. W.B. Whitman, F. Rainey, P. Kämpfer, M. Trujillo, J. Chun,P. DeVos, B. Hedlund and S Dedysh. (pp. 1-27) New York, NY: John Wiley & Sons, Inc.

Vegge, C. S., Jansen van Rensburg, M. J., Rasmussen, J. J., Maiden, M. C., Johnsen, L. G., Danielsen, M., MacIntyre, S., Ingmer, H. and Kelly, D. J. (2016). Glucose metabolism via the Entner-Doudoroff pathway in *Campylobacter*: a rare trait that enhances survival and promotes biofilm formation in some isolates. *Frontiers in Microbiology*, (7), 1877.

Vellinga, A. and Van Loock, F. (2002). The dioxin crisis as experiment to determine poultry- related *Campylobacter enteritis*. *Emerging Infectious Diseases*, (8), 19–

22.

Veron. and Chatelain, R. (1973). "Taxonomic study of the genus *Campylobacter;* Sebald and Veron and designation of the neotype strain for the type species, *Campylobacter fetus* (Smith and Taylor) Sebald and Veron." *International Journal of Systematic Bacteriology,* (23), 122–134.

Vinaiphat, A. and Thongboonkerd, V. (2017). Heat shock proteins in leptospirosis. In Heat shock proteins in veterinary medicine and sciences (pp. 361-374). Springer, Cham.

Vojdani, A. and Vojdani, E. (2019). Reaction of antibodies to *Campylobacter jejuni* and cytolethal distending toxin B with tissues and food antigens. *World Journal of Gastroenterology*, *25*(9), 1050.

Von Wintersdorff, C., Penders, J., Stobberingh, E., Oude Lashof, A., Hoebe, C., Savelkoul, P. and Wolffs, P. F. (2014). High rates of antimicrobial drug resistance gene acquisition after international travel, the Netherlands. *Emerging Infectious Diseases*, (20), 649–657.

Vu, D. (2018). Bacterial infection in children hospitalised with diarrhoea in the South of Vietnam (Doctoral dissertation, University of Oxford).

Wagenaar, J. A., Newell, D. G., Kalupahana, R. S. and Mughini-Gras, L. (2015). *Campylobacter*: Animal reservoirs, human infections, and options for control. In Zoonoses-infections affecting humans and animals (pp. 159-177). Springer, Dordrecht.

Wales, A. D., Vidal, A. B., Davies, R. H. and Rodgers, J. D. (2019). Field interventions against colonization of broilers by *Campylobacter*. *Comprehensive Reviews in Food Science and Food Safety*, *18*(1), 167-188.

Walker, R. I., Schmauder-Chock, E. A., Parker, J. L. and Burr, D. (1988). Selective association and transport of *Campylobacter jejuni* through M cells of rabbit Peyer's patches. *Canadian Journal of Microbiology,* (34), 1142–1147.

Walther, B. A., Boete, C., Binot, A., Bye, Y., Cappelle, J., Carrique-Mas, J., Chou, M., Furey, N., Kim, S., Lajaunie, C., Lek, S., Méral, P., Neang, M., Tan, B. - H., Walton, C. and Morand, S. (2016). Biodiversity and health: Lessons and

recommendations from an interdisciplinary conference to advise Southeast Asian research, society and policy. *Infection, Genetics and Evolution*, (40), 29–46.

Wang, G., Clark, C. G., Taylor, T. M., Pucknell, C., Barton, C., Price, L., Woodward, D. L. and Rodgers, F. G. (2002). Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. *fetus*. *Journal of Clinical Microbiology*, (40), 4744–4747.

Wasfy, M. O., Oyofo, B. A., David, J. C., Ismail, T. F., El-Gendy, A. M., Mohran, Z. S., Sultan, Y. and Peruski, L. F. (2000). Isolation and antibiotic susceptibility of *Salmonella*, *Shigella*, and *Campylobacter* from acute enteric infections in Egypt. *Journal of Health, Population and Nutrition*, (18), 33–38.

Wassenaar, T. M., Bleumink-Pluym, N. M., Newell, D. G., Nuijten, P. J. and van der Zeijst, B. A. (1994). Differential flagellin expression in a flaA flaB+ mutant of *Campylobacter jejuni*. *Infection and Immunity*, (62), 3901–3906.

