PREVALENCE AND MOLECULAR DETECTION OF *CAMPYLOBACTER* SPECIES AMONG DIARRHOEIC HIV-PATIENTS IN SOME HOSPITALS IN KADUNA STATE, NIGERIA

BY

ERNEST OSAMUDIAMEN OGBOMON

DEPARTMENT OF MICROBIOLOGY,
FACULTY OF LIFE SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

APRIL, 2019

PREVALENCE AND MOLECULAR DETECTION OF *CAMPYLOBACTER* SPECIES AMONG DIARRHOEIC HIV-PATIENTS IN SOME HOSPITALS IN KADUNA STATE, NIGERIA

BY

Ernest Osamudiamen OGBOMON
B.Sc. (DELSU) 2011
P15SCMC8025

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY, ZARIA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) DEGREE IN MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY,
FACULTY OF LIFE SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

APRIL, 2019

DECLARATION

I declare that the work in this dissertation entitled, "PREVALENCE AND MOLECULAR DETECTION OF *CAMPYLOBACTER* SPECIES AMONG DIARRHOEIC HIV-PATIENTS IN SOME HOSPITALS IN KADUNA STATE, NIGERIA" has been performed by me in the Department of Microbiology. The information derived from the literature have been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or-any other Institution.

OGBOMON ERNEST OSAMUDIAMEN _____ _____
Signature Date

CERTIFICATION

This dissertation entitled "PREVALENCE AND MOLECULAR DETECTION OF *CAMPYLOBACTER* SPECIES AMONG DIARRHOEIC HIV-PATIENTS IN SOME HOSPITALS IN KADUNA STATE, NIGERIA" by ERNEST OSAMUDIAMEN OGBOMON meets the regulations governing the award of the degree of Master of Science in Microbiology of the Ahmadu Bello University, and is approved for its contributions to knowledge and literary presentation.

Prof. C.M.Z. Whong Signature _____ Date _____
Chairman, Supervisory Committee

Dr M.H.I. Doko Signature _____ Date _____
Member, Supervisory Committee

Prof. I.O. Abdullahi Signature _____ Date _____
Head, Department of Microbiology

Prof. S. Z. Abubakar Signature _____ Date _____
Dean, School of Postgraduate Studies

DEDICATION

This work is dedicated to the Almighty God, for His Grace and Strength upon me throughout the programme, and to my parents for their love and encouragement that kept me going.

ACKNOWLEDGEMENTS

I thank, God who is my redeemer, the Source and Supplier of all knowledge, the Omnipotent One, the Father and Lord of all creation for the special grace and wisdom to write this dissertation.

This work is a synergistic product of many persons. I am grateful to all who contributed in one way or the other that led to the success of this dissertation.

My immense gratitude goes to my supervisors, Prof. C.M.Z. Whong and Dr M.H.I. Doko for their tremendous support, in guidance during the research, and reading and correction of all the write-up.

I am most grateful to Prof. I.O. Abdullahi, Head, Department of Microbiology, Ahmadu Bello University and the entire teaching and non-teaching staff of the Department for their various contributions to the successful completion of this work.

My sincere thanks go to Dr E.E. Ella, Department of Microbiology, Ahmadu Bello University, Zaria for his support and constructive contributions all through the research work and the entire staff of DNA LAB, Kaduna, for their tireless effort and hospitality during my molecular work.

I remain grateful to the Doctors, Nurses, Laboratory Scientists and Technicians of National Tuberculosis and Leprosy Training Centre (NTBLC), Saye, Hajia Gambo Sawaba General Hospitals (HGSGH), Zaria city, Dr Gwamna Awan General Hospital, Kakuri, General Hospital, Sabon and General Hospital, Kachia for their assistance during the collection of samples for the study. May God reward you all! Amen.

I am sincerely grateful to all the patients who volunteered and consented to giving their samples for this work.

My wonderful parents, Mr and Mrs Joseph Ogbomon, my siblings for their patience, understanding and support during this work, I say a very big thank you. God bless you all!

I do not forget the entire member of the Deeper Life Campus Fellowship (Postgraduate and Staff Arm) for their prayers, encouragement and love during the period of this research work and my friend, Stephen Otu, who was always behind me to make sure this work is a success.

I appreciate the following people that were always there for me whenever I call on them; Bitrus Barde, Precious Rufus, Queen Imade and lastly, my mentor, Aaron Ogenyi.

ABSTRACT

Campylobacter diarrhoea is regarded as the most common type of bacterial diarrhoea worldwide but the disease burden among diarrhoeic HIV-patients in Kaduna State, Nigeria had not been ascertained. This study is aimed at determining the prevalence of *Campylobacter* species among diarrhoeic HIV-patients in some hospitals in Kaduna State, Nigeria and evaluates their antibiotic susceptibility patterns. A total of 230 faecal samples were collected and analysed for *Campylobacter* species using membrane filtration method, characterised based on morphological appearance and biochemical testing using standard methods. Positive isolates were further analysed by API Campy kits® (bioMèrieux, France) to identify the isolates to species level. Polymerase Chain Reaction (PCR) targeting the IpxA gene, 23S rDNA and napA gene were used to confirm the isolates. The demographic and risks factors were evaluated respectively, using questionnaires. Susceptibility profiles of the species were carried out using the modified Kirby-Bauer disc diffusion method. Out of the 230 faecal samples that were collected and cultured for *Campylobacter*, 45 samples were culture positive giving a prevalence of 19.6%. The *Campylobacter* species identified were *C. jejuni, C. coli, C. fetus* and *C. hyointestinalis. Campylobacter coli* were mostly isolated with isolation rate of 44.4%, followed by *C. jejuni* (33.3%), *C. fetus* (15.6%) and *C. hyointestinalis* (6.7%). All the isolates produced the expected bands ((757bp, 746bp, 656bp, 835bp) during the PCR confirmation. Age and Sex distribution of campylobacteriosis was established. The prevalence was higher among female (23.9%) than male (13.0%) and the difference was statistically significant ($p = 0.042$). All the risk factors considered such as source of drinking water, consumption of raw milk, eating of under-cooked meat, contact with farm animals in the study were not found to be associated with the disease condition. All the *Campylobacter* species were highly susceptible to imipenem (100%) but showed varying sensitivity to gentamicin, azithromycin and ciprofloxacin but were resistant to erythromycin and the β-lactams (amoxicillin and oxacillin). In this study, all the isolates had Multiple Antibiotic Resistance (MAR) indices greater than 0.2 which is of public health importance.The prevalence of this bacterium among the study population is relatively high. Studies are needed to reassess the effect of Zidovudine with this high prevalence since the risk factors were not statistically significant in this study. The use of antibiotic as prophylaxis against human campylobacteriosis should be discouraged in the management of bacterial diarrhoea among the populace.

TABLE OF CONTENTS

LIST OF TABLES

Table Number **Page**

LIST OF FIGURES

LIST OF PLATES

Plate **Page**

LIST OF APPENDICES

Appendix **Page**

ABBREVIATION

AFLP Amplified Fragment Length Polymorphism

AML Amoxicillin

API CAMPY Appareils et Procedes d'Identification *Campylobacter*

AZM Azithromycin

CIP Ciprofloxacin

CN Gentamicin

DGAGH Dr Gwamna Awan General Hospital, Kakuri

E Erythromycin

GHS General Hospital, Sabo-Tesha

GHK General Hospital, Kachia

HGSGH Haij Gambo Sawaba General Hospital, Zaria city

HIV Human Immunodeficiency Virus

IPM Imipenem

MLST Multi-locus Sequence Typing

NA Nalixidic acid

NTBLTC National Tuberculosis and Leprosy Training Centre

OX Oxacillin

PCR Polymerase Chain Reaction

PFGE Pulsed-Field Gel-Electrophoresis

RAPD Randomly Amplified Polymorphic DNA

RFLP Restriction Fragment Length Polymorphism

SNPD Single-Nucleotide Polymorphism Detection

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Campylobacter is a member of the family Campylobacteraceae that causes campylobacteriosis, a food borne disease that is associated with acute diarrhoea in both immunosuppressed and immunocompromised individuals and is of great public health concern (Gwimi *et al.,* 2015). It is a Gram-negative, small, spiral curved rod measuring 0.2-0.5µm long with one polar unsheathed flagellum and is the leading cause of bacterial diarrhoea in both healthy population and HIV patients (Karikari *et al.,* 2017). This organism occurs as commensal in humans and livestock, but has been found in large numbers in the stool of both HIV infected patients with diarrhoea as well as others with debilitating and wasting disease syndrome (Samie *et al*., 2011).

Campylobacteriosis, the collective name for the disease caused by *Campylobacter* species is a zoonotic disease that affects humans and is manifested by diarrhoea with or without blood, headache, abdominal pain, vomiting, nausea and fever (Uaboi-Egbenni, 2008), and usually bacteremia in HIV infected patients (Fernandez-Cruz *et al*., 2010). Infections with *Campylobacter* are usually sporadic and are associated with eating or handling undercooked poultry, consumption of unpasteurized milk or contamination with water. The occurrence of *Campylobacter* species in locally pasteurized milk may be due to cross contamination of the milk by the milk producers, use of contaminated water during the preparation, unhygienic milking conditions and post-pasteurization contamination (Wysok *et al.,* 2011).

Campylobacteriosis has claimed over 33 million lives since the outbreak of HIV/AIDS and due to this alarming death rate, interests in the study of the potency of *Campylobacter* species

in diarrhoea has increased in both developed and under developed countries (Man, 2011). The occurrence of diarrhoea complicates and secures the advancement of HIV infection to AIDS. A decrease in size of the body and organ is a common complication with HIV diarrhoeic patients in Africa (Rossit *et al.,* 2009).

Diarrhoea, which is the passage of unformed or watery stool at least twice daily, often with enteric symptoms is the leading cause of hospital visits among people with poor hygienic practice (Opintan *et al.*, 2010). In spite of improvements in sanitation, and hygiene over the years, diarrhoea remains one of the leading causes of death in the globe (Kosek *et al*., 2002). Diarrhoea is usually self-limiting in immunocompetent patients, and so, treatment can be by mere rehydration solution with antibiotics. But, it can become chronic and life threatening in immunocompromised host especially in HIV infected patients. The cause of diarrhoea in HIV infected patients is multifactorial, but multiple infectious pathogens have been reported as the cause of diarrhoea and these include bacteria, viruses, fungi and parasites; however, the etiology varies with geographical regions (Rossit *et al.*, 2009).

Diarrhoea is a common disease associated with countless numbers of pathogens and is a great concern among HIV/AIDS patients because these people are susceptible to both classical and opportunistic pathogens (Rossit *et al.,* 2009). MacArthur and DoPont (2012) reported that HIV seropositive patients with diarrhoea while receiving Anti-retroviral Therapy (ART) have a significantly negative Health Related Quality of Life (HRQOL) with reduced treatment efficacy and adherence. The adverse effect of ARTs, vitamin deficiencies, AIDS-associated malignancy, effect of prophylactic antibiotic and many other factors have made diarrhoea a major problem in HIV infection by causing damage to normal gastrointestinal flora, thereby paving way for more opportunistic infection as well as emergence of new multi-drug resistant strains. Thus with the occurrence of ART-associated diarrhoea, gastrointestinal damage

related to HIV infection and occurrence of antimicrobial resistance, the incidence of *Campylobacter*-related diarrhoea has to be readdressed (MacArthur and DuPont, 2012). In addition, understanding the causative agents of diarrhoeal disease is important in developing treatment for people with this debilitating syndrome (Dikman *et al*., 2015).

Campylobacter species which are implicated as gastrointestinal pathogens in HIV infected patients include *Campylobacter jejuni, C. coli, C. lari*, C. *upsaliensis, C. hyointestinalis, C. fetus, C. sputorum, C. mucosalis, C. concisus*, though some are rare (Moore *et al*., 2005). These bacteria are microaerophilic, and are sensitive to light and desiccation. HIV diarrhoeic patients have prolonged, recurring and severe *Campylobacter* infection with bacteremia and this has contributed to the multidrug antibiotic resistance in treatment of bacterial infection among these patients (Fernandez-Cruz *et al.,* 2010).

Campylobacter infection is the major bacterial opportunistic infection in HIV-infected patients and death is usually due to the complications such as bacteremia, hepatitis, meningitis, and arthritis, brain abscess, soft tissue infection, Guillain-Barr syndrome and Miller Fisher syndrome which are associated with the diarrhoea (Kaakoush *et al*., 2015).

Cultural methods have been used commonly in most developing countries for the detection of this bacterium, but the major challenges are its limited sensitivity and lack of specificity (Samie *et al*., 2011). This is because, *Campylobacter* is biochemically inert and discrimination between two species may depend on one biochemical test. However, the advent of Rapid test identification kits and molecular techniques which have acceptable levels of specificity and sensitivity have made *Campylobacter* detection and characterization easier and efficient from faecal samples (Louwen *et al.,* 2012*).*

1.2 Statement of Research Problem

HIV infection is an epidemic that is occurring worldwide and is of great concern to public health. The outbreak of this disease over the last three decades had caused panic in different sectors affecting people of different socio-cultural background. Recently, AIDS has become the greatest threat to human existence, claiming over 25 million lives since its discovery in the 1980s (Samie *et al*., 2011). It has been reported that over 36.9 million people all over the world are living with HIV/AIDS as at 2014 with sub-Saharan Africa being the most affected region with over 24 million (Awofala and Ogundele, 2016). Nigeria has about 3.2 million people living with HIV/AIDS, with a prevalence of 3.4% between 2000 and 2014 (Burlew *et al.*, 2014; Adesiji and Oloke, 2015). Kaduna State is among the most affected states in Nigeria with prevalence of 8.1% as at 2015 (NACA, 2016). Sub-Sahara Africa countries such as Cote d' Ivoire, Cameroon, Senegal, Zambia, and Nigeria have developed measures with aid from international bodies to prevent and lower the spread of this infection (USAID, 2014). However, despite the measures implemented to control this disease, as at 2013, a total of 215,000 new cases were reported in Nigeria (USAID, 2014) and asymptomatic patients were implicated in the spread of these new cases. Because of the high prevalence in Kaduna State, the Government has initiated ways to fight against the spread through Kaduna State Agency for the Control of AIDS (KADSACA) together with National Agency for the Control of AIDS (NACA). However, the spread is still noticed on the life and communities in the State (NACA, 2016).

The major implication of HIV-infection is its destructive effect on the immune system thereby exposing the individual to multiple opportunistic infections. Gastrointestinal diseases have been shown to be the major opportunistic infections in patients with HIV/AIDS and diarrhoea is reported in over 90% of these patients in both developed and under developed countries

(Rossit *et al.,* 2009; Silva *et al*., 2010). HIV/AIDS is the leading cause of death worldwide, and the death rate due to chronic diarrhoea is strongly marked among these patients (Bradshaw *et al.,* 2005).

Campylobacter species are the leading causes of bacterial diarrhoea among HIV/AIDS patients (Tee and Mijch, 1998), but their role in diarrhoea was not known for years due to unavailability of medium used for their growth. The implication of *Campylobacter* species in diarrhoeic HIV patients is not clear, despite the facts that these organisms connote a very important risk factor among this populace (Adesiji and Oloke, 2015). However, it is believed that HIV/AIDS can increase the number of cases of campylobacteriosis in most countries. This observation further supports the need for improved understanding of the epidemiology of campylobacteriosis in these countries (Coker *et al.,* 2002). With the development of selective medium and molecular methods to detect *Campylobacter*, clinicians still face challenges because of their special growth condition. Most *Campylobacter* known to cause diarrhoea do not grow in the available selective medium. These bacteria are highly fastidious with special culture requirement and their growth can be inhibited by exposure of the stool sample to high oxygen content as well as antimicrobial content of the medium used for their isolation (Silver *et al.,* 2011).

Treatment of *Campylobacter* infection in immunocompromised individuals such as HIV infected patients and others with debilitating syndrome has become a challenge due to the occurrence of multidrug resistant strains (Larson and Spickler, 2013). Resistance to fluoroquinolones and tetracycline are most common with emerging resistance to macrolides with no availability of vaccine. Administration of antimotility drugs to these set of people is usually wrong since it has been associated with prolonged symptoms and fatalities (Larson and Spickler, 2013).

1.3 Justification

Since the outbreak of HIV/AIDS, it had become important to observe the distribution of gastrointestinal pathogens such as *Campylobacter* species among HIV seropositive individual (Samie *et al.,* 2011). This is because, this set of people are at a great risk of invasive campylobacteriosis more than those in the general population. Most screening for the causative agents of diarrhoea among HIV infected populace does not include *Campylobacter*. In the proper management of HIV- associated diarrhoea, a key agent such as *Campylobacter* cannot be ignored (Karikari *et al*., 2017). Demographic and occupational risk factors had been reported to be predisposing factors of campylobacteriosis, but these factors had not been studied intensively as a determinant of this disease among diarrhoeic HIV-patients (Chia-ping *et al*., 2017).

Campylobacter species are biochemically inert and discrimination between two species may depend on one biochemical test. Selective medium used for the isolation of *Campylobacter* species inhibits the growth of certain species of this bacterium; hence there is need for an alternative means of isolation such as the use of special culture techniques (membrane filtration method) coupled with genetic characterization (Snijders *et al.,* 1997).

Antibiotics such as erythromycin and gentamicin have been proven to be effective in treatment of campylobacteriosis. However, antibiotic resistance is an overgrowing problem and there is a need to monitor the susceptibility of this bacterium to drugs used in the study area in order to provide guidelines for the empirical treatment of campylobacteriosis (Samie *et al*., 2011).

Studies have shown the efficacy of Crofelemer, a new anti-diarrheic agent used in the treatment of diarrhoea in HIV- infected patients but with increasing antimicrobial resistance, there is a need to evaluate the efficacy of other medication that could be used in combination with this agent against *Campylobacter* diarrhoea among this populace (Crutchley *et al*., 2010).

A study in Nigeria was done in Lagos where a prevalence of 7.0% among HIV patients with diarrhoea was reported using a *Campylobacter* selective medium (Smith *et al.,* 2005).

However, there is a dearth of information on the prevalence of *Campylobacter* species among HIV- infected patients in Kaduna State.

1.4 Aim

The aim of this research was to determine the prevalence of *Campylobacter* species among diarrhoeic HIV- patients in some Hospitals in Kaduna State, Nigeria.

1.5 Specific Objectives

Specific objectives of this research were to:

1.) isolate and characterize *Campylobacter* species from stool samples of HIV-infected patients using conventional method and API Campy kits.

2.) determine some predisposing factors associated with campylobacteriosis among the HIV- infected patients using structured questionnaire.

3.) confirm the *Campylobacter* species by detecting some species-specific genes (IpxA, 23S rDNA and napA gene) using Polymerase Chain Reaction.

4.) determine the antibiotic susceptibility patterns of the various *Campylobacter* isolates.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of *Campylobacter*

The genus *Campylobacter* is believed to have been discovered by Theodore Escherich in 1886, when he observed and described it as a non-culturable spiral-shaped bacterium (Silva *et al.,* 2011). The first identification of this bacterium was done in 1906 when two British veterinarians reported its presence in the uterine mucus of pregnant sheep (Silva *et al.,* 2011). In 1913, Mcfadyean and Stockman successfully isolated this *Vibrio-like* organism from aborted bovine fetus (Debruyne *et al*., 2008; King and Adams, 2008). The name *Vibrio jejuni* was used to refer to a group of bacteria that was isolated from diarrhoeic cattle by Smith and Orcutt in 1919 (Silva *et al*., 2011). Years after, another group of this bacterium was isolated from diarrhoeic pigs by Doyle in 1944, and was named *Vibrio coli* (Vandamme, 2000; Silva *et al.,* 2011). Due to the successful isolation of this organism from blood cultures of women who aborted, as well as diarrhoeic patients, interest in its study became high (Debruyne *et al*., 2008). The genus *Campylobacter* was defined in 1963 by Sebald and Veron after considering their unique characteristics such as low DNA base composition, microaerophilic growth requirement and non-fermentative metabolism, to distinguish it from true *Vibrio* species (On, 2001).

2.2 Taxonomy and Characteristics of the genus *Campylobacter*

Campylobacter species belong to the family Campylobacteraceae of the epsilon subdivision of proteobacteria (Debruyne *et al*. 2008). This family consists of two genera, *Campylobacter* and *Arcobacter* which occur mainly as a commensal in both humans and animals (Silva *et al.,* 2011). The genus *Campylobacter* comprises of over 20 species and 6 subspecies. These

include *Campylobacter jejuni ssp jejuni, ssp doylei, C. coli, C. lari, C. upsaliensis, C. fetus ssp fetus, spp venerealis, C. hyointestinalis ssp hyointestinalis, C. mucosalis, C. concisus C. curvus, C. rectus, C. gracilis, C. showae, C. insulanigrae* and *C. sputorum* which are associated with human gastroenteritis. However the pathogenic roles of some of the species in human gastroenteritis are not well elucidated (Chua *et al.,* 2007). *Campylobacter* species that are not known to be associated with human gastroenteritis are *Campylobacter ovium, C. ureolyticus, C. troglodytic, C. Canadensis, C. cuniculorum, C. subantarticus, C. hominis, C. volucis* and *C. helveticus*.

Campylobacter species are zoonotic pathogens, having their primary reservoirs in animals. For example, *Campylobacter upsaliensis* was first isolated from dogs (Humphries and Linscott, 2015), *Campylobacter ovium* from poultry (Rossi *et al.,* 2009), *Campylobacter troglodytis* from chimpanzees (Kaur *et al.,* 2011), *Campylobacter cuniculorum* from rabbits (Zanoni *et al.,* 2009), *Campylobacter pylori* from molluscs (Debruyne *et al*., 2009) while *Campylobacter subantarticus* was first isolated from birds (Debruyne *et al.,* 2010). However, periodontal cavity of human serves as reservoir for *Campylobacter rectus, C. curvus, C. showae* and *C. concisus* (Lastovica and Allos, 2008).

Campylobacter species are curved, spiral-shaped rods measuring 0.2 – 0.9µm wide and 0.5 - 5µm long. *Campylobacter* species can easily transform from spiral shape to being coccoid in an ageing culture (Griffiths, 1993). They are Gram-negative, non-spore forming rods with one polar unsheathed flagellum used for its motility in a corkscrew-like manner except *Campylobacter showae* that have multiple flagella (Debruyne *et al.,* 2005). Most species have activity on oxidase reagent except *Campylobacter gracilis* (Silva *et al*., 2008). They do not ferment carbohydrate but obtain their energy from amino acids and-or intermediate of tricarboxylic acid cycle (Vandamme, 2000; Silva *et al.,* 2011).