Weber, G. M. and Windisch, W. (2017). Producing sufficient animal-source protein for the growing world population. In Sustainable nutrition in a changing world (pp. 321-334). Springer, Cham.

Weis, A. M., Huang, B. C., Storey, D. B., Kong, N., Chen, P., Arabyan, N., Gilpin, B., Mason, C., Townsend, A. K., Smith, W. A., Byrne, B. A., Taff, C. C. and Weimer, B. C. (2017). Large-scale release of *Campylobacter* draft genomes: Resources for food safety and public health from the 100K pathogen genome project. *Genome Announcements*, *5*(1), e00925-16.

WHO/CIA. (2011). *Critically Important Antimicrobials for Human Medicine [3rd Revision]*. WHO document production services, Geneva, Switzerland. Retrieved from http://apps.who.int/medicinedocs/documents/s22251en/s22251en.pdf (Accessed on: 22th April, 2018).

Wieczorek, K. and Osek, J. (2013a). Characteristics and antimicrobial resistance of *Campylobacter* isolated from pig and cattle carcasses in Poland. *Polish Journal of Veterinary Sciences*, *16*(3), 501–508.

Wieczorek, K. and Osek, J. (2013b). Antimicrobial Resistance Mechanisms among

Campylobacter. *BioMed Research International*. Retrieved from https://doi.org/10.1155/2013/340605%0A

Wieczorek, K., Szewczyk, R. and Osek, J. (2012). Prevalence, antimicrobial resistance, and molecular characterization of *Campylobacter jejuni* and *C. coli* isolated from retail raw meat in Poland. *Veterinary Medicine*, (57), 293–299.

Wijnands, L.M., Delfgou-van Asch, E.H., Beerepoot-Mensink, M.E., van der Meij-Florijn, A., Fitz-James, I., van Leusden, F.M. and Pielaat, A. (2014). Prevalence and concentration of bacterial pathogens in raw produce and minimally processed packaged salads produced in and for the Netherlands. *Journal of Food Protection*, (77), 388-394.

Wilson, I.G. and Moore, J.E. (1996). Presence of *Salmonella* sp. and *Campylobacter* sp. in shellfish. *Epidemiology and Infection*, (116), 147-153.

Wingstrand, A., Neimann, J., Engberg, J., Nielsen, E.M., Gerner-Smidt, P., Wegener, H.C. and Mølbak, K. (2006). Fresh chicken as main risk factor for Campylobacteriosis, Denmark. *Emerging Infectious Diseases*, (12), 280-285.

Wittwer, M., Keller, J., Wassenaar, T., Stephan, R., Howald, D., Regula, G. and Bissig-Choisat, B. (2005). Genetic diversity and antibiotic resistance patterns in a *Campylobacter* population isolated from poultry farms in Switzerland. *Applied and Environmental Microbiology*, *71*(6), 2840–2847.

Wong, J. T., de Bruyn, J., Bagnol, B., Grieve, H., Li, M., Pym, R. and Alders, R. G. (2017). Small-scale poultry and food security in resource-poor settings: A review. *Global Food Security*, (15), 43-52.

Workman, N. S., Mathison, E. G. and Lavoie, C. M. (2005). Pet dogs and chicken meat as reservoir of *Campylobacter* sp. in Barbados. *Journal of Clinical Microbiology*, *43*(6), 2642–2650.

World Health Organisation (WHO). (2013). *The Global View of Campylobacteriosis; World Health Organization.* Geneva, Switzerland.

Wu, Y. L., Lee, L. H., Rollins, D. M. and Ching, W. M. (1994). "Heat shock induced and alkaline pH induced proteins of *Campylobacter jejuni*-characterization and immunological properties." *Infections and Immunology*, *62*(10), 4256–4260.

Yang, R., Abraham, S., Gardner, G. E., Ryan, U. and Jacobson, C. (2017). Prevalence and pathogen load of *Campylobacter* sp., *Salmonella enterica* and *Escherichia coli* O157/O145 serogroup in sheep faeces collected at sale yards and in abattoir effluent in Western Australia. *Australian Veterinary Journal, 95*(5), 143-148.

Zhou, Y., Bu, L., Guo, M., Zhou, C., Wang, Y., Chen, L. and Liu, J. (2013). Comprehensive genomic characterization of *Campylobacter* genus reveals some underlying mechanisms for its genomic diversification. *PLoS ONE,* (8), e70241.

APPENDICES

Appendix 1.1: Gram Staining and Microscopy

Gram staining was performed on the *Campylobacter* isolates using a gram staining kit from HI-MEDIA which contains all the four major reagents needed for performing the stain.

Before the gram staining was performed, a smear was first prepared by dropping two or three loopful of sterile distilled water on a labelled glass slide and, a freshly incubated bacteria scooped and mixed uniformly with the water on the slide. The smear is then heat-fixed and allowed to cool to the touch before stains or gram staining reagents were applied in the steps described below. Also, the smear was carefully prepared with the consideration that the quality of the smear (too heavy or too light cell concentration) will affect the gram stain results.

i. The air-dried heat-fixed smear was flooded with crystal violet staining reagent for 3 minutes. Flooded slides were washed in a gentle and indirect stream of tap water for few seconds.
ii. Excess water was then drained off and again flooded for 2 minutes with the mordant; thus, a second reagent called gram's iodine.
iii. Flooded slides were washed in a gentle and indirect stream of tap water for few seconds.
iv. After excess water was drained off, slides were flooded with a decolorizing agent (Acetone-alcohol decolourizer) for 10-15 seconds.
v. Flooded slides were washed in a gentle and indirect stream of tap water for few seconds.

vi. Excess water was drained and slides were flooded with counterstain, safranin the final reagent, also for 30 seconds.

vii. Flooded slides were washed in a gentle and indirect stream of tap water until no colour appears in the effluent, and then blotted dry with absorbent paper.

viii. Observations for the staining procedure results were made under oil immersion (100x) using a bright field microscope (i.e. prepared microscopic slides were examined using oil immersion phase contrast microscopy).

Slides demonstrating typical *Campylobacter* corkscrew morphology were regarded as presumptively positive.

Appendix 1.2: Latex Agglutination Immunoassay

A rapid confirmatory latex agglutination test for *Campylobacter* was done using the Thermo Scientific *Campylobacter* Test Kit (Oxoid Ltd., Basingstoke, UK) which contains solutions of test latex, control latex, and sample diluent. Each presumptively positive colony was confirmed by testing an additional portion of the colony using the *Campylobacter* Test Kit following the procedure below;

i. All reagents were brought to room temperature and all latex reagents shaken to ensure a homogeneous suspension.

ii. A 50 µL of sample diluent was dispensed onto each of two ovals of the agglutination slide.

iii. With the aid of an inoculating loop, several colonies of *Campylobacter*-like morphology were removed and in observed cases of sparse microbial growth, a broad sweep was taken of the agar surface.

iv. The scooped bacteria were then mixed into each of the two drops of the sample diluent on the slide to form even suspensions.

v. One drop (50 µL) of the control latex reagent was added to one of the bacterial suspensions on the slide and similarly one drop (50 µL) of test latex reagent dispensed to the other bacterial suspension.

vi. All bacterial suspensions with their respective latex reagents were mixed using a mixing stick and mixtures spread to the edges of the oval areas, starting from the control latex reagent.

vii. Slide was gently rocked while keeping the fluid suspensions in constant movement for 2 minutes and observed for agglutination.

viii. Test results were read and interpreted.

Interpretation: Based on the indication by visible aggregation of the latex particles, the strength of the reaction varies and was assessed according to the below guidelines;

- + *reaction*- fine but readily discernible granularity against a milky background.
- ++ *reaction* - coarse granularity against a milky background.
- +++ *reaction* - heavy clumping of particles around the periphery of the test oval against a clear background.

From the Thermo Scientific *Campylobacter* test, results were interpreted as follows;

Reaction with test latex	**Reaction with control latex**	**Interpretation**
+	-	Campylobacters present.
-	-	Campylobacters not present in sufficient numbers detected by the test.
+	+	Non-specific agglutination.