Most *Campylobacter* species are able to grow between 37ºC and 42ºC but cannot grow below 30ºC because they do not have the cold shock protein gene for low- temperature adaptation, and are referred to as thermotolerant not thermophilic since they cannot grow at 55ºC or above (Levin, 2007). This inability of this bacterium to grow in a low temperature reduces its ability to multiply outside human host and in food packaging and processing (Silva *et al.*, 2011). This bacterium cannot grow in an environment of water activity lower than 0.987 and are easily inactivated by heat treatment and light with D-value less than 1 (Silva *et al*., 2011). Freezing and thawing easily inactivate these bacteria but cannot eliminate them from contaminated substrate; however, their cultures are able to survival at 4ºC for a long time (Hazeleger *et al.,* 1995). They are pH sensitive, and thus, cannot grow at pH less than 4.9 and pH above 9.0 with optimum pH requirement at 6.5 – 7.5. These non-spore forming and fastidious bacteria are strictly microaerophilic and require a condition of low oxygen content (6% O_2, 10% CO_2 and 84% N_2) for their growth (Garenaux *et al.,* 2008; Silva *et al.*, 2011).

2.3 *Campylobacter* species found in Diarrhoeic HIV-patients

Campylobacter species known to cause disease in humans include *Campylobacter fetus, C. jejuni, C. hyointestinalis, C. coli, C. upsaliensis, C. lari, C. mucosalis, C. sputorum* and *C. concisus,* though some are rare (Moore *et al.*, 2005).

2.3.1 *Campylobacter fetus*

The strains of *Campylobacter fetus* are divided into two subspecies, *C. fetus* subsp *fetus* and *C. fetus* subsp *venerealis* based on their habitat, biological properties and the diseases they cause, although, they have the same G+C content, closely related in DNA-DNA hybridization test and show agglutination in an antiserum against the same serotype (Penner, 1988). They are susceptible to cephalothin but resistant to nalixidic acid and can grow at both 37 ºC and

42ºC. They are found in the intestinal flora of domestic animals and humans. It enters the intestine through ingestion of food and-or water contaminated with faeces containing the bacteria. Human infection caused by this bacterium is usually septicaemia in immunocompromised or predisposed patients (Penner, 1988). Other complications that may be associated with the disease include meningitis, pericarditis, arthritis, peritonitis. It is reported that strain of *C. fetus* has been implicated as cause of premature labour and neonatal sepsis in human (Penner, 1988).

2.3.2 *Campylobacter hyointestinalis*

They are found in the intestine and are closely related to *C. fetus* having the same DNA hybridization but are differentiated from *C. fetus* by their ability to produce hydrogen sulphide (H_2S) in Triple Sugar Iron agar (TSI) (Penner, 1988). They are resistant to nalixidic acid but susceptible to cephalothin and are capable of growing at 37 ºC.

2.3.3 Thermotolerant *Campylobacter* species

They are have optimum growth temperature at 42 ºC and are the most predominant *Campylobacter* species as agents of human gastroenteritis. It comprises of four species, namely, *Campylobacter coli, C.lari, C.upsaliensis* and *C.jejuni*. They are closely related in both DNA hybridization and pathogenesis. *Campylobacter jejuni* is the most infectious agent of human in this group and are differentiated by its ability to hydrolyse Hippurate. The hippuricase enzymes hydrolyse Hippurate to benzoic acid and glycine, and glycine is detected with a solution of ninhydrin added to the test tube (Penner, 1988). Complications associated with diarrhoea caused by *Campylobacter jejuni* are brain abscess, soft tissue infections, Guillain-Barre syndrome and Miller Fisher syndrome. Strains of *Campylobacter jejuni* are divided into *C. jejuni* subsp *jejuni* and *C. jejuni* subsp *doylei*.

Campylobacter lari are strains of thermotolerant *Campylobacter* that are nalixidic acid resistant, and this resistance is acquired due to previous exposure of the patients to norfloxacin (Penner, 1988). They are capable of anaerobic growth in the presence of 0.1% Trimethylamine N- oxide hydrochloride (TMAO) and production of H_2S in a TSI (Penner, 1988). Human gastroenteritis caused by *C. lari* is usually associated with bacteremia among elderly and immunocompromised individuals.

Campylobacter upsaliensis are strains of thermotolerant *Campylobacter* that are highly implicated as causes of diarrhoea in dogs and children (Penner, 1988). They produce weak or negative catalase reaction. Successful isolation of this bacterium requires the use of membrane filtration technique because their growths are inhibited by the antimicrobial content of most *Campylobacter* selective medium (Lastovica and Roux, 2000).

2.3.4 *Campylobacter sputorum*

These species of *Campylobacter* are able to produce H_2S in a TSI, grow in 1% glycine, and at 37ºC but not at 25ºC. It has three biovars namely; *Campylobacter sputorum* biovar *sputorum*, *C. sputorum* biovar *bubulum and C. sputorum* biovar *fecalis.*

Campylobacter sputorum biovar *sputorum* are responsible for infant diarrhoea and soft tissue abscess in elderly people. They can be differentiated from other *C. sputorum* biovars by their ability to grow in 1% bile and their inability to grow in 3.5% NaCL or 0.1% TMAO (Penner, 1988). Membrane filtration methods are used for successful isolation of this strain from faecal sample.

Campylobacter sputorum biovar *bubulum* occurs as commensals but are normally found in the preputial cavity of man and the genital tract of female (Penner, 1988). *Campylobacter sputorum* biovar *fecalis* is not associated with human infection (Penner, 1988).

2.3.5 *Campylobacter mucosalis*

They are species of *Campylobacter* that require hydrogen or formate (under anaerobic condition) as electron donor and oxygen (under microaerophilic condition) as electron acceptor for their growth (Lastovica *et al*., 2006). They are susceptible to cephalothin; produces dirty yellow colonies and can grow at 37ºC (Penner, 1988). They are associated with human gastroenteritis among immunocompromised patients (Snijders *et al*., 1997).

2.3.6 *Campylobacter concisus*

They are catalase-negative, and require hydrogen or formate as energy source. *Campylobacter concisus* do not form pigmented colonies, cannot grow at 25ºC and are resistant to cephalothin (Penner, 1988). They are responsible for human periodontal diseases (Penner, 1988).

2.4 Epidemiology of *Campylobacter* infection

There is a global rise in the incidence of campylobacteriosis in the past decades (Kaakoush et *al.,* 2015). These incidence and number of cases varies from regions and even within the same country. This variation could be due to sensitivity of methodology, area population, and differences in surveillance bias, food practices and availability of natural reservoirs of *Campylobacter* species in these regions. *Campylobacter* is the leading cause of traveller diarrhoea from 2004 to 2009 accounting for 41.7% of cases (3,445 of 8,270 cases reported), followed by *Salmonella* (36.7%) and 13.0% for *Shigella* (Corry *et al*., 1995). Season affect the incidence of *Campylobacter* infection with the highest number of cases reported during the summer and this seasonal variation may be due to different ecological event. The incidence has a bimodal age distribution with the highest number occurring in children younger than 5 years and at young adult between 20-40 years (Samuel *et al.,* 2006).

The host immune response against an infection within a population can guide against transmission of diseases for an exposed individuals. This population-level immunity is known to be unique on the epidemiology and risk assessment of campylobacteriosis (Havelaar *et al*., 2005). In most countries where *Campylobacter* infection is endemic, infection is usually high among children but the incidence decreases with age, suggesting that exposure in early life might lead to development of protective immunity (Rao *et al.,* 2001). The asymptomatic excretion of this bacterium could have an impact on the transmission of campylobacteriosis in those countries. Outbreak of campylobacteriosis is usually sporadic. Consumption of poultry products or water has been implicated in most outbreak of this disease (Kaakoush *et al*., 2015).

In the United States, the annual number of campylobacteriosis cases, based on 10 years of outbreak data (1998 to 2008), was estimated to be 845,024 cases, resulting in 8,463 hospitalizations and 76 deaths, and this incidence has increased by 14% in 2012 compared to the 2006–2008 period, whereas the incidences of *Cryptosporidium*, *Listeria*, *Salmonella*, *Shigella*, Shiga-toxigenic *Escherichia coli* (STEC) O157, and *Yersinia* infections decreased over the same period (Batz *et al.,* 2012; Kaakoush *et al*., 2015). In Canada, it is reported that over 36% of patients with diarrhoea (31 May to 31 October 2005) were positive for campylobacteriosis (Inglis *et al*., 2011). Although, *Campylobacter* is a major pathogen in the United States and Canada, but its precise impact in regions of Central America is less clear (Kaakoush et al., 2015).

In the Europe, overall estimated cases of campylobacteriosis are about 9.2 million, compared to 6.2 million cases of salmonellosis, in 2009, although, there is compelling evidence that emerging *Campylobacter* species contribute to the etiology of gastroenteritis in this region

(Havelaar *et al*., 2013). In Africa, data from limited countries have indicated that *Campylobacter* infection is most prevalent in the pediatric population (Kaakoush *et al*., 2015).

2.5 Risk factors and Transmission *Campylobacter* infection

A number of risk factors contribute to the susceptibility of humans to campylobacteriosis. A recent meta-analysis revealed that international travel was the most important risk factor for campylobacteriosis, followed by consumption of undercooked chicken, consumption of raw and or contaminated water, environmental exposure, and direct contact with farm animals. In the overall burden of *Campylobacter* transmission, the transfer or spread of exotic or antibiotic-resistant strains to previously unexposed populations is a further concern. In addition, travel-related infections are often associated with consumption of contaminated meat or water Kaakoush *et al*., 2015), suggesting that a complex interrelationship exists between risk factors. Consumption of contaminated food, particularly poultry products, unpasteurized milk, and water, is a risk factor for campylobacteriosis. One approach to estimate the source attribution of campylobacteriosis cases is through the analysis of outbreak data. As expected, *Campylobacter* infections are mainly attributed to poultry products by using these techniques. More recently, however, molecular techniques, such as multi-locus sequence typing, *flaA* typing, and gyrase subunit A typing, have played a more significant role in source attribution, allowing further insights into the contributions of the different reservoirs to the burden of campylobacteriosis in humans and the subsequent transfer of *Campylobacter* species to wildlife through environmental contamination. Immunodeficiency is also a risk factor for campylobacteriosis (Kaakoush *et al*., 2015). HIV-infected patients presenting with diarrhoea are more frequently infected with *Campylobacter* than uninfected individuals with diarrhoea.

In addition, the incidence of *Campylobacter*-related illness among HIV-infected patients is higher than the incidence found in the general population (Coker et al., 2002).

2.6 Sources of *Campylobacter* species

Campylobacter species cause campylobacteriosis, which is a zoonotic disease. This organism is wide spread in nature and is found naturally in the intestinal tract of farm animals such as poultry, pigs, cattle and sheep, in pets, as well as in rodent and wild birds (Meerburg *et al.*, 2006). Poultry and poultry products have the highest prevalence of *Campylobacter* species. This is because, the intestinal tract of chicken can harbour large amount of *Campylobacter* species; during processing, may leak or rupture and the content transferred to the skin carcases (Berrang *et al.*, 2001; Silva *et al.*, 2011). The bacteria may remain in a fluid film in the skin and become entrapped into the cervices and channels (Chantarapanont *et al*., 2003), which provide a favourable environment for cross contamination (Silva *et al.*, 2011). The survival and persistence of *Campylobacter* species are fostered by a suitable microenvironment of the skin. Most campylobacteriosis cases in humans are sporadic, which complicates the identification of infection sources.

2.7 Pathogenesis of *Campylobacter* infection

The precise mechanism *Campylobacter* uses to cause infection is not well known. However, it is believed to involve flagella-mediated motility, adherence to intestinal mucosa and the ability to invade and produce toxins (Silva *et al.*, 2011). The production of lipo-polysaccharide aids this pathogen to enter and survive the gastric juice, bile salt and nonphagocytic cells. Cellular inflammation caused by the bacterial invasion is due to production of cytotoxins and then reducing the absorption capacity of the intestine (Van Deun *et al.*, 2007). Despite the use of flagella for colonization, it's role is important for the bacteria

survival at the different ecological niches it may encounter in the gastrointestinal tract (Jagannathan and Penn, 2005). *Campylobacter* flagella have two components, *fla*A and *fla*B coded by the flagellin genes (Silva *et al.,* 2011). The *fla*A gene is used for adherence, colonization and invasion of the host epithelial cells (Jain *et al.*, 2008). The flagellin genes are able to secrete non-flagellar proteins that may be associated with virulence such as O-linked glycosylated flagellin used for adherence to the epithelial cells.

Campylobacter produces two types of toxins; the Enterotoxins and the Cytotoxins. Enterotoxins are secreted proteins that have the ability to bind a cellular receptor, enter the cells and increase intracellular cyclic AMP level leading to production of watery stool (cAMP). This Enterotoxin called *Campylobacter* Cytotonic toxin (CCT) is similar to the Cytotonic toxin of *Vibrio cholerae* and the heat labile toxins of *Esherichia coli* (Wassenaar, 1997). It has two subunits, the larger A subunit which contains enzymatic activity and the smaller B subunit which binds to the host receptors. The cytotoxins are proteins that kill target cells. This toxin has two parts, the larger A subunits that contains enzymatic activity and the small B subunit that bind to the sphingoglycolipid receptors. *Campylobacter* have six different types of cytotoxins namely; 70-kDa Cytotoxins, Shiga-like toxins, Vero Cytotoxins, Cytolethal distending toxins (CDT), Haemolytic toxins and Hepato-toxins (Wassenaar, 1997). The 70-kDa Cytotoxins are active against HeLa, HEp2, CHO and MRC cells. These toxins are heat labile, trypsin sensitive and the expression of the toxins could be lost upon sub-culturing and long term storage of the strains (Wassenaar, 1997). The use of Anti-clostridium or anti-Stx antitoxins to neutralise this toxins are ineffective. The Vero Cytotoxins are active against both HeLa and Vero cells (Wassenaar, 1997). Like the 70-kDa Cytotoxins, Vero Cytotoxins are not neutralised by anti-clostridium or anti-Stx antitoxins. The Cytolethal distending toxins (CDT) are active against HeLa, Vero, HEp2 and CHO cells. The toxins

cause elevated extracellular cAMP level by extensive distension and deaths are determined by trypan blue sensitivity (Wassenaar, 1997). Antiserum raised by the Vero or HeLa cells cannot neutralise it's activity and the toxins are hydrophobic. Production of cytolethal distending toxins (CDT) is an important virulence factor of *Campylobacter* species. CDT holotoxin composed of three subunits encoded by the *cdt*A, *cdt*B and *cdt*C genes that causes the arresting of the eukaryotic cells at the G2/M phase of their life cycle thereby preventing them from entering mitosis and thus leads to the cell death (Silva *et al*., 2011). Hybridization test shows that CDT genes are present in all *Campylobacter* species but their expression varies among species (Wassenaar, 1997). For instance, *Campylobacter jejuni* strains are strongly related in their *cdtB* gene while other *Campylobacter* species show a weak relatedness during hybridization test. The Shiga-like toxins are cytotoxins that be neutralised by stx antitoxins and are identical to 70-kDa toxins. They are detected in the lysates of *Campylobacter* spp., and are active against HeLa cells. The Haemolytic cytotoxins are responsible for the occurrence of haemolysis observed in an ageing *Campylobacter* culture incubated at 42ºC for 4 days in a blood agar plate. This Haemolytic cytotoxins cause Alpha haemolysis or Beta haemolysis but the haemolysis could be cell-associated or contact haemolysis (Wassenaar, 1997). Alpha haemolysis are the typical green zone that occur around a colony within 48 hrs at 37ºC in a blood agar plate while Beta haemolysis are observed as area of clear zone that occur when the plate is incubated for 4 days and above (Wassenaar, 1997). In a contact haemolysin assay, a bacterial suspension is mixed with erythrocytes and centrifuged to enhance the contact between bacteria and cells, so that cell-bound haemolysin can be detected (Wassenaar, 1997). The Hepatotoxins are Cytotoxins that are able to induce the liver cells and cause hepatitis, the nature and mechanism of activity are not clear (Wassenaar, 1997).

2.7.1 Clinical features of *Campylobacter* infection

Campylobacter infection is manifested by diarrhoea with or without blood, headache, abdominal pain, vomiting, nausea and fever that lasted for several days to weeks (Uaboi-Egbenni, 2008), with infective dose of 5 -10 cells. The incubation period is 4 days but can vary from 20 hours to 6 days. The symptoms can be severe, mild or prolonged which can be self-limiting but relapsing occurs in over 7% of untreated patients. Complications such as bacteremia, hepatitis, meningitis, and arthritis, brain abscess, soft tissue infection, guillain-Barr syndrome and miller fisher syndrome are associated with the diarrhoea especially in elderly and compromised patients (Kaakoush *et al*., 2015). There is Formation of antibodies in the serum from the 5^{th} day of the diarrhoea, and decreases over months (Strid *et al*., 2001).

2.7.2 Complications associated with *Campylobacter* infection

In addition to gastrointestinal infection, *Campylobacter* species also cause a range of clinical manifestations in other parts of the body, as either a local isolated infection, a systemic manifestation after an episode of enteritis, or a post infectious immune disorder. These manifestations include Guillain-Barré syndrome, Miller Fisher syndrome, brain abscesses and meningitis, bacteremia, sepsis, endocarditis and myocarditis, reactive arthritis, and clinical manifestations that result in complications in the reproductive tract.

2.8 Clinical Microbiology of *Campylobacter* infections

2.8.1 Isolation methodologies in clinical settings

There is no gold standard or common method for the isolation of all *Campylobacter* species from clinical samples. The inability of *Campylobacter* species to strive efficiently in the presence of oxygen and some free radicals has led to the development of selective media containing oxygen scavengers and antimicrobial agents (Silva *et al*., 2011). Definitive

diagnosis is used to detect *Campylobacter* species from the faecal sample of patients suffering from gastrointestinal infection. The development of this protocol was well described by Corry *et al.,* (1995); however, the protocol is not the only single method for routine isolation of all *Campylobacter* species. This method of isolation involves incubating the sample at 42ºC in a selective media containing antimicrobial agent in a microaerophilic condition after pre-enrichment. Several selective media have been formulated and tested to be effective in the isolation of *Campylobacter* species. These include Preston, Charcoal Cefoperazone Deoxycholates agar (CCDA), Butzler agar, Skirrow agar and Karmali agar. However, the commonly used blood free selective agar (modified charcoal cefoperazone deoxycholates agar, (CCDA) with 32ng cefoperazone per litre) and incubation at 42ºC has been recommended for the isolation of most *Campylobacter* species from faecal samples including those species that are susceptible to many antimicrobial agents. Lastovica (2006) and Snijders *et al.,* (1997) reported and recommended that for isolation of a wide variety of *Campylobacter* species from human stool samples, a special culture technique such as membrane filtration should be used. This method involves the filtration of stools using filter paper of pore size 0.45 – 0.65μm centrally placed on the surface of antibiotic- free *Campylobacter* sheep blood agar plates and incubated in hydrogen enhanced microaerophilic condition at 37ºC in an anaerobic jar for 4 -7 days.

2.8.2 Laboratory diagnosis

Laboratory diagnosis of *Campylobacter* infection requires the use of culture-dependent and-or culture-independent methodologies. In culture-dependent methodologies, single isolated colonies can be subjected to a range of conventional biochemical tests to identify phenotypic traits. Temperature, incubation time, and atmospheric conditions, for example, an H_2-enriched atmosphere or growth at 42°C, can also be used to favour the growth of *Campylobacter*

species during isolation procedures. The biochemical profile of an unknown organism is then matched to previously defined characteristics of the *Campylobacter* genus or species to enable identification of the organism to the genus or species level. In culture-independent methodologies, DNA or RNA can be isolated from clinical samples or from a pure culture. A genetic signature or marker of the organism can then be determined using sequencing techniques or genus- or species-specific PCR amplification of the gene of interest, for example, the 16S rRNA gene. Culture-independent tests are increasingly being used for the detection of *Campylobacter* species, and while in many cases they can enhance detection sensitivity, it has been proposed that this will have an impact on public health surveillance given that detailed analyses of isolates are required to monitor the distribution of different strains (Kaakoush *et al*., 2015).

2.9 Identification of *Campylobacter* species

The combination of phenotypic and genotypic characterization must be applied for the correct identification of *Campylobacter* species and information obtained can be utilized for epidemiological studies and risk assessment. The phenotypic methods include both biochemical identification and typing while the genotypic method includes molecular identification and typing.

2.10 Phenotypic methods

2.10.1 Biochemical identification

Biochemical tests are applied by most clinical laboratories for identification of *Campylobacter* species. The relevant biochemical tests used to differentiate between members of the *Campylobacter* genus have been reviewed by Lastovica (2009). In that article, a biochemical flowchart to identify *Campylobacter* species is outlined, beginning with growth

with or without supplementation of H_2, followed by an indoxyl acetate test, a hippurate test, growth on MacConkey agar, an aryl sulfatase test, and production of H_2S. Furthermore, detection of L-alanine amino peptidase activity can be employed to differentiate between *Campylobacter*, *Helicobacter*, and *Arcobacter* species and other Gram-negative bacteria (Lastovica, 2009). However, this identification method is complicated because, *Campylobacter* species are biochemically inert and discrimination between two species may depend on one biochemical test (Table 2.1). The only differentiating test that distinguishes *Campylobacter jejuni* phenotypically from other *Campylobacter* species is the hippurate hydrolysis. This test was described by Hwang and Ederer, (Hwang and Ederer, 1980). Most *Campylobacter jejuni* strains are able to hydrolyze hippurate to glycine and benzoic acid, although hippurate negative *Campylobacter jejuni* strains have been reported (Kos *et al.*, 2006).

Martiny *et al.*, (2010) reported the use of matrix-assisted laser desorption ionization time-of-flight mass spectrometry to identify most *Campylobacter* species. This method offers good specificity and sensitivity and is able to correctly identify 100% of *Campylobacter* species in mixed culture more easily than the conventional methods.

API Campy kit (bioMèrieux, France), are a miniaturized identification system that uses 11 enzymatic and conventional tests, and 9 assimilation and inhibition tests. It correctly identified 94% of *Campylobacter jejuni* ssp. *jejuni*, *C. jejuni* ssp. *doylei* strains, 100% of *C. upsaliensis*, and 74% *C coli*; however, it has a lower level of identification for other *Campylobacter* species such as 60% for *C. lari*, 33% for *C. hyointestinalis* and 29% for *C. fetus* (Martiny *et al.*, 2010).

VITEK 2 (bioMèrieux, France) is an automated identification system for various bacterial and fungal species. The Neisseria-Haemophilus card used for identification of *Neisseria*,

Haemophilus and other fastidious gram-negative bacteria can identify most *Campylobacter* species to some level. It identifies correctly 90-95% of *C. jejuni* ssp. *jejuni* strains, 70-90% *C. coli* strains, 100% *C. fetus* (Martiny *et al.*, 2010).

The Phoenix Automated Microbiology system (Becton Dickinson, Franklin Lakes, New Jersey, USA), the RaplD Remel (Remel, Lexena, USA) and BBL Crystal (Becton Dickinson) have a lower identification rate for *Campylobacter* species. Several latex agglutination tests are available for detection of *Campylobacter* species. They use polyclonal antibodies to detect antigenic outer membrane proteins but cannot characterize *Campylobacter* species (Cheesbrough, 2006).

2.10.2 Phenotypic typing

The various methods used for phenotypic typing of *Campylobacter* species include;

The *Penner serotyping technique* – It is the most commonly used phenotypic typing method for typing *Campylobacter* species. Its mechanism of action is based on passive haemogglutination and differentiates strains based on heat stable antigens (Penner and Hennessy, 1980). It involves extraction of antigenic materials by heat or chemical treatment and immersion of the antigen into sheep or chicken erythrocytes. These exposed erythrocytes are then tested with antisera in micro-titration plate with U-shaped wells and examining the wells for agglutination of erythrocytes. Capsular polysaccharides are the major sero-determinants.

The *Lior serotyping scheme* - This method is based on slide agglutination procedure using live bacteria together with unabsorbed and absorbed antisera. It detects the heat-labile antigens. Uncharacterized bacterial surface antigens and flagellar antigens in some serotype are the sero-determinants.

The Phage typing - This method is based on viewing zone of clear growth where the susceptible bacteria have been lysed. It is able to detect different strains of *Campylobacter* within a species. It involves growing a culture of the *Campylobacter* strain onto Columbia blood agar for 18-24 hrs at 37ºC under microaerophilic condition, after which, 3-5 colonies are transferred into 5 ml of sterile physiological saline in a test tube and mixed to match turbidity equivalent to 1.0 MacFarland. The suspension are then cultured into Columbia blood agar and incubated at 37ºC for 4 hrs, after which the phage inoculum are dispensed into it, and then incubate for 18 hrs at 37ºC under microaerophilic conditions.

The Biotyping – It involves the identification of bacteria based on reaction to biochemical test. The biochemical tests used for biotyping *Campylobacter* are catalase test, hippurate hydrolysis test, nitrate reduction, oxidase test e.tc.

Antimicrobial resistotyping (AR) – This technique differentiate *Campylobacter* based on their susceptibility and resistance to antibacterial agents. It is usually done as described by the Clinical Laboratory Standard Institute (CLSI). Organisms are characterized as sensitive or resistant to the antibacterial agent by measuring the zones of clear growth and comparing it with the CLSI standard for that agent on the organism.

However, despite the uniqueness of phenotypic methods for typing *Campylobacter* species, the major limitation in its use is the nontypeability of strains due to lack of antigen detection and the strains are otherwise not atypical (Wassenaar and Newell, 2000).

Table 2.1: Biochemical characterization used for identification of *Campylobacter* species

Species	hippurate	catalase	indoxyl acetate	urease	nitrate	H_2S	aryl sulfatase	oxidase	growth at 37°	growth at 42°
C. jejuni spp *jejuni*	+	+	+	-	+	v	v	+	-	+
C. jejuni spp *doylei*	v	+	+	-	-	-	-	+	+	-
C. lari	-	+	-	-	+	+	-	+	-	+
C. upsaliensis	-	+	+	-	+	-	-	v	v	v
C. fetus	-	+	-	-	+	-	-	+	+	v
C. hyointestinalis	-	+	-	+	+	-	-	+	+	v
C. sputorum	-	+	-	-	+	+	+	+	+	-
C. mucosalis	-	+	-	-	+	-	-	+	+	v
C. coli	-	+	+	-	+	-	-	+	-	+

Lastovica and Allos, (2008)

Legend:
Positive reaction/ +
Negative reaction/ -
Indistinguishable reaction/**v**

2.11 The Genotypic method

2.11.1 Molecular identification

The advent of Genotype-based species identification methods have solved the problem caused by the biochemical inertness of *Campylobacter* species. Different molecular techniques (PCR) based on species-specific genes have been used in species identification of *Campylobacter*. These species- specific genes include *hipO*, 23S rDNA, *cadF, ipxA, napA, ceuE, cdtA* for *C. jejuni; aspA,* 23S rDNA, *cdtB, glyA, ceuE, cadF, ipxA* for *C. coli; ipxA* and *glyA* for *C.lari; ipxA* and *glyA* for *C.upsaliensis*; *cdtC, sapB2* and 16S rDNA for *C. fetus* (Eyers *et al*., 1997; Linton *et al*, 1997; Gonzalez *et al.,* 1997; Oyarzabal *et al.,* 1997; Houng *et al.,* 2001; Wang *et al.,* 2002; Best *et al*., 2003; Klena *et al.,* 2004; Persson and Olsen, 2005; Asakura *et al.,* 2008; Cummingham *et al.,*2010). However, the sensitivity and specificity of this PCR assay (Molecular techniques) in detecting *Campylobacter* species depend on the number of strains and so, do not work well with different set of strains. Partial sequencing of the 16S rDNA is widely used for species identification of many enteric bacteria. However, it is less useful for identification of *Campylobacter* because of the lack of discrimination between *Campylobacter jejuni, C. coli*, *C. lari* and *C. upsaliensis* strains (Gorkiewick *et al.,* 2003). Sequencing a house keeping gene groEL/cpn60 has been used for successful identification of *Campylobacter* species (Kärenlampi *et al*. 2004; Hill *et al.,* 2006).

2.12 Genotypic typing

The various methods used for genotypic typing of *Campylobacter* species are classified into three groups based on their principles. These include: Those that work on restriction analysis of the bacterial DNA. Examples are the Pulsed-field gel electrophoresis (PFGE), Restriction Fragment Length Polymorphism (RFLP), and Ribotyping; those that work on identification of

DNA sequence polymorphism. Examples are the Multi-locus Sequence typing (MLST), Single-Nucleotide Polymorphism (SNP) detection and Sequencing the *fla*A-gene; those that work on PCR amplification of a particular gene. Examples are the Amplified Fragment Length Polymorphism (AFLP) and the Randomly Amplified Polymorphic DNA (RAPD).

2.12.1 Pulsed-field gel Electrophoresis (PFGE)

It involved the entire bacterial genome been digested with an enzyme and the large DNA (20 – 200kb) molecules separated by the use of an alternating electrical field. Various banding patterns are utilized to compare the genetic materials between isolates with availability of software used for the analysis. Insertion, deletion and point mutations are responsible for the difference that occurs in the PFGE profile. While insertion or deletion creates a 2-band difference, a point mutation causes a 3-band difference. A PFGE profile that differs from each other in a position of 3-bands is closely related and this criterion is used for the investigation of nosocomial outbreak. However, when investigating food outbreak, isolates that displays indistinguishable PFGE pattern are used for detecting infection clusters. When interpreting the result, the prevalence of a specific PFGE pattern, the diversity of the organism, the quality of the PFGE gel and the reproducibility of the PFGE method used for a specific bacterium must be considered. PFGE is a good differential genotypic typing for *Campylobacter* species and is a standard guild for molecular typing of foodborne pathogens. The PulseNet protocol is one of the standardized PFGE protocol used for *Campylobacter* species.

2.12.2 Restriction Fragment Length Polymorphism (RFLP)

It involves the use of Polymorphic genes (*gyr*A and *pfl*A) for PCR-RFLP analysis. However, to obtain a higher level of discriminatory power, many of the polymorphic genes with *flaA* as a third target gene must be used. Other gene candidates that could be used for RFLP are the

*fla*E that codes for flagellar hook proteins and outer membrane protein. The identification of a new hypervariable genetic loci and lipopolysaccharide phenotypes by Multilocus Enzyme Electrophoresis (MEE) and HS antigen-dependent serotyping respectively could serve as target for RFLP (Wassenaar and Newell, 2000).

2.12.3 Ribotyping

It involves the use of rRNA gene for subtyping purposes. This technique uses agarose gel electrophoresis of the digested DNA followed by southern blot hybridization with probe specific for the rRNA gene; although, the discriminatory power is low, but it is most useful for determining *Campylobacter* species that are difficult to analyze phenotypically (Wassenaar and Newell, 2000). There is a comparative difference of the Ribotyping profile based on the restriction enzyme and probe used. The rehybridization of the southern blots with probes for other hypervariable region of the chromosome and automation has been used to increase the level of discrimination (Wassenaar and Newell, 2000).

2.12.4 Multi-Locus Sequence Typing

It involves comparing the gene sequence with nucleotide base change. A strain is allocated a particular sequence on the alleles from each of the sequence gene. This technique is highly reproducible with internet-based MLST database that makes it easy to standardize the naming and fast conversion of MSLT results. MLST typing of *Campylobacter* species depends on the genetic differences present in the housekeeping gene location and is appropriate to analyse the genetic number, to understand the overall genetic structure of *Campylobacter* during outbreak investigation. However, the use of this technique for typing *Campylobacter* species is limited because of the cost.

2.12.5 Single-Nucleotide Polymorphism Detection (SNPD)

It occurs due to nucleotide changes at a certain locus in the bacterial genome. Understanding of the relatedness of strain can be obtained by considering multiple SNPs (Cebula *et al*., 2005). Detection of SNPs at specific loci of an MLST gene has been applied for genotyping of *Campylobacter* isolates (Best *et al*., 2004). A combination of SNPs analysis with sequencing of short variable regions of *fla*A gene gives a discrimination index similar to that of MLST analysis for genotyping *Campylobacter* species (Price et al., 2006).

2.12.6 Sequencing the *fla*A gene

It involves the direct sequencing of the *fla*A gene in the flagellin locus with or without PCR amplification (Wassenaar and Newell, 2000). It has become an alternative method for genotyping bacterial isolates because of its high reproducibility. However, the complex data and superb discriminatory power is dependent on computerized programs and parameters set by the software packages used (Wassenaar and Newell, 2000).

2.12.7 Amplified Fragment Length Polymorphism (AFLP)

It involves complete digestion of a bacterial genome by restriction or cutting enzymes (endonucleases) to create a large number of fragments and PCR amplification of the fragments (Vos *et al*., 1995). The various restriction site fragments caused by changes (mutation) in the genome leads to the discrimination of the isolates. This method was developed by Duim *et al.*, (1999) for *Campylobacter* species. It uses radioactive or fluorescent labeled primers to make the analysis and facilitate discrimination. However, complexity of this technique limits it application.

2.12.8 Randomly Amplified Polymorphic DNA (RAPD)

It involves PCR amplification of a segmented genomic DNA using certain primers in a harsh reaction conditions with the amplicon separated by agarose gel electrophoresis and using the banding pattern to compare relatedness of the bacterial isolates (Franklin *et al.*, 1999). Despite the cost-effectiveness of this technique, its poor reproducibility limits it application.

2.12.9 Limitation to Genotypic typing of *Campylobacter* species

Despite the good discriminatory power of genotypic typing, certain factors limit its usefulness. Some of these factors as enlisted by Wassenaar and Newell, (2000) are;

Unavailability of standard sets of strains for comparative evaluation- This is because some members of certain groups of strains are closely related phenotypically and genotypically than members of other group; Different levels of discrimination among the various genotypic techniques within heterogeneous groups of strains; Occurrence of genetic instability among strains due to recombination and rearrangement of genetic segments; the cost of obtaining some of the genotypic typing techniques are generally expensive.

2.13 Treatment of *Campylobacter* infection

Most *Campylobacter* infections are self-limiting and require no therapeutic intervention other than supportive therapy, such as maintenance of hydration and electrolyte balance. However, antibiotics are employed in immunocompromised patients, patients whose symptoms are severe or persistent, and those with extra-intestinal infections (Kaakoush *et al*., 2015). Macrolide (azithromycin) has been the best drug of choice for the management of campylobacteriosis worldwide. The reason for the increasing importance of this macrolide for

the treatment of *Campylobacter* infections is that the rates of ciprofloxacin resistance are relatively high for *Campylobacter* species due to the use of antibiotics in the poultry industry and animal husbandry operations and, to a lesser extent, the indiscriminate use of ciprofloxacin for treatment of human campylobacteriosis. *Campylobacter* species which are resistant to antibiotics have been associated with a longer duration of illness, an increased risk of invasive disease and death, and increased health care costs. In the case of severe *Campylobacter* infection in humans, treatment with aminoglycosides (e.g., gentamicin or kanamycin) is commonly employed.

2.13.1 Antibiotic Resistance of *Campylobacter* species

Antibiotic resistance in *Campylobacter* infection is recognized by World Health Organization (WHO) as a problem of public health importance (Moore *et al.*, 2006; Silva *et al*., 2011). Patients infected with *Campylobacter* species are treated by replacing the fluid lost. However, antibiotics such as tetracycline, macrolide, fluoroquinolones and gentamycin may be administered in severe cases. Although comparable standardised procedures for susceptibility testing are available for a wide range of organisms, based on the guidelines defined by the Clinical Laboratory Standards Institute (CLSI), no internationally accepted criteria is available for susceptibility testing of *Campylobacter* species (Moore *et al.*, 2006). The indiscriminate use of antibiotic in animal production to control, prevent and treat infection may be responsible for the outbreak of multi-drug resistance among *Campylobacter* species (Gallay *et al*., 2007).

2.14 Prevention and Control of *Campylobacter* infection

Campylobacter species are known to be sensitive to the environment but they have some degree of resilience and can retain the state of VBNC where they can become non-detectable by culture-based methods, yet can infect susceptible cells. However, since *Campylobacter* are sensitive to desiccation, freezing, low humidity and exposure to oxygen, the control strategies should be based on these aspects where relevant (Silva *et al.*, 2011). Eating of poultry meat (Chicken) is regarded as the main source of human campylobacteriosis, so, effort must be made to reduce human disease (Silva *et al.*, 2011).

2.14.1 On Farm

Since chicken is regarded as a good reservoir of *Campylobacters*, effort should be geared at minimizing contamination of chicken rearing houses by practicing strict hygienic protocols and installing hygienic barriers. Strict laws should be enforced on the use of antibiotics in food animal production in order to reduce the increase in antibiotic resistant strains. The use of pre-and probiotics, application of bacteriocin producing bacteria, application of bacteriophages to treat chicken could help in the reduction of *Campylobacter*; subsequently, leading to reduction in human infection.

2.14.2 In Processing Plants

The strategy involves using a rapid test protocol to separate *Campylobacter* positive livestock from negative livestock at the slaughter house in order to reduce spread of contamination (Havelaar *et al*., 2007; Silva *et al.*, 2011). Adherence to strict hygienic protocol such as washing, de-feathering, and submersion chilling during processing line reduces the level of *Campylobacter* in the meat (Silva *et al*., 2011).

2.14.3 In Home and Kitchen

Good sanitary protocols and proper cleaning of working surface and utensils coupled with the cooking temperature are able to eliminate *Campylobacter* from our domestic homes and kitchens if not compromised. Cross contamination must be avoided by wrapping the poultry when transferring from the oven. Hot water with detergent must be used when cleaning kitchen surfaces, utensils, and during hand washing (Silva *et al.,* 2011). Public enlightenment campaign must be carried out to inform customers, cooks and buyers of chicken about the burden of campylobacteriosis and how to avoid this disease in our environment.

2.14.4 In Water

Campylobacter species are highly predominant in untreated water contaminated with faecal materials, human sewage and run-off manure. Therefore, as an important aspect of HACCP program, drinking water must be chlorinated to reduce the burden of *Campylobacter* in processing plant. Also, water used in the farm (animal house), slaughter houses and kitchens must be chlorinated to avoid water contaminating the process.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in some selected hospitals in Kaduna State. Kaduna State is located between latitude 10º20′N and 7º45′E and Longitude 10º33′N and 7º75′E. According to the 2006 National Population Census, the population of Kaduna State is six million one hundred and thirteen thousand, five hundred and three (6,113,503). Kaduna State has 23 Local Government Areas (LGAs) in the three senatorial districts. The people of Kaduna State are mostly farmers, business men and women, and civil servants.

3.2 Study design and Selection of Study sites

The study was cross sectional, having a representative of the three senatorial districts but hospital-based. The selected hospitals for the study were General Hospital Kachia, Dr Gwamna Awan General Hospital, Kakuri, General Hospital Sabon, Gambo Sawaba General Hospital, Zaria city, Zaria, and National Tuberculosis and Leprosy Training Centre (NTBLTC) Saye, Zaria.

3.3 Ethical Clearance

Ethical approval was obtained from the State Ministry of Health and the Ethical Committee of the various selected hospitals prior to the study. The consent of all participants were sought and obtained prior to collection of specimens

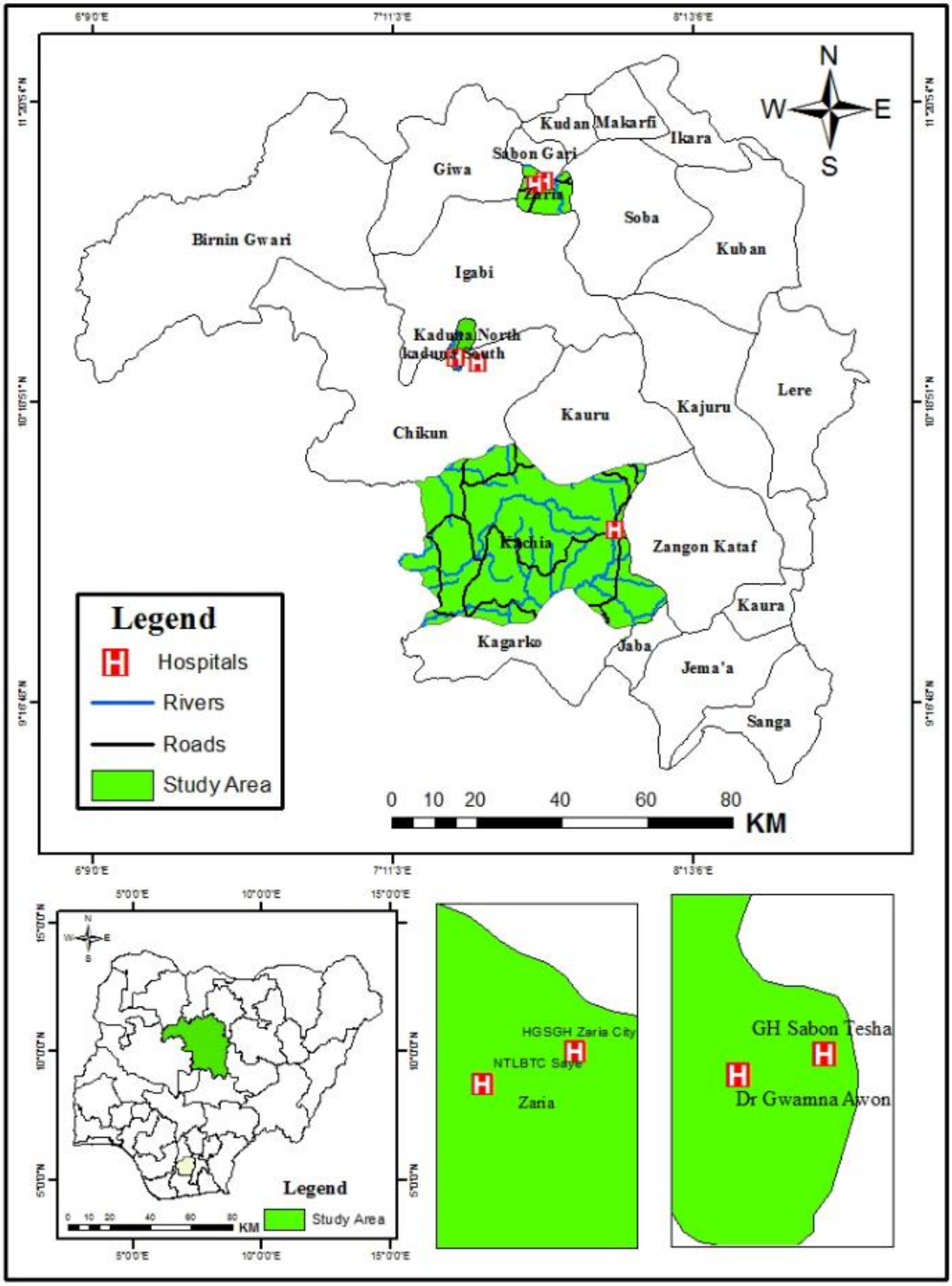


Figure 3.1: Map of Kaduna State showing the sampling sites.

3.4 Determination of Sample size

A prevalence of 7.0% as reported in a study carried out in Lagos (Smith *et al*., 2005) was used to determine the sample size using the equation below as described by Naing *et al.,* (2006)

$n= Z^2p (1-p)/ d^2$

Where n= sample size

Z = statistics for a level of 95% confidence interval = 1.96

p = prevalence of 7.0% (0.07) from Smith *et al*., (2005)

d = precision of 5% =0.05

Therefore,

$n = 1.96^2 \times 0.07 (1-0.07)/ 0.05^2$

n = 100.04

The calculated sample size of 100 was the minimum sample size that was used for the study. However, statistical credence was given by increasing the sample size to 230 in order to increase the possibility of getting positive samples. The samples were collected based on convenience.

3.5 Study Population

These were HIV-infected patients with diarrhoea attending the selected Hospitals for their Anti-retroviral drugs.

3.5.1 Inclusion Criteria

All diarrhoeic HIV -infected patients attending the selected hospitals during the study period.

3.5.2 Exclusion Criteria

These were not consenting diarrhoeic HIV-infected patients.

3.5.3 Questionnaire administration

A Well-structured questionnaire (Appendix 1) was administered to consenting patients to obtain relevant data on the frequency and consistency of their stools during the week, their age, sex, educational status, occupation, and rick factors such as source of drinking water, type of sewage disposal system, contacts with farm animals, consumption of raw milk, eating of chicken that predispose them to campylobacteriosis. This was done before sample collection.

3.6 Collection of Samples and processing

Sterile stool sample containers with applicator were given to the HIV- infected patients whose questionnaires were satisfactorily filled to bring fresh stool samples. The samples were transported in ice-pack container immediately to the Department of Microbiology Laboratory, Ahmadu Bello University, Zaria, Nigeria. One (1 ml) of the faecal sample was emulsified in 9 ml 0.1% peptone water to a 1:10 dilution. The samples were inoculated onto prepared antibiotic-free blood agar (*Campylobacter* blood agar base with 7% sheep blood) within 4 h of collection.

3.7 Laboratory Analysis

3.7.1 Isolation of *Campylobacter* species from the faecal sample

Ten (10) drops of the homogenous faecal suspension was inoculated onto a 0.45 µm cellulose triacetate membrane filter of diameter 50mm (Sartorius, Goettigen, Germany) on an antibiotic-free blood agar plate (*Campylobacter* blood agar base with 7% sheep blood) using a Pasteur pipette with the filter placed on the centre of the agar medium. The filter was removed with forceps after 30 minutes of inoculation and the plates were kept in a 3.5 L anaerobic jar and Campy-Gen gas generating sachet (Campygen N25 Oxoid) was introduced to maintain

the microaerophilic condition (6% O_2 and 10% CO_2 in 84% N_2) and incubated at 37ºC for 6 days (Lastovica, 2006).

Presumptive isolates (colonies that were gray, flat, irregular and spreading) were sub-cultured unto a *Campylobacter* blood free agar base with SR155E supplement (modified Charcoal Cefoperazone Deoxycholate Agar, mCCDA) prepared according to the manufacturer's instruction. The plates were kept in a 3.5L anaerobic jar and Campy-Gen gas generating sachet (Campygen N25 Oxoid) was introduced to keep the microaerophilic condition (6% O_2 and 10% CO_2 in 84% N_2) and incubated at 42^{o}C for 2 days.