Appendix 1.3: Steps Followed in Performing the Antibiotic Susceptibility Test

Procedure for performing the antibiotic susceptibility test for the isolated *Campylobacter* sp. are as below:

i. A range of 3-5 isolated colonies from freshly incubated *Campylobacter* on blood agar were scooped or touched using a sterile inoculating loop.

ii. The scooped colonies were then suspended in 2 ml of sterile saline and vortexed while in the tube to create a smooth/uniform suspension.

iii. The uniform suspension was kept in a densitometer (DEN-1B McFarland densitometer) to measure for 0.5 McFarland turbidity.

iv. With the aid of a dropper, suspensions whose turbidity were too heavy were adjusted to a 0.5 McFarland standard by diluting with sterile saline and if suspension created was too light, it was adjusted by adding more organism to achieve the turbidity of a 0.5 McFarland standard.

v. Prepared suspensions were used not more than 5 minutes of preparation.

vi. A sterile swab was dipped into the inoculum tube and since the swab should not be dripping wet, it was rotated against the side of the tube (i.e. above the fluid level) using firm pressure to remove excess fluid.

vii. The Mueller-Hinton agar plates supplemented with 5% defibrinated sheep blood were inoculated dried by streaking the swab three times over the entire agar surface while rotating the plate approximately 60 degrees each time to ensure an even distribution of the inoculum.

viii. The plate was finally rimmed with the swab to pick up any excess liquid and swab discarded into an appropriate container.

ix. Inoculated plates were allowed to sit at room temperature for at least 3 to 5 minutes for the surface of the agar plate to dry before proceeding to the placement of the antibiotic disks.

x. The dried plates were filled with the antibiotic disks by carefully removing disks from the cartridge and adding disks one at a time to the agar plate using sterile forceps.

xi. Once all disks were in place, plates were covered and in inverted position incubated at 42 °C microaerophilically 24 hours.

xii. Readings or measurements and recordings of zone of inhibition were done after incubation.

Appendix 1.4: Approved Ethical Clearance From Tamale Teaching Hospital

TAMALE TEACHING HOSPITAL ETHICAL REVIEW COMMITTEE

In case of reply the number and date of this letter should be quoted

My Ref. NO:TTH/ERC/18
Your Ref. No:

Tamale Teaching Hospital
P O Box 16
Tamale
03720-22545/22483

3rd July 2018

Dr. Akosua Bonsu Karikari
University of Development Studies
Tamale

Dear Dr. Karikari,

ETHICS APPROVAL– ID NO: TTHERC/19/06/18/03.

The Tamale Teaching Hospital Ethical Review Committee (TTHERC) reviewed and approved your study protocol titled: **'Genetic Diversity of Campylobacter'** during the committee's full board meeting on 19th June 2018.

Please note that this approval is for a period of 12 months, beginning **4th July 2018 to 4th July 2019.** Any modification to your approved research protocol requires TTHERC approval.

You are required to report all serious adverse events related to the ERC within seven days verbally and fourteen days in writing and to submit a final report to the TTHERC at the end of the research project.

Please always quote the protocol identification number in all future correspondence in relation to this approved protocol.

Sincerely,

THE CHAIR
TAMALE TEACHING HOSPITAL
ETHICAL REVIEW COMMITTEE
NORTHERN REGION-GHANA

Dr. Paulina Tindana
TTH ERC Chairperson
Cc. Head, Research and Development, Tamale Teaching Hospital Northern Region - Ghana

Appendix 1.5: *Campylobacter* Isolates` Multidrug Resistant Levels in Poultry and Humans According to Number of Antibiotics

Of the total 105 strains of *Campylobacter*, 101 were multidrug resistant. In overall, resistance to 11 of the 12 antibiotics used was observed, the highest frequencies of 21 strains each were resistant to 4 and/or 5 of the antibiotics were revealed. In poultry, a highest of 16 strains were resistant to 5 antibiotics while 6 strains each were resistant to 4 and 6 antibiotics, respectively in humans (Table A).