3.7.2 Identification and Characterization of *Campylobacter* isolates

Presumptive *Campylobacter* colony was picked and tested biochemically using standard methods based on the morphology and motility of the isolates. Colonies that were small, curved Gram-negative rods and positive to oxidase, catalase test and reduce Nitrate were further analysed by API Campy kit® (bioMèrieux, France) to identify the isolates to species level.

3.8 Biochemical tests

The following primary biochemical tests were carried out to characterize the *Campylobacter* isolates.

3.8.1 Gram Staining

Principle: It is based on the ability of the bacterial cell wall to retain certain primary stain (crystal violet).

Procedure: A smear of all the presumptive *Campylobacter* isolate was made by taking a distinct colony from the culture and emulsifying on clean grease-free glass slides. The smears

were allowed to air-dry and heat-fixed by passing them over the blue flame of a Bunsen burner. The fixed smears were Gram-stained by flooding them with Crystal violet (primary stain). They were allowed to stand for 60 seconds after which they were washed off with clean water. The water on the smears was tipped off and thereafter covered with Lugols iodine for 60 seconds, after which they were washed off using clean water. The smears were decolorized rapidly by gently washing each smear with acetone which was immediately washed off with clean water. The smears were finally covered with Carbol Fuchsin for 60 seconds after which the smears were washed off with clean water and air-dried on a draining rack. The Gram-stained smears were examined under the microscope using × 40 objective and ×100 (oil immersion) objective.

Interpretation: The Gram-stained smears that showed Gram negative curved rods were regarded as positive for presumptive *Campylobacter* species (Cheesbrough, 2006).

3.8.2 Oxidase test

Principle: It is based on the ability of the bacteria to produce the enzyme cytochrome oxidase.

Procedure: A loopful of the fresh culture was placed on a filter paper pad and then flooded with oxidase reagent.

Interpretation: The formation of a dark purple colour within 10 seconds was presumed positive for oxidase production (Cheesbrough, 2006).

3.8.3 Catalase test

Principle: It is based on the ability of the bacteria to produce the enzyme catalase needed to breakdown hydrogen peroxide to oxygen and water.

Procedure: Hydrogen peroxide solution was prepared, after which 3ml were dispensed into a test tube. A sterile wooden stick was used to take several colonies of the isolates and immersed into the test tubes.

Interpretation: The formation of bubbles is regarded as positive catalase test (Cheesbrough, 2006).

3.8.4 Nitrate reduction test

Principle: It is based on the ability of the bacteria to reduce Nitrate to Nitrites.

Procedure: Nitrate broth was inoculated with a fresh culture of the isolate and incubated at 37ºC for 24 – 48 h. Five (5) drops of Nitrate A reagent and 5 drops of Nitrate B reagents were added. The tubes were inverted gently to mix reagents.

Interpretation: The development of red colour within 2 minutes was checked for, indicating the reduction of Nitrate to Nitrites (Cheesbrough, 2006).

3.8.5 Hippurate hydrolysis test

Principle: It is based on the ability of the bacteria to produce the enzyme hippuricase needed to hydrolyse hippurate to benzoic acid and glycine.

Procedure: Small amount of the pure culture was inoculated into 0.4 ml of 1% Sodium hippurate prepared by dissolving 1g of Sodium hippurate into 99 ml of distilled water in a tube. The tube was capped and incubated at 37ºC for 2 h. Thereafter, 0.2 ml of 2% Ninhydrin reagent was added and then incubated again at 37ºC for 15 minutes.

Interpretation: The development of a purple-violet colour was regarded as hippurate positive (Cheesbrough, 2006).

3.9 Identification of *Campylobacter* isolates using API Campy kits

Principle: It is based on the ability of the bacteria to utilise corresponding substrate for growth.

Procedure: Biochemically confirmed *Campylobacter* isolates were subcultured onto Columbia sheep blood agar plates, prepared according to the Manufacturer's instructions and incubated at 37ºC for 24 h in a microaerophilic condition. The strips were removed from the packaging and separated into two parts (first part and second part strips), along the centre fold without touching the cupules. Two incubation boxes were prepared using the tray and lip, and 3 ml of distilled water were distributed into the honeycombed wells of the trays. The strips were placed in the incubation boxes with the desiccant discarded. Thereafter, a sterile wireloop was used to harvest all the culture into an ampule of API NaCL 0.85% medium (3 ml). The suspension was mixed thoroughly to match 6 McFarland turbidity standards.

On the first part of the strip, 100 µl of the suspension was inoculated onto the tests URE to PAL, and test H_2S of the second part of the strip, gently avoiding bubble formation using a sterile Pasteur pipette. The cupule of the test URE was overlaid with mineral oil forming a slight convex meniscus. The incubation box of the first part of the strip was closed and incubated at 37ºC for 24 h in an aerobic condition. Thereafter, 150 µl of the remaining suspension was transferred into an ampule of API AUX medium and mixed gently.

On the second part of the strip, the test cupules GLU to ERO was inoculated with the suspension from the AUX medium, avoiding the formation of meniscus. The incubation box was closed and was incubated at 37ºC for 24 h under microaerophilic condition. Thereafter, 1 drop of Nitrate reagents (NIT 1 and NIT 2) was added into the test NIT. Three (3) drops of Ninhydrin reagent (NIN) were added to the test HIP while 1 drop of Fast Blue reagent was added into the tests GGT, PyrA, ArgA, AspA and PAL.

Interpretation: The formation of colours and bubbles within 5- 10 minutes were compared with the API Campy colour chart and the result was interpreted using the API web (software).

3.10 PCR detection of the *Campylobacter* species

Eight (8) out of the 45 culture positive samples characterized by conventional biochemical tests and API Campy kit were selected for molecular analysis. Two (2) samples were chosen randomly to represent the various *Campylobacter* species that were characterized by API Campy kits. However, during optimization, we lost the entire eluded DNA, having only 4 samples to fall back for the analysis.

3.10.1 DNA Extraction

The DNA of the bacterial isolates from the pure cultures were extracted using the Phenol-Chloroform method. A two hundred microliters (200 µL) phosphate buffer solution was added to the plates containing 24 – 48 h grown *Campylobacter* isolates; and the plates were washed using a sterile small plastic hockey stick. The bacterial cultures were dispensed into 1.5 ml eppendorf tube containing four hundred microliters (400µL) of lysis buffer and ten microliters (10 µL) of Proteinase K. The tubes were vortexed at high speed for 10 seconds to ensure proper mixing of the culture with the mixtures, after which, they were placed on a heat block at 55ºC for 1 h. Four hundred microliters (400 µL) of Phenol-Chloroform (1:1) was added to the lysate and vortexed briefly for 5 seconds to mix, after which, it was spinned at 13000 rpm for 10 minutes to separate the phase. The upper phase of the tube was removed gently and dispensed into a new 1.5 ml eppendorf tube using a pipette, leaving the white interphase to be discarded. Four hundred microliters (400 µL) of Chloroform was added into the tubes to recover a clear layer, after which, it was vortexed briefly for 5 seconds. It was then spinned at 13000 rpm for 5 minutes to separate the phases. The upper layer was removed

gently without touching the white interphase using a pipette and dispensed into a new 1.5 ml eppendorf tubes. A mixture containing 100% ethanol and 40 µL of 3M sodium acetate was added to the tubes to precipitate the DNA. The tubes were then incubated over night at -20ºC using a refrigerator, after which, they were spinned at 1300rpm using a refrigerated centrifuge for 15 minutes. The ethanol was discarded and 400 µL of 70% ethanol was added. The tubes were spinned at a maximum speed at 4ºC for 5 minutes using refrigerated centrifuge and spun at 13000 rpm for 30 seconds, after which, a pipette was used to remove the ethanol completely. The eppendorf tubes were opened to air-dry the DNA, after which, the pellets were re-suspended in 200 µL molecular grade water. The eluded DNA was stored at -20ºC for amplification

3.10.2 DNA Amplification (PCR)

Species-specific primers that have variability at the *ipx*A gene of the thermotolerant *Campylobacter* species were used to identify *Campylobacter jejuni* and *C. coli*. Because of the relatedness of these two thermotolerant *Campylobacter* species, an additional primer, that target the *nap*A gene (specific for *C. jejuni*) was used in combination with the primer that detect *ipx*A of *Campylobacter jejuni*; while species-specific primers which have variability at the 23S rDNA gene of the *Campylobacter* species were used for the identification of *Campylobacter fetus* and *C. hyointestinalis* (Table 3.1) (Bastyn *et al.*, 1994; Klena *et al.*, 2004; Miller *et al.*, 2007). The primers were obtained from Bioneer (USA), and were reconstituted by adding molecular grade water according to the Manufacturer's instruction.

PCR amplification was performed on a Gene Amp® PCR system 2700 (Applied Biosystem) following the Manufacturer's instruction. The PCR reaction mixture of 20 µl containing 10 mM of Tris-HCL (pH 8.3), 50 mM of KCL, 1.5 mM of $MgCL_2$, 100 µg/mL of bovine serum

albumin, 1µL of both primers, 200 µM of dNTPs, 1.25 U of Amplitaq DNA polymerase (Perkin-Elemer-Cetus), and 5 µL of extracted DNA were spun at high speed for 3 seconds.

The samples were subjected to 35 amplification cycles for 1 minute at 95ºC, Annealing at 50ºC for 1 minute (primers IpxACj/KK2 and IpxACc/KK2), at 53ºC for 30 seconds (primer napAIF3/napAIR3), at 54ºC for 1 minute (primers HYO1/69ar and FET1/69ar), Extension at 72ºC for 2 minutes and Final extension at 72ºC for 8 minutes.

The PCR products were separated by gel-electrophoresis on 1.2% Agarose gel with 1 µg/ml Ethidium bromide at 90V for 1½ h. The sizes of the PCR products (amplicons) were estimated by using a 500^{+}bp marker (Miller *et al.*, 2007).

3.11 Antibiotic Susceptibility Testing

The susceptibility testing of the isolates was carried out using the modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar (CM0337, Oxoid) plate supplemented with 5% hydrolysed sheep blood (Cheesbrough, 2006). A sterile swab stick was used to inoculate the plate with 0.5 MacFarland suspensions (100 µL) of the isolates ensuring even distribution. After 15 mins, commercial antibiotic disc purchased from Oxoid, UK such as Tetracycline (30 µg), Gentamicin (10 µg), Nalixidic acid (30 µg), Amoxicillin (25 µg), Azithromycin (15 µg), Erythromycin (30 µg), Oxacillin (1 µg), Imipenem (10 µg) and Ciprofloxacin (5 µg) were placed on the surface of the plate (90 mm) using sterile forceps and incubated at 37 ºC for 18 h under microaerophilic condition using Campy-Gen gas generating sachet (Campygen N25, Oxoid) in a 3.5 L anaerobic jar. The diameter of the zones of inhibition were measured in mm using a ruler and interpreted as sensitive, intermediate, or resistant according to Clinical Laboratory Standard Institute guidelines (CLSI, 2016). *Escherichia coli* was used as quality control of the susceptibility testing.

Table 3.1: Primers sequence used for PCR amplification of *Campylobacter* species from Diarrhoeic HIV- patients

species	Gene	Primer	Sequence (5' - 3')	Size	Reference
C. jejuni	*ipx*A	IpxACj	(F)ACAACTTGGTGACGATGTTGTA	757bp	Klena
		IpxAR	(R)CAATCATGDGCDATATGASAATAHGCCAT		*et al*., 2004
	*nap*A	napAF3	(F)TAGAACAAATAATATCGATCCAAATCG	1454bp	Miller
		napAR3	(R)AAAAGTGTATCATCTTCGCTATAACCC		*et al.*, 2007
C. coli	*ipx*A	IpxACc	(F)AGACAAATAAGAGAGAATCAG	746bp	Klena
		IpxAR	(R)CAATCATGDGCDATATGASAATAHGCCAT		*et al*., 2004
C. fetus		FET1	(F)CTCATAATTTAATTGCACTCATA	835bp	Bastyns
	23S rDNA	69ar	(R)CTTAGGACCGTTATAGTTAC		*et al*., 1994
C. hyointestinalis		HYO1	(F)ATCTAGGTGAGAATCCTAG	656bp	Bastyns
		69ar	(R)CTTAGGACCGTTATAGTTAC		*et al.*, 1994
	23S rDNA				

3.12 Multiple Antibiotic Resistance (MAR) index

Multi-resistance of the *Campylobacter* isolates was determined using the MAR indexing method as described by Krumperman, (1983).

$$\text{MAR index} = \frac{\text{Number of antibiotic to which a particular isolate was resistant}}{\text{Total number of antibiotics for which the isolates were tested}}$$

3.13 Data Analysis

Data obtained from the questionnaire and results were analysed using Statistical Program SPSS version 20.0. Chi square test was used to test for various associations between the *Campylobacter* species isolated and the risk factors that may be associated with the campylobacteriosis among the study population. Results were presented in tables, figures and charts.

CHAPTER FOUR

4.0 RESULTS

4.1 Biochemical identification of the *Campylobacter* isolates

All the isolates were catalase positive, oxidase positive and able to reduce Nitrate. *Campylobacter jejuni* and *C. coli* were able to utilise citrate while *C. fetus* and *C. hyointestinalis* were non-citrate utilizing. Some isolates of *Campylobacter jejuni* were able to hydrolyse hippurate. The result is as presented in Table 4.1. Details of the biochemical tests are in the Appendix **C**

4.2 API Campy kits (BioMèrieux, France) identification of the *Campylobacter* isolates

Forty five (45) out of the 230 faecal samples collected and analysed were found to be positive for *Campylobacter* species giving a prevalence of 19.6%. The API Campy kits offer a good specificity and sensitivity, and it correctly identified 99.9% of the *Campylobacter* species. Four species of *Campylobacter* were identified with the API Campy kits. These were *Campylobacter jejuni* subsp *jejuni* 1; subsp *doylei*, *C. fetus* subsp *fetus, C. coli* and *C. hyointestinalis*. The result is as presented in Table 4.3. Details of the API Campy kits characterizations are in Appendix **D - E**

4.3 Prevalence of *Campylobacter* species in stool samples of Diarrhoeic HIV-patients in Kaduna State based on the study sites

This is presented in Table 4.3. Eighteen (18) out of the 65 samples collected from Hajia Gambo Sawaba General Hospital (HGSGH), Zaria city were positive for *Campylobacter* species giving a prevalence of 27.7%. Fifteen (15) out of the 60 samples collected from National Tuberculosis and Leprosy Training Centre (NTBLTC), Saye were positive for *Campylobacter* species giving a prevalence of 25.0%. Three (3) out of the 30 samples collected from Dr Gwamna Awan General Hospital (DGAGH), Kakuri were positive for

Campylobacter species giving a prevalence of 10.0%. Five (5) out of the 40 samples collected from General Hospital (GH), Sabo-Tesha were positive for *Campylobacter* species giving a prevalence of 12.5%. Four (4) out of the 35 samples collected from General Hospital (GH), Kachia were positive for *Campylobacter* species giving a prevalence of 11.4%. However, the differences in prevalence based on the study sites were not statistically significance (p-value < 0.05). The location of these hospitals is shown in figure 3.1.

4.4 Distribution of *Campylobacter* species among Diarrhoeic HIV-patients in Kaduna State based on study sites

This is presented in Table 4.4. *Campylobacter coli* had the highest prevalence (44.4%) from the Diarrhoeic HIV-patients followed by *C. jejuni* (33.3%), *C. fetus* (15.6%) while *C. hyointestinalis* had the lowest prevalence (6.7%), but there was no statistical differences in the distribution of the *Campylobacter* species based on the study sites (p-value < 0.05).

4.5 Age and Sex distribution of campylobacteriosis among diarrhoeic HIV-patients in Kaduna State

In this study, all age groups were affected by the infections. However, patients in the age group 30- 39 were mostly infected 20(49.3%) followed by the age group 20-29 (42.6%) while the least cases (25.0%) were observed in the age group 10-19. There was no significant association between campylobacteriosis and age. In this study, the infection was more common among female (23.9%) than male (13.0%). There was significant difference in the distribution of campylobacteriosis with sex in this study (p-value 0.04). The result is as presented in Table 4.5.

Table 4.1: Biochemical characterization of the *Campylobacter* isolates

Campylobacter Species	Catalase test	Citrate utilization	Oxidase test	Nitrate reduction	Indoxyl-Acetate test	Hippurate hydrolysis	Frequency (f)
C. jejuni	+	+	+	+	+	-/+	15
C. coli	+	+	+	+	+	-	20
C. fetus	+	-	+	+	+	-	7
C. hyointestinalis	+	-	+	+	+	-	3

Legend:
Positive/+
Negative/-

Table 4.2: API Campy kits identification of the *Campylobacter* isolates

PROFILE	%ID	REFERENCE
6400567	99.8	*Campylobacter coli*
4400567	99.7	*Campylobacter coli*
6400567	99.8	*Campylobacter coli*
6600577	88.7	*Campylobacter coli*
6400756	81.4	*Campylobacter fetus subsp fetus*
6504555	99.8	*Campylobacter jejuni subsp jejuni* 1
4504555	99.2	*Campylobacter jejuni subsp jejuni* 1
4500004	95.3	*Campylobacter jejuni subsp doylei*
4402774	99.1	*Campylobacter hyointestinalis*
6600577	88.7	*Campylobacter coli*
4400567	99.7	*Campylobacter coli*
4400577	99.7	*Campylobacter coli*
4400577	99.7	*Campylobacter coli*
6600577	88.7	*Campylobacter coli*
6400537	99.9	*Campylobacter coli*
6400556	80.4	*Campylobacter coli*
4400577	99.7	*Campylobacter coli*
6400777	98.4	*Campylobacter coli*
6400757	53.7	*Campylobacter fetus subsp fetus*
6400656	80.7	*Campylobacter fetus subsp fetus*
6400646	74.9	*Campylobacter fetus subsp fetus*
6505755	99.9	*Campylobacter jejuni subsp jejuni* 1
6505555	99.9	*Campylobacter jejuni subsp jejuni* 1
4500005	99.4	*Campylobacter jejuni subsp doylei*
6500005	95.8	*Campylobacter jejuni subsp jejuni* 1
6500105	99.2	*Campylobacter jejuni subsp jejuni* 1
6500155	99.3	*Campylobacter jejuni subsp jejuni* 1
6402774	86.3	*Campylobacter hyointestinalis*
4602774	83.6	*Campylobacter hyointestinalis*
6400577	99.8	*Campylobacter coli*
6400577	99.8	*Campylobacter coli*
6400777	98.4	*Campylobacter coli*
6400766	95.9	*Campylobacter coli*
6400467	99.5	*Campylobacter coli*
6400767	99.9	*Campylobacter coli*
6400557	88.7	*Campylobacter coli*
6400746	76.6	*Campylobacter fetus subsp fetus*
6400656	80.7	*Campylobacter fetus subsp fetus*
6400756	81.4	*Campylobacter fetus subsp fetus*
4500005	99.4	*Campylobacter jejuni subsp doylei*
6500005	95.8	*Campylobacter jejuni subsp jejuni* 1
4500004	95.3	*Campylobacter jejuni subsp doylei*
6505755	99.9	*Campylobacter jejuni subsp jejuni* 1
6504555	99.8	*Campylobacter jejuni subsp jejuni* 1
4500005	99.4	*Campylobacter jejuni subsp doylei*

Table 4. 3 Prevalence of *Campylobacter* species in stool samples of Diarrhoeic HIV-patients in Kaduna State based on the study sites

Study sites	Number Examined	Number positive (%)	χ^2	P value
NTBLTC	60	15 (25.0)	8.340	**0.080**
HGSGH	65	18 (27.7)		
DGAGH	30	3 (10.0)		
GHS	40	5 (12.5)		
GHK	35	4 (11.4)		
Total	230	45 (19.6)		

P-value significant at <0.05
$\chi^{2:}$ Chi-square

KEY:
NTBLTC: National Tuberculosis and Leprosy Training Centre
HGSGH: Hajia Gambo Sawaba General Hospital
DGAGH: Dr Gwamna Awan General Hospital
GHS: General Hospital, Sabo-Tesha
GHK: General Hospital, Kachia

Table 4.4: Distribution of *Campylobacter* species from Diarrhoeic HIV-patients in some hospitals in Kaduna State based on study sites

***C*. species**	**HGSGH**	**NTBLTC**	**DGAGH**	**GHS**	**GHK**	**Total**	**χ^2**	**p-value**
C. jejuni	6 (33.3)	4 (26.6)	2 (66.7)	2 (40.0)	1 (25.0)	15 (33.3)	13.701	**0.621**
C. coli	9 (50.0)	7 (46.7)	0 (0.0)	2 (40.0)	2 (50.0)	20 (44.4)		
C. fetus	1 (5.56)	3 (20.0)	1 (33.3)	1 (20.0)	1 (25.0)	7 (15.6)		
C. hyointestinalis	2 (11.1)	1 (6.67)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.7)		
Total	18 (100)	15(100)	3 (100)	5 (100)	4 (100)	45 (100)		

P-value significant at <0.05
$\chi^{2:}$ Chi-square

KEY:
NTBLTC: National Tuberculosis and Leprosy Training Centre
HGSGH: Hajia Gambo Sawaba General Hospital
DGAGH: Dr Gwamna Awan General Hospital
GHS: General Hospital, Sabo-Tesha
GHK: General Hospital, Kachia

Table 4.5 Age and Sex distribution of campylobacteriosis among diarrhoeic HIV-patients in Kaduna State

	Male			Female				
Age (yrs)	**No. examined**	**No. examined**	**No. (%) Positive**	**No. examined**	**No. (%) Positive**	**Total (%) Positive**	χ^2	**P - value**
10 - 19	8	4	0 (0.0)	4	1(25.0)	1(25.0)	**4.144**	**0.042***
20 - 29	45	18	3 (16.7)	27	7 (25.9)	10(42.6)		
30 - 39	78	33	6 (18.2)	45	14(31.1)	20(49.3)		
40 - 49	66	25	2 (8.0)	39	6 (15.4)	8 (23.4)		
≥ 50	33	12	1 (8.3)	23	5 (21.7)	6 (30.0)		
Total	230	92	12(13.0)	138	33(23.9)	45(19.6)		

***p- value significant at < 0.05**

4.6 Prevalence of *Campylobacter* species in relation to risk factors among Diarrhoeic HIV-patients in Kaduna State

The relationship of *Campylobacter* species with respect to risk factors of campylobacteriosis is presented in Table 4.6. Factors that were considered were; type of sewage disposal system, source of drinking water, contact with farm animals, taking of raw milk, eating of chicken, eating of undercooked meat and-or meat product. Under the sewage disposal system, pit latrine had the highest prevalence of 31 (22.7%) while septic tank had a lowest prevalence of 14 (14.8%). On the source of drinking water, participants that drank water from river had the highest prevalence of 8 (34.9%) while participants that drank water from well had the lowest prevalence of 15 (14.8%). Under contacts with farm animals, participants that have had contact with farm animals had a high prevalence of 29 (19.8%) while those that had no contact with farm animal had a low prevalence of 16 (19.0%). Under eating of chicken, participants that eat chicken had a high prevalence of 31 (20.8%) while those that did not eat chicken had a low prevalence of 14 (17.2%). Among those that consume raw milk, participants that take raw milk had a high prevalence of 43 (20.5%) while those that did not consume raw milk had a low prevalence of 2 (9.5%). Participants that ate under cooked meat and meat products had a high prevalence of 20 (19.6) while participants that did not eat under cooked meat and meat products had a low prevalence of 25 (19.5%). However, there were no significant association between *Campylobacter* isolates and the risk factors to campylobacteriosis at a p-value of 0.05.

Table 4.6: Prevalence of *Campylobacter* species in relation to risk factors among Diarrhoeic HIV-patients in Kaduna State

Variable	Number examined	Positive (%)	p- value	Odds Ratio	CI
Sewage disposal					
Pit latrine	136	31 (22.7)	**0.138**	1.687	0.842 – 3.380
Septic tank	94	14 (14.8)			
Water sources					
Tap	64	12 (18.7)	**0.150**		
River	23	8 (34.9)			
Well	101	15 (14.8)			
Borehole	42	10 (23.8)			
Contact with Farm animal					
Yes	146	29 (19.8)	**0.881**	1.053	0.534 – 2.079
No	84	16 (19.0)			
Taking of Raw Milk					
Yes	209	43 (20.5)	**0.383***	2.462	0.552–10.975
No	21	2 (9.5)			
Eating of Chicken					
Yes	149	31 (20.8)	**0.520**	1.257	0.625 – 2.529
No	81	14 (17.2)			
Eating under-cooked meat and-or-meat product					
Yes	102	20 (19.5)	**0.988**	0.995	0.517 – 1.917
No	128	25 (19.6)			

CI: Confidence Interval, *Fischer's Exact test, p-value significant at .0.05

4.7 PCR confirmation of *Campylobacter* species isolated from Diarrhoeic HIV-patients in some Hospitals in Kaduna State

The result of the molecular detection of *Campylobacter* species using Polymerase Chain Reaction (PCR) is presented in Plate 4.1. From the plate, lane M contains the molecular marker while lane 1, 2, 3 and 4 contain the samples. Lane N is the negative control (molecular grade water). All the four samples were positive for *Campylobacter* species.

Lane 1: two amplicons at 1454 and 757bp detecting the *nap*A gene and the *ipx*A gene of *Campylobacter jejuni*

Lane 2: one amplicon at 656bp detecting the 23S rDNA gene of *Campylobacter hyointestinalis*

Lane 3: one amplicon at 746bp detecting the *ipx*A gene of *Campylobacter coli*

Lane 4: one amplicon at 835bp detecting the 23S rDNA gene of *Campylobacter fetus*

4.8 Susceptibility of the isolated *Campylobacter* species to selected antibiotics

The result of the antibiotic susceptibility test is presented in Table 4.7. Among the antibiotics tested, Imipenem had a 100% efficacy against all the *Campylobacter* species isolated while Oxacillin was not effective (0%). Gentamicin had 93%, activity on all *Campylobacter jejuni* followed by azithromycin with 67%, tetracycline with 60%, ciprofloxacin with 47%, amoxicillin with 40% and then nalixidic acid and erythromycin with a 20% activity. Gentamicin was found to have 100% activity against all *C. coli* followed by nalixidic acid with 55%, azithromycin with 50%, ciprofloxacin with 30%, tetracycline with 25% and then amoxicillin with 10% activity. Ciprofloxacin and gentamicin were found to have 100% activity against all *C. fetus* followed by azithromycin with 86%, amoxicillin with 71% and then nalixidic acid and tetracycline with a 14% activity. All *C. coli* and *C. fetus* were resistant to erythromycin. Azithromycin, tetracycline, gentamicin, ciprofloxacin and amoxicillin were found to have a 100% activity against all *C. hyointestinalis* followed by nalixidic acid with 67% and then erythromycin with 33% activity.

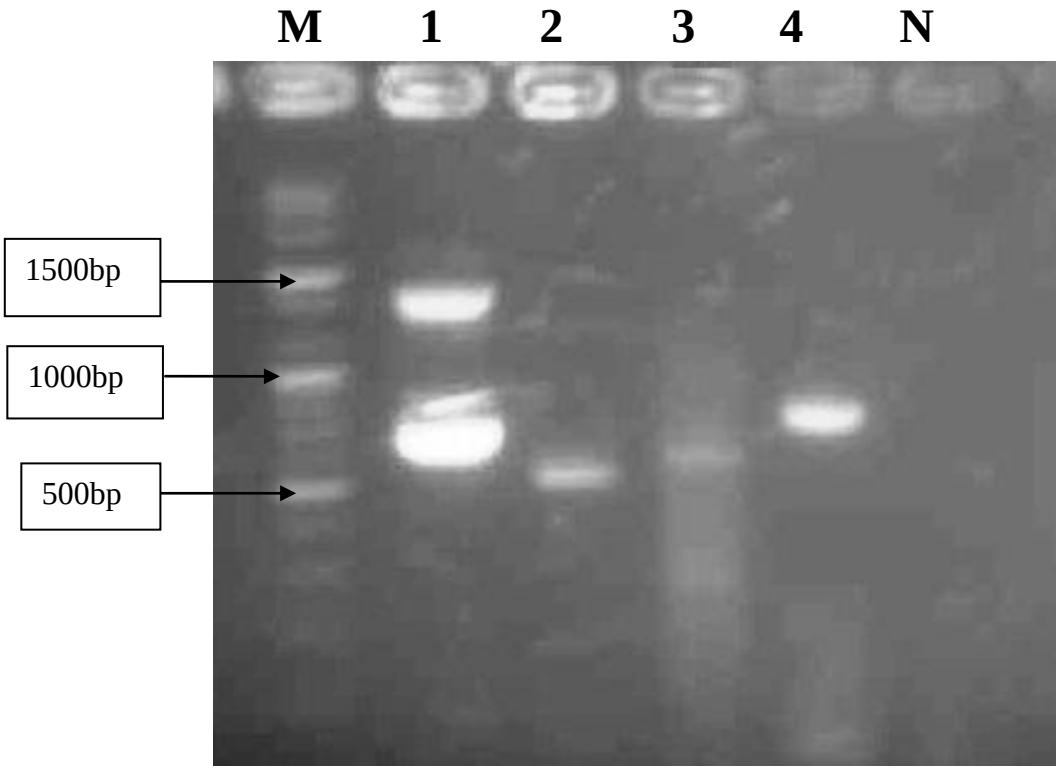


Plate I: Agarose gel electrophoresis of *Campylobacter* species isolated from Diarrhoeic HIV-patients in some Hospitals in Kaduna State.

Lane M: molecular marker

Lane 1: two amplicons at 1454 and 757bp detecting the *nap*A gene and the *ipx*A gene of *Campylobacter jejuni* respectively.

Lane 2: one amplicon at 656bp detecting the 23S rDNA gene of *Campylobacter hyointestinalis*

Lane 3: one amplicon at 746bp detecting the *ipx*A gene of *Campylobacter coli*

Lane 4: one amplicon at 835bp detecting the 23S rDNA gene of *Campylobacter fetus*

Lane N: Negative control

Table 4.7 Susceptibility of the isolated *Campylobacter* species to selected antibiotics

Antibiotics	*C. jejuni* (n=15)	*C. coli* (n= 20)	*C. fetus* (n= 7)	*C. hyointestinalis* (n= 3)
AZM (15 µg)	10 (67%)	10 (50%)	6 (86%)	3 (100%)
IPM (10 µg)	15 (100%)	20 (100%)	7 (100%)	3 (100%)
NA (30 µg)	3 (20%)	11 (55%)	1 (14%)	2 (67%)
TE (30 µg)	9 (60%)	5 (25%)	1 (14%)	3 (100%)
CN (10 µg)	14 (93%)	20 (100%)	7 (100%)	3 (100%)
CIP (5 µg)	7 (47%)	6 (30%)	7 (100%)	3 (100%)
E (10 µg)	3 (20%)	0 (0%)	0 (0%)	0 (0%)
AML (25 µg)	6 (40%)	2 (10%)	5 (71%)	3 (100%)
OXL (1 µg)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Key:

AZM: Azithromycin; IPM: Imipenem; TE: Tetracycline; NA: Nalixidic acid; CN: Gentamicin; CIP: Ciprofloxacin; E: Erythromycin; AML: Amoxicillin; OX: Oxacillin

4.9 Multiple Antibiotic Resistance (MAR) index of the *Campylobacter* species

The MAR index of the *Campylobacter* species isolated from diarrhoeic HIV-patients in the study is show in Table 4.8. *Campylobacte*r species in this study exhibited different antibiotic resistance patterns with MAR indices ranging from 0.33 to 0.88. The highest MAR index of 0.88 was observed in *C. jejuni* with resistance to eight different antibiotics while the lowest MAR index of 0.33 was observed among *Campylobacter* isolates that were resistance to three different antibiotics. The overall percentage resistance was 49.13% of which 54% of *C. coli* were resistant to three or more antibiotics, followed by *C. jejuni* (50%), *C. fetus* (46%) while *C. hyointestinalis* (22%), demonstrating an increasing trend in *Campylobacter* Multidrug Resistance (MDR).

Table 4.8 Multiple Antibiotic Resistance (MAR) index of the *Campylobacter* species

Campylobacter species	Antibiotic resistant profiles	No of Isolate	MAR index	Percentage of Isolates (%)
C. jejuni	**AZM,-OXL,-E**	5	0.33	3.29
	AZM- OXL-CIP	5	0.33	3.29
	AZM-OXL-CN	1	0.33	0.66
	AZM-OXL-E-TE	5	0.44	3.29
	AZM-OXL-E-AML	5	0.44	3.29
	AZM-OXL-E- CN	1	0.44	0.66
	AZM- OXL- E- AML- NA	5	0.55	3.29
	AZM –OXL- E- AML- TE	5	0.55	3.29
	AZM- OXL- E- AML- CIP	5	0.55	3.29
	AZM-OXL-E-AML-NA-TE	5	0.66	3.29
	AZM-OXL-E-AML-NA-CN	1	0.66	0.66
	AZM-OXL-E-AML-NA-TE-CIP	5	0.77	3.29
	AZM-OXL-E-AML-NA-TE-CIP-CN	1	0.88	0.66
C. coli	**AZM-OXL-E**	10	0.33	6.57
	AZM-OXL-NA	9	0.33	5.92
	AZM-OXL-AML	10	0.33	6.57
	AZM-OXL-E-TE	10	0.44	6.57
	AZM-OXL-E-NA	9	0.44	5.92
	AZM-OXL-E-AML-TE	10	0.55	6.57
	AZM-OXL-E-AML-NA	9	0.55	5.92
	OXL-E-TE-CIP-NA-AML	9	0.66	5.92
	AZM-OXL-E-AML-NA-TE-CIP	9	0.77	5.92
C. fetus	**AZM-OXL-E**	1	0.33	0.66
	AZM-OXL-TE	1	0.33	0.66
	AZM-OXL-NA	1	0.33	0.66
	NA-OXL-E-TE	6	0.44	3.95
	AML-OXL-E-NA	2	0.44	1.32
	OXL-E-AML-TE	2	0.44	1.32
	OXL-E-AML-NA-TE	2	0.55	1.32
	AZM-OXL-E-AML-TE	1	0.55	0.66
C. hyointestinalis	AZM-OXL-E-AML-NA-TE	1	0.66	0.66
	NA-E-OXL	1	0.33	0.66

KEY:

MAR – Multiple Antibiotics Resistance

Azithromycin –AZM; Oxacillin – OXL; Erythromycin – E; Tetracycline – TE; Nalixidic acid-NA; Amoxicillin – AML; Ciprofloxacin – CIP; Gentamicin – CN.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Prevalence of *Campylobacter* species among Diarrhoeic HIV-patients in some Hospitals in Kaduna State

The 19.6% prevalence obtained in this study is within the documented range of *Campylobacter* species among diarrhoeic HIV-patients in Nigeria and other parts of the World. This finding agrees with the work of Snijders *et al.,* (1997) who report a prevalence of 16% in Netherland; Grant and Tee, (1998) who reported 18.5% prevalence in Australia; Lastovica and Roux, (2000) who reported a prevalence of 21.8% in Cape Town; Coker *et al*., (2002) who reported a prevalence of 20% in Lagos. This high prevalence could be due to the resistance of *Campylobacter* to Zidovudine (anti-retroviral drug) makes *Campylobacter* to be persistent among the populace (Salmon, 1991). This bacterium has several extracellular and cell-mediated factors that may be important virulence determinants in its infection. However, the study was at variance with Smith *et al.,* (2015) who reported a low prevalence of 7% in Lagos and Samuel *et al.,* (2006) who reported a prevalence of 8.2% in Ilorin. In their study, Butzler virion medium was used to isolate *Campylobacter* species. This *Campylobacter* selective medium can only allow the growth of *Campylobacter jejuni* and few strains of *Campylobacter coli*. Additionally, their primary incubation temperature was 42 ºC, which allows the growth of *C. jejuni* subsp *jejuni* and *C. coli* but never allow the growth of other (emergent) *Campylobacter* species (Lastovica, 2006).

5.2 Prevalence of *Campylobacter* species in stool samples of Diarrhoeic HIV-patients in Kaduna State based on the study sites

From the result, high prevalence values of 27.7% and 25.0% were observed in HGSGH and NTBLTC respectively, both in Zaria metropolis. This high prevalence seen in Zaria could be

due to the fact that most of the samples were collected from Zaria. Additionally, even though, the risk factors were not statistically associated with campylobacteriosis in this study, lack of adequate potable drinking water in Zaria metropolis could as well be responsible for this high prevalence. This opinion is also shared by Aboh *et al.,* (2015)

5.3 Distribution of *Campylobacter* species among Diarrhoeic HIV-patients in Kaduna State

The most frequently isolated *Campylobacter* species from the study was *C. coli* with prevalence of 44.4% compared to *C. jejuni* with a prevalence of 33.3%, *C. fetus*, 15.6% and *C. hyointestinalis*, 6.7%. This high prevalence of *Campylobacter coli* could be due to proven facts that *C. coli* are more resistant to antimicrobial agents than other *Campylobacter* species and can transfer this resistance gene to other bacteria leading to the high multi-drug resistance observed among this sets of people (Payot *et al.,* 2004). This observation was in an agreement with the findings of Gurtler *et al*., (2005) who reported that *C. coli* were more prevalent than *C. jejuni* in Leipzig, Germany. This work also agrees with the work of Adekunle *et al.,* (2009) and Gwimi *et al.,* (2015) who reported a higher prevalence of *C. coli* than *C. jejuni*. However, this observation was not in accordance with previous studies that *Campylobacter jejuni* is the most common *Campylobacter* species (Ugboma *et al*., 2013). These differences may be due to the fact that their *Campylobacter* species characterisation was based only on primary biochemical tests which tend to overestimate the *Campylobacter jejuni* involvement because multiplex PCR does not detect most primary biochemically characterised *C. jejuni* (Payot *et al*., 2004; Gurtler *et al.,* 2005). The low isolation rate of *Campylobacter fetus,* (15.6%) and *C. hyointestinalis*, (6.7%) may be associated with the Campygen gas generating sachet used in this study which may have suppressed the growth of

this group of *Campylobacter* species because it does not create a hydrogen-enriched atmosphere which is needed for their growth (Workman *et al.*, 2005; Ugboma *et al*., 2013).

5.4 Age and Sex distribution of campylobacteriosis among diarrhoeic HIV-patients in Kaduna State

Campylobacter is normally recovered from children less than 2 years in most developing countries (Samuel *et al*., 2006); this study rather had higher prevalence in the 30 - 39 age groups. This may be attributed to the design of this study which did not focus on children with acute diarrhoea and also a reflection of the infection sources which were mostly obtained from the outpatient department which is usually dominated by these age groups in the study hospitals. *Campylobacter* infections were more prevalent in female (23.9%) than in male (13.0%) patients. The results from this study probably speculate the gender distribution of *Campylobacter* infections in patients attending the selected hospitals in Kaduna State. Despite the fact that there were more female participants in the study than male, no obvious reason has been reported on the impact of gender in *Campylobacter* acquisition. However, it is believed that infants and females had an increased risk of acquiring *Campylobacter* infection (Gillespie *et al.,* 2006). This finding was in line with the work of Gwimi *et al.,* (2015) who reported a higher prevalence of 64.79% among the female than male (60.7%). It also agrees with Samie *et al.,* (2011) who reported a prevalence of campylobacteriosis of 54% among the female than male (46%). A prevalence of 67.6% among female compared to a prevalence of 32.4% among men was reported by Karikari *et al.,* (2017) in his study at Ghana.

5.5 Prevalence of *Campylobacter* species in relation to risk factors among Diarrhoeic HIV-patients in Kaduna State

Even though the risk factors considered in this study were not statistically associated with campylobacteriosis, these factors are still implicated in *Campylobacter* infection.

Under the sewage disposal system, pit latrine users had a prevalence of 22.7%. This could be serving as a direct transmission of the bacteria to drinking water source as sewage is been washed to water source during raining period. A prevalence of 34.9% was found among participants that drank water from river. This could be as a result of pollution from untreated sewage disposed into the water bodies (rivers). Prevalence of 19.8% was recorded among the participants that have had contact with farm animals. This observation agrees with previous studies that contact with farm animal is a good source of contamination by *Campylobacter* species. For instance, Kaakoush *et al*., (2015) reported a high prevalence in Nigeria due to contact with animals; such as, 58% from contact with dogs, 33% from contact with Cattle, 85% from contact with pigs etc. This could be due to the fact that most people that have had contacts with farm animals are agricultural workers; viz; Cultivation of crops, rearing and selling of animal products. Most farmers use animal faecal materials as manure without adhering to strict precautionary measures. These farmers may have cuts in their body while manuring their farms leading to contamination with this bacterium. Meat sellers with open wound or cut are at risk of infection with *Campylobacter*. This finding was in agreement with the work of Chia-ping *et al.*, (2017) who reported a higher prevalence (17%) of campylobacteriosis among farmers in Maryland, Ohio and Virginia, 2014. A prevalence of 20.5% was also observed among participants that drank locally pasteurized milk product (nono). This could be due to direct transmission from contaminated personnel during milk processing, unhygienic milking, use of contaminated water and post-pasteurization contamination. This was in agreement with Longenberger *et al.,* (2013) who reported a prevalence of 12.3% of *Campylobacter* species from raw milk. However, it disagrees with Lovett *et al*., (1983) who reported a lower prevalence of 1.5% in Ohio, USA. Eating of chicken has been implicated as a good source of campylobacteriosis. The study observed a

prevalence of 20.8% among participants that eat chicken. This high prevalence found in chicken could be due to direct contamination from unhygienic chicken handling during processing, cross contamination during evisceration, unhygienic rinsing of multiple carcasses in the same water and cross contamination of the meat slab by meat handlers with the organisms carried from different farms (Olatoye and Ogunsemoyin, 2016). This is because, the intestinal tract of chickens can harbour large amount of *Campylobacter* species; during processing, may leak or rupture and the contents transferred to the skin of the carcases (Silva *et al.*, 2011). The bacteria will remain in films on the skin and become entrapped into the crevices and channels which provide a favourable environment for cross contamination (Silva *et al.*, 2011). This was in agreement with Olatoye and Ogunsemoyin, (2016) who reported a higher prevalence of 96% from retail chicken sold in Oyo State. Salihu *et al.,* (2009) also reported a prevalence of 81.9% in Sokoto.

5.6 PCR confirmation of *Campylobacter* species isolated from Diarrhoeic HIV-patients in Kaduna State

The result of the molecular analysis confirms the isolates to be *Campylobacter jejuni, C. coli, C. fetus* and *C. hyointestinalis* by giving the expected bands. The genes that were detected are i*pxA*, *nap*A and 23S rDNA. The *Ipx*A gene is needed for the biosynthesis of lipid A, a phosphorylated glycolipid that anchors the lipopolysaccharide to the outer membrane of the cell. The *nap*A gene is a part of the nitrate reductase which code for the biosynthesis of the napAB complex needed to catalyse the reduction of nitrate to nitrites. The 23S rDNA gene is a part of the 50S rDNA needed for the biosynthesis of the bacteria RNA.This shows the suitability of molecular approach for correct identification of *Campylobacter* species and the result was in agreement with Bastyns *et al*., (1995); Klena *et al.,* (2004); Parker *et al.,* (2007) and Miller *et al.,* (2007). The primers used for the PCR confirmation is presented in Table 3.1.

5.7 Susceptibility pattern of *Campylobacter* isolates to selected antibiotics

The *Campylobacter* species in this study showed varying sensitivity to selected antibiotics. Imipenem had 100% activity against all *Campylobacter* species. Its mechanism of action involves binding to penicillin-binding proteins, thereby stopping the formation of the bacterial cell wall. This susceptibility observed in Imipenem is due to the fact that Carbapenem is able to resist hydrolysis by β-lactamases, modification in penicillin-binding proteins and decrease in porin uptake leading to inhibition of *Campylobacter* cell wall synthesis. This finding agrees with the work of Melo *et al*., (2013), Man, (2011) and Karikari *et al*., (2017) who reported a 100% susceptibility of all *Campylobacter* species to Imipenem.

Campylobacter resistance to Macrolides (Erythromycin and Azithromycin) could be due to point mutation in the peptidyl-encoding region in the domain V of the 23S rRNA gene leading to modification of the target ribosomal proteins as pointed out in earlier studies (Vlieghe *et al*., 2008) while *Campylobacter* resistance to tetracycline could be due to protection of the ribosomal binding sites of tetracycline and mutations in the 16S rDNA mediated by the *tet* (O) plasmids (Frank and Engberg, 2001). The *C. jejuni* strains in the study had 93% susceptibility to gentamicin while nalixidic acid and erythromycin had the least efficacy on *C. jejuni* with a 20%. This finding agrees with the work of Moore *et al.,* (2006), Melo *et al.,* (2013), Rodrigues *et al*., (2015) and Karikari *et al*., (2017) where a high resistance of *C. jejuni* was reported to erythromycin and nalixidic acid. Coker and Adefeso, (1994), reported a 79% resistance of *C. jejuni* to erythromycin. *Campylobacter coli* strains were highly susceptible to gentamicin (100%) and resistant to erythromycin. This was in line with the findings of Moore *et al.,* (2006) who reported a 0% resistance of *C. coli* to gentamicin.

Campylobacter resistance to quinolones and fluoroquinolones (nalixidic acid and ciprofloxacin) could be due to mutation in the *gyrA* gene encoding subunits of DNA *gyrase*

and Topoisomerase IV subunit, *parC* as reported in earlier studies (Vlieghe *et al*., 2008). *Campylobacter fetus* was 100% susceptible to ciprofloxacin and gentamicin and 100% resistant to erythromycin. *Campylobacter hyointestinalis* were 100% susceptible to ciprofloxacin, gentamicin, azithromycin and tetracycline but 100% resistant to amoxicillin and Oxacillin. This resistance of *Campylobacter* to amoxicillin and oxacillin could be due to the high production of β-lactamase by *Campylobacter* species that break the β-lactam ring of the antibiotics (Frank and Engberg, 2001). The susceptibility of *Campylobacter* species to gentamicin, imipenem and azithromycin could be due to the fact that these antibiotics are not commonly used in animal production hence not subject to much abuse.