Table A: Multidrug Resistant Levels in Poultry and Human Sources

SN	NUMBER OF ANTIBIOTICS	POULTRY (N=71)	HUMANS (N=30)	TOTAL (N=101)
1	Resistance to 3 antibiotics	10 (14.08%)	0	10 (9.9%)
2	Resistance to 4 antibiotics	15 (21.13%)	6 (20%)	21 (20.8%)
3	Resistance to 5 antibiotics	16 (22.54%)	5 (16.7%)	21 (20.8%)
4	Resistance to 6 antibiotics	14 (19.72%)	6 (20%)	20 (19.8%)
5	Resistance to 7 antibiotics	8 (11.27%)	3 (10%)	11 (10.9%)
6	Resistance to 8 antibiotics	5 (7.04%)	5 (16.7%)	10 (9.9%)
7	Resistance to 9 antibiotics	3 (4.22%)	2 (6.7%)	5 (4.9%)
8	Resistance to 10 antibiotics	0	2 (6.7%)	2 (2%)
9	Resistance to 11 antibiotics	0	1 (3.2%)	1 (1%)
	TOTAL	71 (100%)	30 (100%)	101 (100%)

Values in bracket indicate percentage.

Key; N=Number of multidrug isolates

In poultry sub-sources, the study showed one strain of commercial source that was resistant to 9 of the antibiotics used while 2 strains were resistant to the same maximum number of antibiotics in domestic/household source. However, most strains (11 strains each) were resistant to 5 and/or 6 antibiotics in commercial while a maximum of 7 strains were resistant to 4 of the antibiotics used (Table B).

Also, in the human sub-sources, one strain from the hospital sub-source resisted 11 of the antibiotics used while a maximum of 9 antibiotics were also resisted by one strain in domestic/household sub-source (Table B).

Table B: Multidrug Resistant Levels in Poultry and Human Sub-Sources

	MULTIDRUG	POULTRY		HUMANS	
SN	Number Of Antibiotics	Commercial (N=47)	Domestic (N=24)	Hospital (N=20)	Households (N=10)
1	Resistance to 3 antibiotics	7 (14.89%)	3 (12.50%)	0	0
2	Resistance to 4 antibiotics	8 (17.03%)	7 (29.20%)	4 (20%)	2 (20%)
3	Resistance to 5 antibiotics	11 (23.40%)	5 (20.80%)	2 (10%)	3 (30%)
4	Resistance to 6 antibiotics	11 (23.40%)	3 (12.50%)	3 (15%)	3 (30%)
5	Resistance to 7 antibiotics	5 (10.64%)	3 (12.50%)	3 (15%)	0
6	Resistance to 8 antibiotics	4 (8.51%)	1 (4.20%)	4 (20%)	1 (10%)
7	Resistance to 9 antibiotics	1 (2.13%)	2 (8.30%)	1 (5%)	1 (10%)
8	Resistance to 10 antibiotics	0	0	2 (10%)	0
9	Resistance to 11 antibiotics	0	0	1 (5%)	0
	TOTAL	47 (100%)	24 (100%)	20 (100%)	10 (100%)

Values in bracket indicate percentage.

Key; N=Number of multidrug isolates

Of the 72 *C. jejuni* strains, 68 (94.4%) were multidrug resistant. With the 68 multidrug resistant isolates in general, *C. jejuni* biotype-I recorded the highest rate of 42.6% (29 strains) followed by 39.7% (27 strains) of biotype-II, 14.7% (10 strains) of biotype-III and least of 2.9% (2 strains) of biotype-IV (Table C).

Table C: Multidrug Resistant Levels in the Various *C. jejuni* Biotypes of Poultry and Human Sources

SN	BIOTYPES OF *C. JEJUNI*	POULTRY (N=54)	HUMANS (N=14)	TOTAL (N=68)
1	Biotype-I	19 (35.2%)	10 (71.4%)	29 (42.6%)
2	Biotype-II	26 (48.1%)	1 (7.2%)	27 (39.7%)
3	Biotype-III	7 (13%)	3 (21.4%)	10 (14.7%)
4	Biotype-IV	2 (3.7%)	0	2 (2.9%)
	TOTAL	54 (100%)	14 (100%)	68 (100%)

Values in bracket indicate percentage.

Key; N=Number of multidrug isolates