5.8 Multiple antibiotic resistance (MAR) index of the *Campylobacter* species

The occurrence of multiple antibiotic resistant strains among *Campylobacter* species is a problem in the treatment of campylobacteriosis. In this study, *Campylobacter* species exhibited different antibiotic resistance patterns; *Campylobacter jejuni* showed 13, *C. coli* showed 9, *C. fetus* showed 9 while *C. hyointestinalis* showed 1. The highest MAR index of 0.88 (0.66%) was found with the resistant profile AZM-OXL-E-AML-NA-TE-CIP-CN. The number of antibiotic an isolate was resistant to increases with the MAR indices. Altogether, 49.1% of the positive *Campylobacter* isolates demonstrated multiple antibiotic resistances which were more pronounced among the thermotolerant *Campylobacter* species (*C. coli* 54% and *C. jejuni* 50%) than among the emerging *Campylobacter* species (*C. fetus* 46% and *C. hyointestinalis* 22%). This problem is further compounded by the circulation of substandard drugs, use of antibiotics in livestock production as well as misuse and indiscriminate of antibiotics. The most important is the high resistance of *Campylobacter* species to erythromycin, which has been used to treat human campylobacteriosis. The use of antibiotics in the treatment of non-gastrointestinal infection also contributes to this high *Campylobacter*

resistance, thereby limiting the usefulness of these antibiotics in treatment of campylobacteriosis. The MAR indices for isolated *Campylobacter* species in this study were high, suggestive of public health implications. In this study, all the isolates had a MAR index greater than 0.2. An isolate with MAR index greater than 0.2 is considered to have originated from a high risk source of contamination such as animal and-or human faecal material while an isolate with MAR index less than 0.2 is considered to have originated from low risk source of contamination in which antimicrobial is not commonly used (Marian *et al*., 2012).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

Campylobacteriosis is the most common form of acute bacterial diarrhoea characterized by passage of watery stools with or without blood, fever, abdominal pain, vomiting, headache and weakness. It is a zoonotic disease caused by *Campylobacter* species. This disease varies from mild gastrointestinal disorder to severe relapsing colitis in human, but most are self-limiting and death is due to complications associated with the diarrhoea. Immunocompromised and Immunosuppressed individuals are highly vulnerable to this disease. The major routes of transmission of this disease are through consumption of contaminated or undercooked meat, raw or unpasteurized milk, untreated water and contact with animals. The intestinal floral of warm-blooded animal are the habitats of this bacteria.

Untreated campylobacteriosis results in relapsing; leading to autoimmune complications such as Guillain-Barre Syndrome, Reactive arthritis and bacteremia especially in immunocompromised individuals. Understanding the pathogenesis of the disease will give a good predictive index in prescribing drugs to patients. This study examined the disease burden of campylobacteriosis among diarrhoeic HIV-patients in some hospitals in Kaduna State, Nigeria. This was carried out by isolation and characterization of *Campylobacter* species from the stool samples of diarrhoeic HIV-patients using the conventional and molecular method, determination of the antibiogram of the *Campylobacter* isolates to some selected antibiotics, and the statistical analysis of all the data and results obtained from the study.

The isolation of *Campylobacter* species was carried out using membrane filtration technique culturing feacal suspension of the study participants onto a *Campylobacter* non-selective agar plate, then, subculturing onto *Campylobacter* selective agar plates, characterizing using both conventional biochemical tests and API Campy kits, and molecular confirming of the isolates using PCR. The antibiotic study was done using Kirby-Bauer disc diffusion method.

A total of 230 faecal samples were cultured, and characterized for *Campylobacter*. Forty five (45) were found to be positive for *Campylobacter* species giving a prevalence of 19.6%. The faecal samples were collected based on convenience from the selected hospitals. Based on study sites, HGSGH had the highest prevalence (27.7%) while DGAGH had the lowest prevalence (10.0%) but the differences were not statistically significant. After characterization of the isolates, *Campylobacter coli* had the highest prevalence of 44.4% while *C, hyointestinalis* had the lowest prevalence (6.7%) but their differences were not statistically significant. Age and Sex distribution with campylobacteriosis were established. Risk factors considered in this study not statistically significant with campylobacteriosis. Among the antibiotics tested, Imipenem had a 100% efficacy on all the *Campylobacter* isolates followed by Gentamicin (93 -100%). The problem of antibiotic resistance was also reaffirmed in this study and higher in *Campylobacter coli* (54%) compared to other *Campylobacter* species such as *C. jejuni* (50%), *C. fetus* (46%) and *C. hyointestinalis* (22%).

6.2 Conclusion

The findings from this study showed a relatively high prevalence of campylobacteriosis among diarrhoeic HIV-patients. A prevalence of 19.6% was recorded in the study population. Also, a high isolation rate of *Campylobacter* species was obtained. *Campylobacter coli* had the highest isolation rate of 44.4%, followed by *C. jejuni* (33.3%), *C. fetus* (15.6%) and then *C. hyointestinalis* (6.7%). Age and Sex distribution with *Campylobacter* infection was

established. The highest prevalence of 49.3% was found among age group 30- 39 years. The prevalence was more in female (23.9%) than male (13.0%). The association between *Campylobacter* species and risk factors to campylobacteriosis was not established in this study. The results obtained showed that imipenem, azithromycin and gentamicin are recommendable as first antibiotics in the management of *Campylobacter* infections. Multiple antibiotic resistance was observed among mostly *Campylobacter jejuni* and *C. coli* isolates with resistance of 50% and 54% respectively. This is because these species can express different antimicrobial resistance genes. The increasing prevalence of antimicrobial resistance among *Campylobacter* have great public health implication because people that consume raw milk, undercooked chicken, livestock farmers and meat processors are always exposed to this pathogenic organism (Olatoye and Ogunsemoyin, 2016).

6.3 Recommendation

Based on the findings from this research work, the followings are recommended to reduce the high prevalence of campylobacteriosis in the State.

1. There should be vigorous public awareness campaign on campylobacteriosis especially on those whose occupation predispose them to the diseases by the State Ministry of Health

2. Encouragement of more research on campylobacteriosis that will lead to development of Regional Centre of Excellence that will effectively monitor this disease by the State Ministry of Health

3. Discouraging the use of antibiotics as prophylaxis against human campylobacteriosis by the State Ministry of Health

4. The use of antibiotic in animal feed production should be controlled by the State Ministry of Agriculture

5. Susceptibility testing should be carried out before administering drugs to patients with campylobacteriosis, and Imipenem should be among the first line drug of choice

References

Aboh, E.A., Giwa, F.J. and Giwa, A. (2015). Microbiological Assessment of well water in Samaru, Zaria, Kaduna, State, Nigeria. *Annals of African Medicine,* **14** (1): 32-38

Adesiji, Y.O. and Oloke, J.K. (2015). Challenges of the control of opportunistic infections zoonotic origin in HIV/AIDS patients. *International Journal of Immunology*, **3**(2):1-7

Awofalo, A.A., and Ogundele, O.E. (2016). HIV epidemiology in Nigeria. *Saudi Journal of Biological Sciences,* **2**:55-68

Adekunle, O.C., Coker, A.O. and Kolawole, D.O. (2009). Incidence, Isolation and Characterization of *Campylobacter* species in Osogbo. *Biology* and *Medicine*, **1**: 24-27

Asakura, M., Samosornsuk, W., Hinenoya, A., Misawa, N., Nishimura, K., Matsuhisa, A., Yamasaki, S. (2008). Development of a cytolethal distending toxin (cdt) gene based species-specific multiplex PCR assay for the detection and identification of *Campylobacter jejuni, Campylobacter coli* and *Campylobacter fetus. FEMS Immunology Medical Microbiology*, **52**: 260-5

Bastyns, B., Chapelle, S., Vandamme, P., Goosens, H. and De Wachter, R. (1994). Species-specific detection of *Campylobacters* important in veterinary medicine by PCR amplification of 23S rDNA. *Systematic and Applied Microbiology*, **17**:5563-85

Batz, M.B., Hoffmann, S., Morris, J.G, **(**2012). Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. *Journal of Food Protection,* **75:**1278–1291.

Berrang, M.E., Buhr, R.J., Cason, J.A. and Dickena, J.A. (2001). Broiler carcass contamination with *Campylobacter* from faeces during defeathering. *Journal of Food Protection,* **64**: 2063-2066

Best, E.L., Powell, E.J., Swift, C., Grant, K.A., Frost, J.A. (2003). Applicability of a rapid duplex real time PCR assay for speciation of *Campylobacter jejuni* and *Campylobacter coli* directly from culture plates. *FEMS Microbiology letter* **229**: 237-41

Bradshaw, D., Nannan, N., Groenewald, P., Joubert, J., Laubscher, R., Nojilana, B., Norman, R., Pieterse, D., and Scheider, M. (2005). Provincial mortality in south Africa, 2000; priority setting for now and a benchmark for the future. *South Africa Medical Journal*, **95**(7):496-503

Burlew, R., Puckett, A., Bailey, R., Margarate, C., and Brantley, S. (2014). Assessing the Relevant efficiency and Sustainability of HIV/AIDS in-service Training in Nigeria. *Human Resources for Health,* **12**(1):1-5

Chantarapanont, W., Berrang, M. and Frank, J.F. (2003). Direct Microscopic Observation and viability determination of *Campylobacter jejuni* on chicken skin. *Journal of Food Protection,* **66**:2222-2230

Cebula, T.A., Jackson, S.A., Brown, E.W., Goswami, B., Leclerc, J.E. (2005). Chips and SNPs, bugs and Thugs: a molecular sleuthing perspectives. *Journal of Food Protection,* **68**: 1271-84

Cheebrough, M. (2006). *District Laboratory Practice in Tropical Countries*; part 2. Cambridge University Press. Pg 36-42.

Chia-ping, S., Marie, A.d-P., Kathleen, F., Meghan, I.S., Elleb, S., Seth, L., Kareen, G. and Sara, E.L. (2017). Occupational distribution of Campylobacteriosis and Salmonellosis cases – Maryland, Ohio and Virginia, 2014. *Morbidity and Mortality Weekly Report*, **66** (32): 850-853

Chua, K., Gurtler, V., Montgomery, J., Fraenkel, M., Mayall, B.C., Grayson, M.L. (2007). *Campylobacter insulanigrae* causing septicaemia and enteritis. *Journal of Medical Microbiology,* **56**: 1565-7

Clinical and Laboratory Standards Institute (CLSI) (2016). *Performance Standards for Antimicrobial susceptibility testing; Twenty two informational supplement*. Vol **31**(1): 45-57, 74

Coker, A.O., Isokpehi, R.D., Thomas, B.W., Amisu, K.O. and Obi, C.L. (2002). Human Campylobacterosis in Developing Countries. *Emerging Infectious Disease,* **8**:237-243

Coker, A.O. and Adefeso, A.O. (1994). The changing pattern of *Campylobacter jejuni* and *coli* in Lagos, Nigeria after ten years. *East Africa Medical Journal,* **71**: 437-40

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

Cummingham, S.A., Sloan, L.M., Nykre, L.M., Vetter, E.A., Mandrekar, J., Patel, R. (2010). Three hour molecular detection of *Campylobacter, Salmonella, Yersinia* and *Shigella* species in Faeces with accuracy as high as that of culture. *Journal of Clinical Microbiology,* **48**:2929-2933

Crutchly, R.D., Miller, J. and Garey, K.W. (2010). Crofelemer, a Novel Agent for Treatment of Secretory Diarrhoea. *The Annual Pharmacotherapy,* **44**: 40-45

Debruyne, L., Gevers, D. and Vandamme, P. (2005). "Taxonomy of the family *Campylobactereacea"* in *Campylobacter*, 3rd Edn, eds I. Nachamkin and M.J. Blaser (Washington, D.C.: American Society of Microbiology, 3-27).

Debruyne, L., Gevers, D. and Vandamme, P. (2008). *Taxonomy of the Family Campylobacteracea.* In *Campylobacter, 3rd ed*, ed. I Navhamkim, C.M. Szymanski, M.J. Blaser. Pp. 3-26. Washington, D.C: American Society for Microbiology

Debryne, L., On, S.L., De Brandt, E., Vandamme, P. (2009). Novel *Campylobacter lari*- like bacteria from humans and molluscs: description of *Campylobacter peloridis* spp nov., *Campylobacter lari* subsp. *Concheus* subsp. nov. and *Campylobacter lari* subsp. nov. *International Journal of Systemic Evolution in Microbiology*, **59**: 1126-32

Debryne, L., Broman, T., Bergstrom, S., Olsen, B., On, S.L., Vandamme, P. (2010a). *Campylobacter volucris* susp nov., isolated from black-heated gull (*Larus ridibundus)*. *International Journal of Systemic Evolution Microbiology*, **60**:1870-5

Dikman, A.E., Emily, S., Nalinee, C.S. and Michael, A.P. (2015). Human Immunodefficiency Virus Associated-Diarrhoea: Still an issue in the Era of Antiretroviral Therapy. *Digestive Disease of Science*, **60**:2236-2245

Duim, B., Wassennaar, T.M., Rigter, A., Wagnaar, J. (1999). High-resolution genotyping of *Campylobacter* strains isolated from poultry and humans with amplified fragment length polymorphism fingerprinting. *Applied of Environmental Microbiology*, **65**: 2369-75

Eyers, M., Chapelle, S., Camp, G.V., Goosens, H. and De Wachter, R. (1993). Dissemination among Thermophilic Campylobacter species by Polymerase Chain Reaction Amplification of 23S RNA Gene fragments. *Journal of Clinical Microbiology*, **32**(12):3340-3343

Fernandez-Cruz, A., Patricia, M., Rosa, M., Maricela, V., Mercede, M., Luis, A., Rodriguez-Creixem, M., Emilia, C. and Emilio, B. (2010). *Campylobacter* Bacteremiae: Clinical Characterisatics, Incidence and Outcome over 23 years. *Medicine*, **89**(5):319-330

Franklin, R.B., Taylor, D.R., Mills, A.L. (1999). Characterization of microbial communities using randomly amplified polymorphic DNA (RAPD). *Journal of Microbiology Methods*, **35**: 225-35

Frank, M.A. and Engberg, J. (2001). Antimicrobial resistance of thermophilic *Campylobacter* species. *Veterinary Research*, **32**:311-321

Gallay, A., Prouzet-Mauleon, V., Kempf, I., Lehours, P., Lebadi, L., Camou, C., Denis, M., de valk, H., Desenclos, J.C. and Megraud, F. (2007). *Campylobacter* drug resistance among Humans, broiler chicken and pigs, France. *Emerging Infectious Diseases*, **13**:259-266

Garenaux, A., Jugiau, F., Rama, F., Jonge, R., Denis, M., Federighi, M. and Ritz, M. (2008). Survival of *Campylobacter jejuni* strains from origins under oxidative stress conditions: effect of temperature. *Current Microbiology*, **56**:293-297

Gillespie, I.A., O'Brien, S.J., Frost, J.A., Tam, C., Tompkins, D., Neal, K.R. (2006) The *Campylobacter* Sentinel Surveillance Scheme Collaborators: Investigating Vomiting and-or Bloody Diarrhea in *Campylobacter jejuni* Infection. *Journal of Medical Microbiology*, **55**: 741 - 746

Grant, A.J. and Tee, W. (1998). Prevalence of *Campylobacter upsaliensis*-Associated diarrhoeic in HIV-infected patients. *Clinical Infectious Diseases,* **27**:816-21

Griffiths, P.L. (1997). Morphological changes of *Campylobacter jejuni* growing in liquid culture. *Letter Applied of Microbiology*, **17**: 152-5

Gonzalez, I., Grant, K.A., Richardson, P.T., Park, S.F., Collins, M.D. (1997). Specific Identification of the enteropathogenic *Campylobacter jejuni* and *Campylobacter coli* by using a PCR test based on the ceuE gene encoding a putative virulence determinants. *Journal of Clinical Microbiology,* **35**: 759-63

Gorkiewicz, G., Feierl, G., Schobar, C., Dieber, F., Kofer, J., Zechner, R., Zechner, E.L. (2003). Species-specific Identification of *Campylobacter* by partial 16S rRNA gene sequencing. *Journal of Clinical Microbiology,* **41**: 2537-46

Gurtler, W., Arter, T., Kasimir, S. and Fehlhaber, K. (2005). The Importance of *Campylobacter coli* in human Campylobacteriosis: Prevalence and genetic Characterization. *Epidemiology and Infection,* **133**: 1081-1087

Gwimi, P.B., Faleke, O.O., Salihu, M.D., Abubakar, M.B., Nwankwo, I.O. and Ibitoye, E. (2015). Prevalence of *Campylobacter* species in Fecal Sample of Pigs and Humans from Zuru, Kebbi State, Nigeria. *International Journal of One Health,* **1**:1-5

Havelaar, A.H., Nauta, M.J., Mangen, M.J., de Koiejer, A.G., Bogaardt, M.J., Evers, E.G., Jacobs-Reitsma, W.F., van Pelt, W., Wagenaar, J.A., de Wit, G.A. and van der Zee, H. (2005). Cost and benefits of controlling *Campylobacter* in the Netherlands; Integrating Risk analysis, Epidemiology and Economics. RIVM report 250911009/2005. Available at: http://www.rivm.ni/bibliotheek/rapporten/250911009.pdf.

Havelaar, A.H., Ivarsson, S., Lofdahl, M., Nauta, M.J. **(**2013). Estimating the true incidence of campylobacteriosis and salmonellosis in the European Union, 2009. *Epidemiology Infection*, **141:**293–302.

Hazeleger, W.C., Janes, J.D., Koenraad, P.M., Beumer, R.R., Rombout, F.M. and Abeg, T. (1995). Temperature-dependent membrane fatty acid and cell physiology changes in coccoid forms of *Campylobacter jejuni. Applied Environmental Microbiology*, **61**:2713-2719

Houng, H.S., Sethabutr, O., Nirdnoy, W., Katz, D.E., Pang, L.W. (2001). Development of ceuE-based Multiplex Polymerase Chain Reaction (PCR) assay of direct detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli* in Thailand. *Diagnostic Microbiology of Infectious Diseases,* **40**: 11-9

Humphries, R.M. and Linscott, A.J. (2015). Laboratory diagnosis of Bacterial Gastroenteritis. *Clinical Microbiology Review,* **28**: 3-31

Hwang, M.N., Ederer, G.M. (1975). Rapid Hippurate hydrolysis method for presumptive identification of group B streptococci. *Journal of Clinical Microbiology,* **1**: 114-5

Inglis, G.D., Boras, V.F., Houde, A. **(**2011). Enteric *Campylobacter* and RNA viruses associated with healthy and diarrheic humans in the Chinook Heath Region of Southwestern Alberta, Canada. *Journal of Clinical Microbiology*, **49:**209–219.

Jagannathan, A. and Penn, C. (2005). *Motility in Campylobacter. Molecular and Cellular Biology*, eds J.M. Ketley and M.E. Konkel. (Norfolk Horizon Bioscience), pp. 331-347

Jain, D., Prasad, K.N., Sinha, S. and Husain, N.(2008). Differences in virulence attributes both Cytolethal distending toxin positive and negative *Campylobacter jejuni* strains. *Journal of Medical Microbiology,* **57**: 267-272

Kaakoush, N.O., Castano-Rodriguez, N., Mitchell, H.M. and Man, S.M. (2015). Global epidemiology of *Campylobacter* infection. *Clinical Microbiology Review,* **28** (3): 687-720

Karikari, A.B., Danso, K.O., Frimpong, E.H. and Krogfelt, K.A. (2017). Antibiotic Resistance in *Campylobacter* species isolated from patients with Gastroenteritis in a Teaching Hospital in Ghana. *Open Journal of Medical Microbiology,* **7**: 1-11

Kaur, T., Singh, J., Huffman, M.A., Petrzelkova, K.J., Taylor, N.S., Xu, S., Dewhirst, F.E., Paster, B.J., Debryne, L., Vandamme, P., Fox, J.G. (2011). *Campylobacter troglodytis*-sp. nov., isolated from faeces of human habituated wild Chimpanzees in Tanzania. *Applied Environmental Microbiology,* **77**: 2366-73

King, S. and Adams, M.C. (2008). Incidence of *Campylobacter* in processed poultry. Is it a concern for human health? *Journal of Food Safety*, **28**: 376-388.

Klena, J.D., Parker, C.T., Knibb, K., Ibbitt, C., Devane, M.L.P., Horn, S.T., Miller, W.G. and Konkel, M. (2004). Differentiation of *Campylobacter coli, Campylobacter jejuni, Campylobacter lari* and *Campylobacter upsaliensis* by a multiplex PCR Developed from then nucleotide sequence of the lipid A gene *Ipx*A. *Journal of Clinical Microbiology,* **42** (12): 5549-5557

Kos, V.N., Gibreel, A., Keelan, M., Taylor, D.E. (2006). Species Identification of erythromycin-resistant *Campylobacter* isolates and optimization of duplex PCR for rapid detection. *Research in Microbiology,* **157**: 503-7

Kosek, M., Bern, C., Lozano, R., Bryce, J., and Guerrant, R.L. (2002). *The magnitude of the global burden of diarrhoeal diseases from studies published from 1992-2000.* Bulletin of the World Health Organization; In press.

Krumperman, P.H. (1983). Multiple Antibiotic Resistance indexing of *Escherichia coli* to identify high-risk sources of faecal contamination of food. *Applied Environmental Microbiology,* **46**: 165-170

Larson, K.R. and Spickler, A.R. (2013). *Zoonotic campylobacteriosis.* http://*www.cfsph.iastate.edu/DiseasInfo/Factsheets.php*

Lastovica, A. (2006). Emerging *Campylobacter* species: The tip of the iceberg. *Clinical Microbiology Newsletter,* **28**: 49-55

Lastovica, A.J. and Roux, L.E. (2000). Efficient Isolation of *Campylobacter* from stools. *Journal of Clinical Microbiology,* **38**:2798-2802

Lastovica, A.J., Allos, M.B. (2008). *Clinical significance of Campylobacter related species other than Campylobacter jejuni and Campylobacter coli. In Campylobacter,* 3rd ed, ed. I., Nachamkin, C.M., Szymanki, M.J. Blaser. Pp. 123 -50. Washington D.C.: *American Society for Microbiology*

Levin, R.E. (2007). *Campylobacter jejuni*: a review of its characteristics, pathogenicity, ecology, distribution, subspecies characterization and molecular methods of detection *Food Biotechnology,* **21**: 271-347

Linton, D., Lawson, A.J., Owen, R.J. Stanley, J. (1997). PCR detection, identification to species level and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* directly from diarrhoeic samples. *Journal of Clinical Microbiology,* **35**: 2568-72

Longenberger, A.H., Palumbo, A.J., Chu, A.K, Moll, M.E., Weltman, A, Ostroff, S.M. (2013). *Campylobacter jejuni* infections associated with unpasteurized milk-multiple state, 2012. *Clinical Infectious Diseases,* **57**: 263-266

Louwen, R., Van Baarlen, P., van Vliot, A.H.M., van Belkum, A., Hays, J.P. and Endtz, H.P. (2012). Campylobacter bacteremia: a rare and under-reported event? *European Journal of Microbiology and Immunology,* **2**(1):76-87

Lovett, J., Francis, D.W. and Hunt, J.M. (1983). Isolation of *Campylobacter jejuni* from Raw Milk. *Applied and Environmental Microbiology,* **46** (2):459-462

MacArthur, R.D. and DuPont, H.L. (2012). Etiology and Pharmacologic management of non-infectious diarrhoeal in HIV-infected individual in the Highly Active Antimicrobial. Therapy Era. *Clinical Infectious Diseases,* **55**(6):860-867

Man, S.M. (2011).The Clinical importance of emerging *Campylobacter* species. *Natural Review Gastroenterology Hepatology,* **8**:665-685

Marian, M.N., Aminah, S.M., Zuraini, M.I., Son, R., Maimunah, M., Lee, H.Y. and Wong, W.C. (2012). MPN-PCR detection and antimicrobial resistance of *Listeria monocytogenes* isolated from raw and ready-to-eat foods in Malaysia. *Food Control,* **28**: 309- 314

Martiny, D., Dediste, A., Debruyne, L., Vlaes, L., Ben Haddou, N., Vandamme, P., Vandenberg, O. (2010). Accuracy of API Campy system, the Vitek 2 Neisseria-Haemophilus (NH) card and the Matrix-Assisted laser Desorption / ionisation Time-of-flight mass spectrometry (MALDI-TOFMS) for the identification of *Campylobacter* and Related organisms. *Clinical Microbiology Infection,* Jul 29 Epub ahead of print.

Meerbury, B.G., Jacob-Reitsma, W.F., Wagenaar, J.A., Kijlstra, A. (2006). Presence of *Salmonella* and *Campylobacter* species in wild Mammals on organic farms. *Applied Environmental Microbiology,* **72**: 960-2

Melo, R.T., Nalevaiko, P.C., Mendonca, E.P., Borges, L.W., Fonseca, B.B., Beletti, M.E. and Rossi, D.A. (2013). *Campylobacter jejuni* strains isolated from chicken meat harbour several virulence factors and a potential risk to humans. *Food Control*, **33**:227-31

Miller, W.G., Health, S., Parker, G. and Lastovica, A.J. (2007). Identification of genomic differences between *Campylobacter jejuni* subsp *jejuni* and *Campylobacter jejuni* subsp *doylei* at the nap locus leads to the development of a *Campylobacter jejuni* subspeciation multiplex PCR method. *BioMedical Centre Microbiology,* **7**: 11 -18

Moore, J.E., Corcoran, D., Dooley, J.S.G, Fanning, S., Lucey, B., Matsuda, M., Mcdowell, D.A., Megraud, F., Millar, C. B., Mahony, R.O., Riordan, L.O., Rourke, M.O., Rao., J.R., Rooney, P.J., Sailsi, A. and Whyte, P. (2005). C*ampylobacter*. *Veterinary Research* **36**: 351-382

Moore, J.E., Barton, M.D., Blair, I.S., Corcoran, D., Dooley, J.S.G., Fanning, S., Kempf, I., Lastovica, A.J., Lowery, C.J., Matsuda, M., McDowell, D.A., McMahon, A., Miller, B.C., RaO, J.R., Rooney, P.J., Seal, B.S., Snelling, W.J. and Tolba, O. (2006). The epidemiology of antibiotics resistance in *Campylobacter* species. *Microbes and Infections*, **8**: 1955-1966

Naing, L., Winn, T. and Rusli, B.N. (2006). *Sample size calculator for prevalence studies.* Available at: http://www.kck.usm.my/ppsg/stats_resources.htm

National Action Committee on AIDS (NACA) 2015: *Federal Republic of Nigeria Global AIDS Response*; Country progress Report, Abuja, Nigeria

National Action Committee on AIDS (NACA) 2016: *Federal Republic of Nigeria Global AIDS Response*; Country progress Report, Abuja, Nigeria

Olatoye, O. and Ogunsemoyin, O. (2016). Prevalence and Antibiotics Resistance of *Campylobacter jejuni* in Retailed Chicken in Oyo State, Nigeria. *Food Science and Quality Management*, **48**:7-12

On, S.L.W. (2001). Taxonomy of *Campylobacter, Arcobacter, Helicobacter* and related bacteria: current status, future prospects and immediate concerns. *Journal of Applied Microbiology,* **90**:15-20

Opintan, J.A., Newmann, M.J., Ayeh-kumi, P.F., Affrim, R., Gepi-Allee, R., Sevilleja, J.E., Roche, J.K., Nataro, J.P., Warren, C.A., and Guerrant, R.L. (2010). Pediatric diarrhoea in Southern Ghana; Etiology and Association with Intestinal Inflammation and Malnutrition. *American Journal of Tropical Medicine and Hygiene,* **83**(4):936-943

Oyarzabel, O.A., Wesley, W., Harmon, K.M., Schroeder-Trucker, L., Barbares, J.M., Lauerman, L.H., Backert, S., Conner, D.E. (1997). Specific Identification of *Campylobacter fetus* by PCR targeting variable regions of the 16S rDNA. *Veterinary Microbiology,* **58**: 61-71

Parker, C.T., Miller, W.G., Horn, S.T. and Lastovica, A.J. (2007). Common genomic features of *Campylobacter jejuni* subsp *doylei* strains distinguish them from *Campylobacter jejuni* subsp *jejuni*. *Biomedical Centre Microbiology*, **5**: 50 -59

Payot, S., Dridi, S., Laroche, M., Federighi, M. and Magras, C. (2004). Prevalence and antimicrobial resistance of *Campylobacter coli* isolated from flattening pigs in France. *Veterinary Microbiology,* **101**: 91-99

Penner, J.L. (1988). The genus *Campylobacter*: a decade of progress. *Clinical Microbiology Reviews,* **1**(2):157-172

Penner, J.L. and Hennessy, J.N. (1980). Passive haemogglutination technique for serotyping *Campylobacter fetus* subsp *jejuni* on the basis of soluble heat-stable antigens. *Journal of Clinical Microbiology,* **12**: 732-7

Persson, S. and Olsen, K.E. (2005). Multiplex PCR for identification of *Campylobacter coli* and *Campylobacter jejuni* from pure cultures and directly on stool samples. *Journal of Medical Microbiology,* **54**: 1043-7

Price, E.P., Thiruvenkataswamy, V., Mickan, L., Unicomb, L., Rios, R.E., Huygena, F., Giffard, P.M. (2006). Genotyping of *Campylobacter jejuni* using seven simple-nucleotide polymorphisms in combination with flaA short variable region sequencing. *Journal of Medical Microbiology,* **55**: 1061-70

Popowski, J., Lekowska, K.A. and Korsak, D. (1997). The incidence of heat tolerant *Campylobacter* in rivers and lakes of Warsaw region. *Roczniki Panstwowego Zakladu Higieni,* **48**: 253-262

Rao, MR, Naficy, AB, Savarino, SJ, Abu-Elyazeed, R, Wierzba, TF, Peruski, LF, Abdel-Messih, I, Frenck R, Clemens JD**.** 2001. Pathogenicity and convalescent excretion of *Campylobacter* in rural Egyptian children. *America Journal of Epidemiology*, **154:**166–173

Rossit, A.R.B., Goncalves, A.C.M., Fransco, C., and Machido, R.L.D. (2009). Etiological agents of Diarrhoea in Patients infected by the Human Immunodeficiency Virus. *Revelutional International Medical Tropical Southern Paulo,* **51**(2):59-65

Rodrigues, C.G., Melo, R.T., Fonseca, B.B., Martins, P.A., Ferreira, F.A., Arauyo, M.B.J. and Rossi, D.A. (2015). Occurrence and Characterization of *Campylobacter* species Isolated in Dogs, Cats and Children. *Pesquista Veterinaria Brasileira,* **35** (4): 365-370

Sample, D., Detrchis, P., Leport, C., Bouvet, E., Karam, D. & Mevohas, M- C.

(1991).Efficacy of Zidovudine in Preventing Relapses of *Salmonella* Bacteremia in AIDS. *Journal of Infectious Diseases,* **163**: 415-416.

Samie, A., Bessong, P.O., Obi, C.L., Dillingham, R. and Guerrant, R.L. (2011). *Bacterial and Parasitic Agents of Infectious Diarrhoea in the Era of HIV/AIDS- The cause of a semi-Rural community in South Africa. Microbes, Viruses, and Parasites in AIDS process;* Prof. Vladin Ar Zajae (Ed). ISN: 978-938-307-601-0

Samuel, M.C., Vugia, D.C., Shallow, S, Marcus, R., Begler, S., McGiven, T., Kassenborg, H., Reilly, K., Kennedy, M, Angulo, F., Tauxe, R.V. (2004). Epidemiology of sporadic *Campylobacter* infection in the United State and declining trend in incidence, foodNet 1996-1999. *Clinical Infectious Diseases,* **58**: 165-74

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

Silva, R.C., Benati, F.J., Pena, G.P.A., and Santos, N. (2010). Molecular Characterization of Viruses associated with gastrointestinal infection in HIV-positive patients. *Brazil Journal of Infectious Disease,* **4**(6):549-552

Silva, J., Leite, D., Fernandes, M., Mena, C., Gibbs, P.A. and Teixeira, P. (2011). *Campylobacter* species as a foodborne pathogens; a review. *Frontiers in Microbiology*, **22**:1-13

Smith, S., Otuoye, N., Omonigbehin, E., Ariyo, F., and Nkoth, N. (2005). Prevalence of *Campylobacter* species during HIV/AIDS patients in Nigeria. *Retrovirology,* **2**:76-78

Snijders, F., Kuijper, E.J., De Wever, B., Van der Hoek, L., Danner, S.A. and Dankert, J. (1997). Prevalence of *Campylobacter*-Associated diarrhoea among patients infected with Human Immunodeficiency Virus. *Clinical Infectious Diseases,* **24**:1107-13

Strid, M.A., Engberg, J., Larsen, L.B., Begtrup, K., Molbak, K., Krogfelt, K.A. (2001). Antibody responses to *Campylobacter* infections determined by enzyme-linked immunosorbent assay: 2 years follow-up study of 210 patients. *Clinical Diagnostic Laboratory Immunology,* **8**:314-9

Tee, W., and Mijch, A. (1998). *Campylobacter jejuni* Bacteremia in HIV infected patients. Comparison of Clinical features and Review. *Clinical Infections Disease,* **26**:91-6

Uaboi-Egbenni, P.O., Okolie D.N., Adesanya, O.D. and Omonigbehi, E. (2008). Epidemiological studies of the incidence of pathogenic *Campylobacter* species amongst animal in Lagos metropolis. *Africa Journal of Biotechnology,* **7**(6):2852-56

United State Agency for International development: *USAID/Nigeria Strategy 2014 World Health Organization. WHO Report 2004: Changing History. Geneva: World Health Organization*, **2004**:200

Vandamme, I. (2000). *Taxonomy of the family Campylobacteracae. In Campylobacter,* 2nd ed, ed. I. Nachamkin, M.J. Blaser, pp. 3-26. Washington, D.C.: American Society for Microbiology

Van Deum, K., Haesebrouck, F., Hendricke, M., Favoreel, H., Dewulf, J., Ceelen, L., Dumez, L., Messena, W., Leleu, S., Van Immersal, F., Ducatelle, P. and Pasmans, F. (2007). Virulence properties of *Campylobacter jejuni* isolates of poultry and human origin. *Journal of Medical Microbiology,* **56**: 1284-1289

Vlieghe, E.R., Jacobs, J.A., Esbroeck, M.V., Koole, O. and Gompel, A.V. (2008). Trends of Norfloxacin and Erythromycin Resistance of *Campylobacter jejuni* and *C. coli* isolates recovered from intestinational travellers, 1994 to 2006. *Journal of Travel Medicines*, **15**: 419-425

Vos, P., Hogers, R., Blecker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. (1995). AFLP: a new technique for DNA Fingerprinting. *Nucleic Acids Resolution,* **23**: 4407-14

Wang, G., Clark, C.G., Taylor, M., Pucknell, C., Price, L., Woodward, D.L. and Rodger, F.G. (2002). Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni, C. coli, C. upsaliensis* and *C. fetus* subsp *fetus. Journal of Clinical Microbiology,* **40**:4744-7

Wassenaar, T.M. (1997). Toxin production by *Campylobacter* species. *Clinical Microbiology Review,* **10** (3): 466-476

Wassenaar, J.A. and Newell, D.G. (2000). "*Poultry infection and their control at the farm level" in Campylobacter*. 2nd Edn, Eds I. Nachamkin and M.J. Blaser (Washington, D.C.: American Society of Microbiology press) 497-510

Wysok, B., Wiszniewska-Laszczych, A., Uradzinski, J. and Szteyn, J. (2011). Prevalence and antimicrobial resistance of *Campylobacter* in raw milk in some selected area of Poland. *Polish Journal of Veterinary Science,* **14** (3): 473-477.

Workman, N.S., Mathison, E.G. and Lavoie, C.M. (2005). Pet dogs and Chicken meats as Reservior of *Campylobacter* species in Barbados. *Journal of Clinical Microbiology*, **43**(6): 2642-2650

Zanomi, R.G., Debruyne, L., Rossi, M., Revez, J., Vandamme, P. (2009). *Campylobacter cuniculorum* sp. nov., from rabbits. *International Journal of Systemic Evolution Microbiology,* **59**: 1666-71

APPENDIX A

QUESTIONNAIRE

A research on Prevalence and Molecular Detection of *Campylobacter* species among Diarrhoeic HIV- Patients in some Hospitals in Kaduna State, Nigeria. Please do honestly fill this questionnaire as this will go a long way in affecting the result of the research.

Thank you very much in anticipation of your cooperation.

SECTION A: BIODATA

1. Reference number: …….
2. Age (years): 1 – 10 ( ), 11 – 20 ( ), 21 – 30 ( ), 31 – 40 ( ), 41 – 50 ( ), 51 and above ( ).
3. Gender: Male ( ), Female ( ).
4. Educational level: Tertiary ( ), Secondary ( ), Primary ( ), Arabic ( ).
5. Occupation: Civil servant ( ), Self-employed ( ), others ( ).

SECTION B: CLINICAL DATA

Presenting symptoms of ailment

1. Fever, Yes ( ) No ( )
2. Headache, Yes ( ) No ( )
3. Nausea, Yes ( ) No ( )
4. Weakness, Yes ( ) No ( )
5. Abdominal discomfort, Yes ( ) No ( )
6. Diarrhoea Yes ( ) No ( )
7. Vomiting, Yes ( ) No ( )

SECTION C: RISK FACTORS

1. Which sewage disposal method do you use at home? Pit latrine ( ) Septic tank ( )

2. What is your source of drinking water? Tap ( ) River ( ) Well ( ) Others ( )

3. Have you ever been in close contact with farm animals? Yes ( ) No ( )

4. Do you like taking raw milk (nono da fura)? Yes ( ) No ( )

5. How often do you eat chicken? Always ( ), Occasionally ( ), Never ( )

7. How often do you eat *suya* or under-cooked meat? Always ( ), Occasionally ( ), Never ( )

APPENDIX B

ETHICAL CLEARANCE

MINISTRY OF HEALTH AND HUMAN SERVICES
KADUNA STATE, NIGERIA

MOH/ADM/744/VOL.1/524 28TH JULY, 2017

NOTICE OF APPROVAL AFTER FULL COMMITTEE REVIEW

PREVALENCE AND MOLECULAR DETECTION OF Campylobacter Species AMONG HIV DIARRHEIC PATIENTS IN SOME PARTS OF KADUNA STATE

Name of Principal Investigator :	**OGBOMON ERNEST**
Address of Ethical Approval :	**Department of Microbiology,**
	Faculty of Life Sciences,
	Ahmadu Bello University,
	Zaria.
Date of receipt of Application :	**8th June, 2017**
Date of Ethical Approval :	**26th July, 2017**

This is to inform you that the Research described in the submitted Protocol, the Consent Forms, advertisements and other participant information materials have been reviewed and given full approval by the the Health Research Ethics committee (HREC).

If there is delay in starting the research or any change, inform the HREC so that the dates of approval can be adjusted accordingly.

However, Researcher is kindly requested to submit a copy of his/her findings to the State Ministry of Health, please.

Dr. BUTAWA NN

Secretary

For: Chairman.

SMOH Resource Centre Kaduna. Date 31/8/2017

Independence Way, P.M.B 2014, Kaduna, Kaduna State-Nigeria
Tel: +234(0) 818 407 8693 | Website: www.kdsg.gov.ng | Email: health@kdsg.gov.ng

APPENDIX C

API CAMPY KIT reaction during characterization of isolates

APPENDIX D

API CAMPY kit before Characterization

APPENDIX E

API Campy kit Identification of *Campylobacter* species

BIOMÉRIEUX

AHMADU BELLO UNIVERSITY - ZARIA

apiweb™

API CAMPY V3.0 | Printout | Export | New test | Modify

REFERENCE

DATE 6/4/18

COMMENT

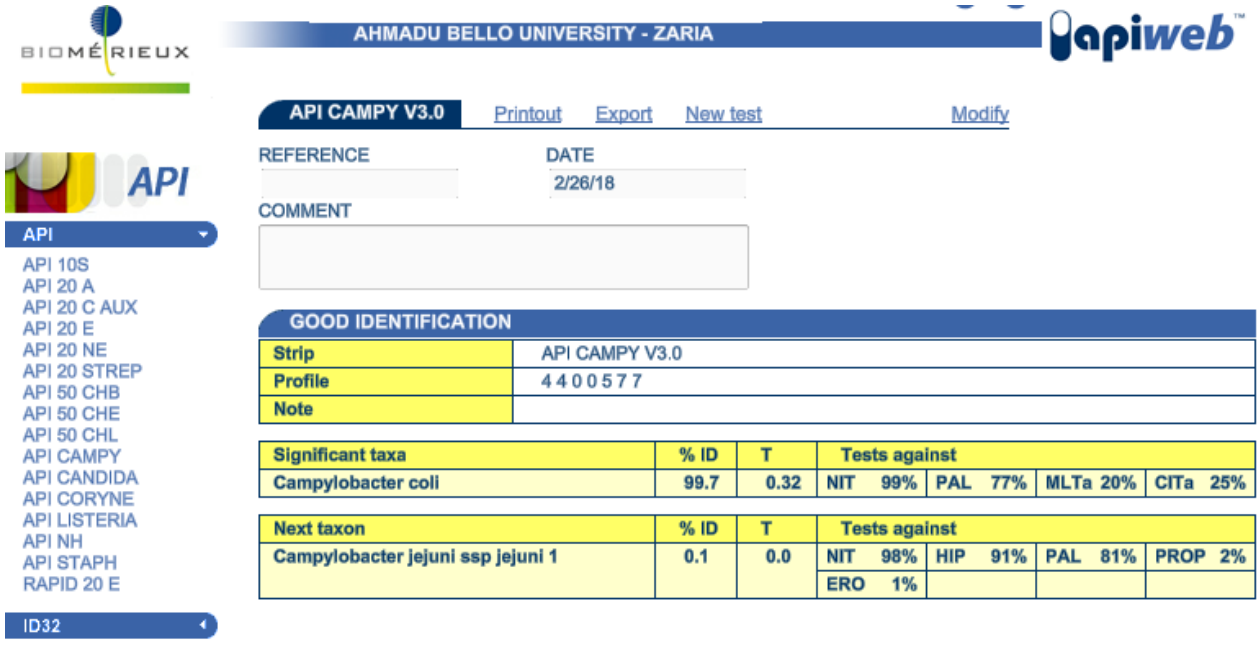
API
10S
20 A
20 C AUX
20 E
20 NE
20 STREP
50 CHB
50 CHE
50 CHL
CAMPY
CANDIDA
CORYNE
LISTERIA
NH
STAPH
ID 20 E

GOOD IDENTIFICATION

Strip	API CAMPY V3.0
Profile	6 5 0 4 5 5 5
Note	

Significant taxa	% ID	T	Tests against			
Campylobacter jejuni ssp jejuni 1	99.8	0.49	PAL 81%	GLUa 1%		

Next taxon	% ID	T	Tests against			
Campylobacter jejuni ssp jejuni 2	0.1	0.0	GGT 100%	GLUa 0%	CITa 25%	



BIOMÉRIEUX

AHMADU BELLO UNIVERSITY - ZARIA

apiweb™

API CAMPY V3.0 | Printout | Export | New test | Modify

REFERENCE

DATE 2/26/18

COMMENT

API
API 10S
API 20 A
API 20 C AUX
API 20 E
API 20 NE
API 20 STREP
API 50 CHB
API 50 CHE
API 50 CHL
API CAMPY
API CANDIDA
API CORYNE
API LISTERIA
API NH
API STAPH
RAPID 20 E
ID32

GOOD IDENTIFICATION

Strip	API CAMPY V3.0
Profile	4 4 0 0 5 7 7
Note	

Significant taxa	% ID	T	Tests against			
Campylobacter coli	99.7	0.32	NIT 99%	PAL 77%	MLTa 20%	CITa 25%

Next taxon	% ID	T	Tests against			
Campylobacter jejuni ssp jejuni 1	0.1	0.0	NIT 98%	HIP 91%	PAL 81%	PROP 2%
			ERO 1%			

AHMADU BELLO UNIVERSITY - ZARIA

apiweb™

API CAMPY V3.0 Printout Export New test Modify

REFERENCE

DATE 2/26/18

COMMENT

ACCEPTABLE IDENTIFICATION TO THE GENUS

Strip	API CAMPY V3.0
Profile	6 4 0 2 5 1 7
Note	

Significant taxa	% ID	T	Tests against							
Campylobacter coli	96.2	0.31	PAL	77%	H2S	1%	CITa	25%		
Campylobacter hyointestinalis	3.6	0.06	EST	9%	NAL	75%	CFZ	5%	CITa	4%
			ERO	4%						

Next taxon	% ID	T	Tests against							
Campylobacter fetus ssp fetus	0.1	0.0	H2S	0%	NAL	78%	CITa	7%	ERO	2%

Complementary test(s)	ANA+TMAO	CFTR R	25°C	
Campylobacter coli	-	+	-	
Campylobacter hyointestinalis	+	-	+(-)	

BIOMÉRIEUX

API

- API
  - API 10S
  - API 20 A
  - API 20 C AUX
  - API 20 E
  - API 20 NE
  - API 20 STREP
  - API 50 CHB
  - API 50 CHE
  - API 50 CHL
  - API CAMPY
  - API CANDIDA
  - API CORYNE
  - API LISTERIA
  - API NH
  - API STAPH
  - RAPID 20 E

AHMADU BELLO UNIVERSITY - ZARIA

apiweb™

API CAMPY V3.0 Printout Export New test Modify

REFERENCE

DATE 2/26/18

COMMENT Nono

VERY GOOD IDENTIFICATION

Strip	API CAMPY V3.0
Profile	6 4 0 0 5 3 7
Note	

Significant taxa	% ID	T	Tests against							
Campylobacter coli	99.9	0.7	PAL	77%	CITa	25%				

Next taxon	% ID	T	Tests against							
Campylobacter fetus ssp fetus	0.1	0.05	NAL	78%	PROP	3%	CITa	7%	ERO	2%

AHMADU BELLO UNIVERSITY - ZARIA

API

API 10S
API 20 A
API 20 C AUX
API 20 E
API 20 NE
API 20 STREP
API 50 CHB
API 50 CHE
API 50 CHL
API CAMPY
API CANDIDA
API CORYNE
API LISTERIA
API NH
API STAPH
RAPID 20 E

ID32

API CAMPY V3.0 Printout Export New test Modify

REFERENCE

DATE 2/26/18

COMMENT

GOOD IDENTIFICATION

Strip	API CAMPY V3.0
Profile	4 4 0 0 5 7 7
Note	

Significant taxa	% ID	T	Tests against			
Campylobacter coli	99.7	0.32	NIT 99%	PAL 77%	MLTa 20%	CITa 25%

Next taxon	% ID	T	Tests against			
Campylobacter jejuni ssp jejuni 1	0.1	0.0	NIT 98%	HIP 91%	PAL 81%	PROP 2%
			ERO 1%			

AHMADU BELLO UNIVERSITY - ZARIA

API

API 10S
API 20 A
API 20 C AUX
API 20 E
API 20 NE
API 20 STREP
API 50 CHB
API 50 CHE
API 50 CHL
API CAMPY
API CANDIDA
API CORYNE
API LISTERIA
API NH
API STAPH
RAPID 20 E

ID32

API CAMPY V3.0 Printout Export New test Modify

REFERENCE k2

DATE 2/26/18

COMMENT

ACCEPTABLE IDENTIFICATION

Strip	API CAMPY V3.0
Profile	4 5 0 4 5 5 5
Note	

Significant taxa	% ID	T	Tests against			
Campylobacter jejuni ssp jejuni 1	99.2	0.24	NIT 98%	PAL 81%	GLUa 1%	

Next taxon	% ID	T	Tests against			
Campylobacter jejuni ssp jejuni 2	0.4	0.0	NIT 92%	GGT 100%	GLUa 0%	CITa 25%

AHMADU BELLO UNIVERSITY - ZARIA

API

API 10S
API 20 A
API 20 C AUX
API 20 E
API 20 NE
API 20 STREP
API 50 CHB
API 50 CHE
API 50 CHL
API CAMPY
API CANDIDA
API CORYNE
API LISTERIA
API NH
API STAPH
RAPID 20 E

ID32

API CAMPY V3.0 Printout Export New test Modify

REFERENCE: B
DATE: 2/26/18
COMMENT:

GOOD IDENTIFICATION TO THE GENUS

Strip	API CAMPY V3.0
Profile	6 4 0 0 7 5 6
Note	

Significant taxa	% ID	T	Tests against			
Campylobacter fetus ssp fetus	81.4	0.59	ERO 2%			
Campylobacter coli	15.1	0.45	PAL 77%	NAL 10%	MLTa 20%	
Campylobacter hyointestinalis	3.0	0.33	EST 9%	CFZ 5%	ERO 4%	

Next taxon	% ID	T	Tests against			
Campylobacter jejuni ssp jejuni 1	0.3	0.15	HIP 91%	PAL 81%	NAL 4%	ERO 1%

Complementary test(s)	ANA+TMAO	CFTR R	25°C	
Campylobacter coli	-	+	-	
Campylobacter fetus ssp fetus	-	-	+	
Campylobacter hyointestinalis	+	-	+(-)	

AHMADU BELLO UNIVERSITY - ZARIA

API

API 10S
API 20 A
API 20 C AUX
API 20 E
API 20 NE
API 20 STREP
API 50 CHB
API 50 CHE
API 50 CHL
API CAMPY
API CANDIDA
API CORYNE
API LISTERIA
API NH
API STAPH
RAPID 20 E

ID32

API CAMPY V3.0 Printout Export New test Modify

REFERENCE:
DATE: 2/26/18
COMMENT: K3

GOOD IDENTIFICATION

Strip	API CAMPY V3.0
Profile	4 4 0 2 7 7 4
Note	

Significant taxa	% ID	T	Tests against			
Campylobacter hyointestinalis	99.1	0.33	EST 9%	CFZ 5%	PROP 4%	

Next taxon	% ID	T	Tests against			
Campylobacter coli	0.6	0.0	NIT 99%	PAL 77%	H2S 1%	NAL 10%
			MLTa 20%			

BIOMÉRIEUX

AHMADU BELLO UNIVERSITY - ZARIA

apiweb™

API

API
API 10S
API 20 A
API 20 C AUX
API 20 E
API 20 NE
API 20 STREP
API 50 CHB
API 50 CHE
API 50 CHL
API CAMPY
API CANDIDA
API CORYNE
API LISTERIA
API NH
API STAPH
RAPID 20 E

API CAMPY V3.0 Printout Export New test Modify

REFERENCE 9SA

DATE 2/26/18

COMMENT

VERY GOOD IDENTIFICATION

Strip	API CAMPY V3.0
Profile	6400577
Note	

Significant taxa	% ID	T	Tests against			
Campylobacter coli	99.8	0.6	PAL 77%	MLTa 20%	CITa 25%	

Next taxon	% ID	T	Tests against			
Campylobacter jejuni ssp jejuni 1	0.1	0.05	HIP 91%	PAL 81%	PROP 2%	ERO 1%

BIOMÉRIEUX

AHMADU BELLO UNIVERSITY - ZARIA

apiweb™

API

API
API 10S
API 20 A
API 20 C AUX
API 20 E
API 20 NE
API 20 STREP
API 50 CHB
API 50 CHE
API 50 CHL
API CAMPY
API CANDIDA
API CORYNE
API LISTERIA
API NH
API STAPH
RAPID 20 E

API CAMPY V3.0 Printout Export New test Modify

REFERENCE

DATE 6/5/18

COMMENT

GOOD IDENTIFICATION

Strip	API CAMPY V3.0
Profile	4500004
Note	

Significant taxa	% ID	T	Tests against			
Campylobacter jejuni ssp doylei	95.3	0.69	PAL 92%			

Next taxon	% ID	T	Tests against			
Campylobacter upsaliensis	4.1	0.38	NIT 78%	EST 7%	HIP 8%	

APPENDIX F

Zones of inhibition of the antibacterial susceptibility testing of the *Campylobacter* isolates

Isolates Code	Antibiotics zones of inhibition (mm)								
	AZM	IPM	NA	TE	CN	CIP	E	AML	OX
CT14	20	28	12	18	17	25	21	13	10
CT11	10	25	21	10	20	18	18	10	12
CT24	15	30	20	10	22	25	12	12	19
CT29	23	30	14	10	22	23	10	18	12
CT06	18	26	19	12	28	20	10	10	18
CT31	17	26	14	12	18	18	15	12	18
PM16	19	28	12	12	27	20	15	15	12
CT19	23	25	15	13	28	22	12	12	15
CT44	16	25	22	10	20	15	10	16	19
CT30	10	26	20	14	18	22	15	10	15
CT59	18	30	17	21	12	15	15	10	12
PM11	20	28	10	18	20	22	10	15	17
PM21	19	25	20	19	22	22	10	19	12
TI23	15	24	19	15	19	23	13	15	10
PI03	10	25	22	17	20	15	16	18	14
PM24	22	23	19	20	22	25	19	18	12
CT18	12	26	20	10	18	20	10	12	18
CT71	10	25	16	16	17	26	10	12	10

Isolates Code	Antibiotics zones of inhibition (mm)								
S036	10	25	20	10	22	25	10	10	10
S018	30	26	19	12	28	15	10	12	15
S062	16	28	15	10	20	15	15	12	18
S042	12	30	12	10	18	20	18	10	15
S071	20	28	12	19	20	13	22	20	10
S026	10	25	14	10	28	20	16	10	12
S056	18	30	20	10	20	20	10	16	19
S014	10	26	15	10	18	20	16	19	10
S077	23	30	14	10	22	23	12	18	19
S003	19	26	12	12	23	25	15	18	19
S065	18	28	14	21	19	24	28	17	10
S037	20	24	19	12	20	25	15	18	12
S066	10	25	14	10	18	10	15	18	25
S059	19	25	18	19	18	22	10	19	12
S070	23	30	14	10	22	23	12	18	19
CT109	18	28	14	21	19	14	28	17	10
CT116	12	30	15	15	18	20	18	10	15
PM005	10	25	10	15	28	20	16	10	15
TI171	15	26	20	22	25	25	15	18	22
CT250	20	24	18	18	20	25	15	18	13

Isolates Code	Antibiotics zones of inhibition (mm)								
CT025	10	25	14	10	18	20	15	18	10
CT015	13	30	14	12	22	13	12	15	10
TI022	12	24	20	10	20	15	10	12	15
TI020	30	26	22	15	28	15	10	12	15
G23	10	25	16	10	18	20	15	10	12
G13	20	22	14	15	20	24	15	12	10
G05	12	24	12	10	19	24	12	10	12

KEY:*Campylobacter jejuni*: CT14, CT11, CT31, CT59, TI23, CT71, S071, S065, S037, S066, CT250, CT109, CT025, G23, G13.

Campylobacter coli: CT24, CT06, CT30, CT19, PM11, CT18, PI03, CT44, PM16, S036, S018, S062, S042, S026, S056, S014, PM005, CT116, TI022, TI025.

Campylobacter fetus: CT29, S077, S003, S070, TI171, CT015, G05

Campylobacter hyointestinalis: PM24, PM21, S059

APPENDIX G

Antibiotic resistance profile of Campylobacter species isolated from Diarrhoeic HIV-patients in some Hospitals in Kaduna state

Antibiotics	*Campylobacter* species			
Resistance profile	*C. jejuni*	*C. coli*	*C. fetus*	*C. hyointestinalis*
OXL	15	20	7	3
IPM	0	0	0	0
AZM	5	10	1	0
NA	12	9	6	1
TE	6	15	6	0
CN	1	0	0	0
CIP	8	14	0	0
E	12	20	7	2
AML	9	18	2	0
NA-E	12	9	6	0
OXL-E	12	20	7	3
NA-AML	9	9	2	0
AZM-CN	1	0	0	0
NA-E-OXL	12	9	6	1
NA-E-AML	9	9	2	1
NA-TE-E-AML-OXL	6	9	1	0
NA-CIP-E-AML-OXL	8	9	0	0
AZM-NA-TE-AML-OXL	5	9	1	0
NA-TE-CIP-E-AML-OXL	6	9	0	0
AZM-E-CIP-OXL-E-NA-TE	5	8	0	0
AZM-NA-TE-CN-CIP-E-AML-OXL	1	0	0	0

APPENDIX H

Interpretation of zones of inhibition

Antibiotic	Diameter to the nearest susceptibility (S)	Immediate (I)	Diameter to the nearest resistance (R)
AZM	≥ 13	-	≤ 12
IPM	≥ 23	20 - 22	≤ 19
NA	≥ 19	14 -18	≤ 13
TE	≥ 15	12 -14	≤ 11
CN	≥ 15	13 -14	≤ 12
CIP	≥ 21	16 -20	≤ 15
E	≥ 19	16 - 18	≤ 15
AML	≥ 18	14 -17	≤13
OX	≥ 22	-	≤ 21

CLSI, (2016)

APPENDIX I

Plates showing the various zones of inhibition of the Campylobacter isolates to the tested antibiotics

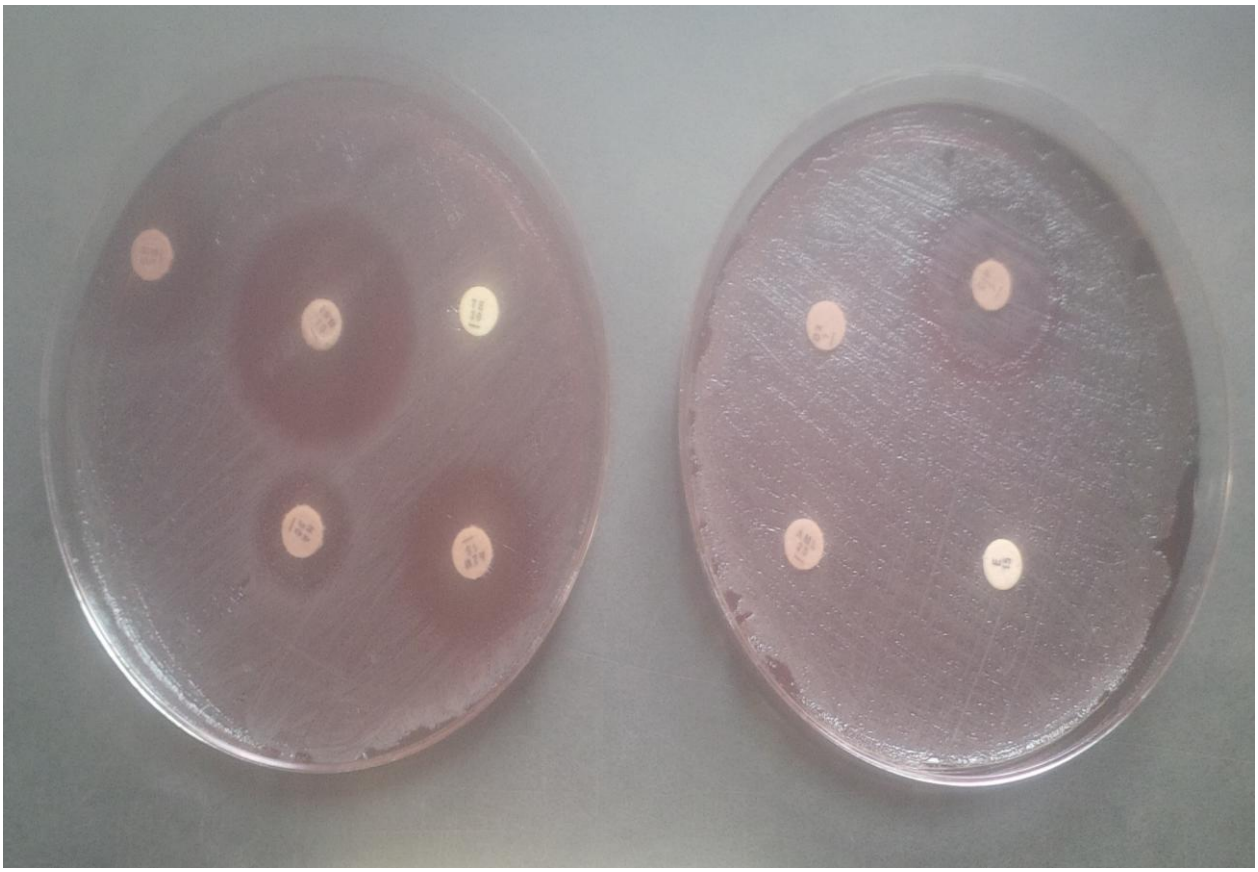


Plate II: showing the zones of inhibition of *Campylobacter fetus* to the tested antibiotics

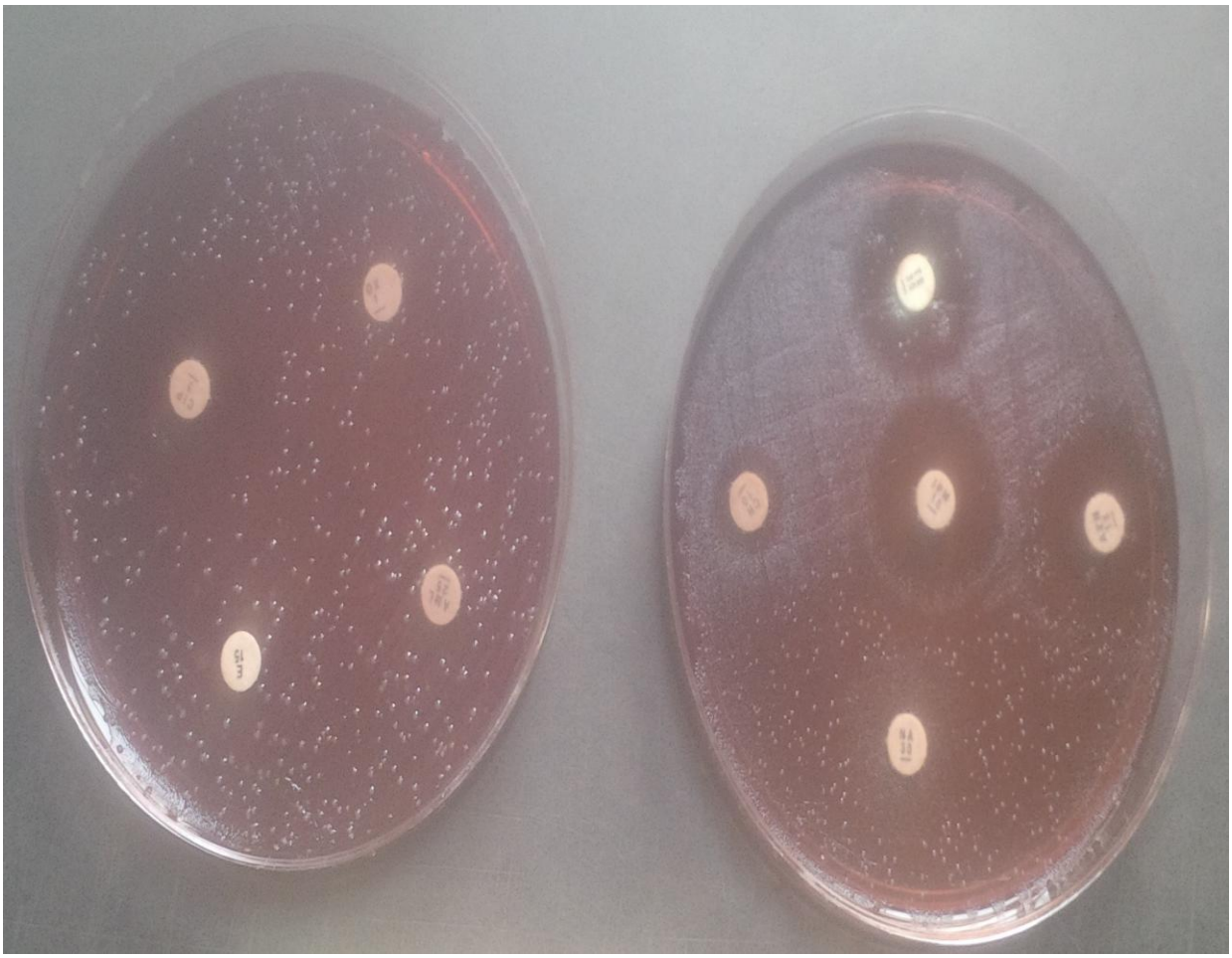


Plate III: showing the zones of inhibition of *Campylobacter hyointestinalis* to the tested antibiotics

Plate IV: showing the zones of inhibition of *Campylobacter coli* to the tested antibiotics

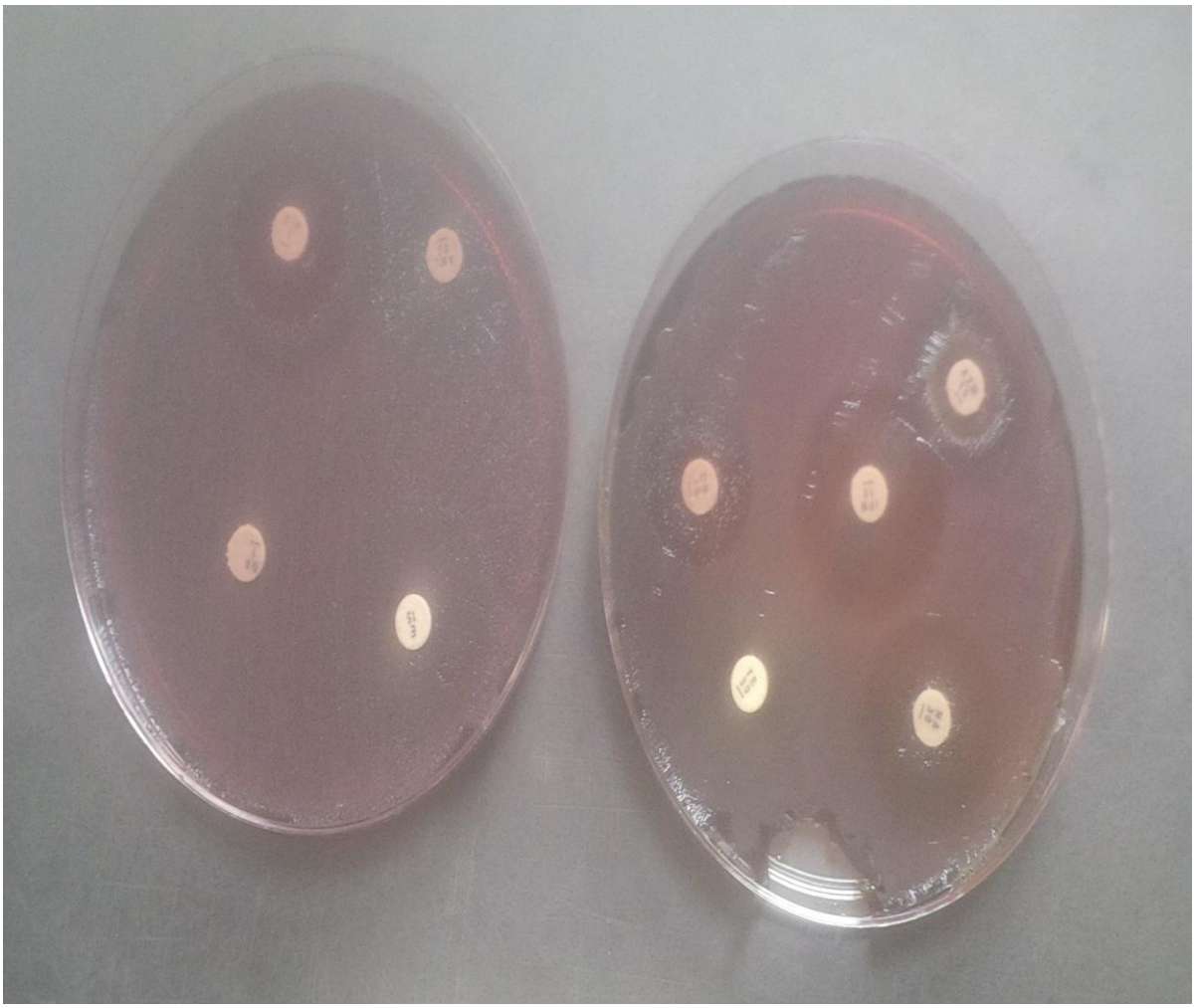


Plate V: showing the zones of inhibition of *Campylobacter jejuni* to the tested antibiotics