Occurrence and antimicrobial resistance of *Campylobacter* spp. isolates from beef cattle in Gauteng and North West provinces, South Africa.

BY

KATEMBUE KAMBUYI

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(Veterinary Science)

Veterinary Public Health Section

Department of Paraclinical Sciences,

Faculty of Veterinary Sciences, University of Pretoria,

Pretoria

SUPERVISOR: Dr. MUSAFIRI KARAMA

Co-supervisor: Dr. Annelize Jonker

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DECLARATION

This dissertation is my original work and has not been presented for any award or degree in another University.

DEDICATION

To my loving parents **Mr. Jean Bosco Katembue Dianda** and **Mrs. Perpetue Mwanza Kanyanya**, my uncle **Dr. John Kafunyi Mwamba** for their constant support and prayers through this long journey.

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LIST OF ABREVIATIONS

AMR: Antimicrobial resistance

AMS: Antimicrobial Stewardship

AZI: Azithromycin

Bp: base pairs

CAMHBT: Cation Adjusted Mueller-Hinton Broth with TES buffer

CDC: Centers for Diseases Prevention and Control

CDT: Cytolethal Distending Toxin

CIP: Ciprofloxacin

CLI: Clindamycin

CLSI: Clinical Laboratory Standard Institute

CVA: Cefoperazone, Vancomycin, Amphotericin

DNA: Deoxyribonucleic acid

dNTPs: Deoxyribonucleotide triphosphates

EDTA: Disodium ethylenediaminetetra-acetic acid

ERY: Erythromycin

EtBr: Ethidium bromide

EU: European Union

FFN: Florfenicol

FIDSSA: Federation of infectious Diseases Societies of Southern Africa

g: gram

GBS: Guillain-Barre Syndrome

GEN: Gentamicin

h: hour(s)

HBA: Horse Blood Agar

l: Liters

IDT: Integrated DNA Technologies

IPC: Improved infection Prevention and Control

LOS: Lipo-oligosaccharide

MDR: Multi-Drug Resistance

MFS: Miller Fisher Syndrome

mg: Milligram

MHB: Mueller-Hinton Broth

MIC: Minimum Inhibitory Concentration

Min: Minute(s)

ml: Milliliter

NAHMS: National Animal Health Monitoring System

NAL: Nalidixic acid

***n*:** number

NARMS: National Antimicrobial Resistance Monitoring System

PCR: Polymerase chain reaction

s: Second (s)

SAASP: South African Antimicrobial Stewardship Program

SANVAD: South African National Veterinary Surveillance and Monitoring Programme for Resistance to Antimicrobial Drugs

TEL: Telithromycin

TET: Tetracycline

Tm: melting temperature

UK: United Kingdom

USA: United States of America

WHO: World Health Organization

THESIS SUMMARY

Introduction: *Campylobacter* spp. is the most frequent cause of bacterial gastroenteritis in humans globally. *Campylobacter* spp. infections are characterized by acute watery or bloody diarrhoea, fever, weight loss and abdominal cramps. Campylobacteriosis complications include extra-intestinal diseases such as Guillain-Barre Syndrome (GBS) or its variant the Miller Fisher Syndrome (MFS). Consumption of contaminated foods of animal origin including undercooked meat, contaminated dairy products has been associated with foodborne campylobacteriosis in humans. Cattle are considered an important reservoir of *Campylobacter* spp. and a source of foodborne Campylobacteriosis. Antimicrobial treatment failure in most bacterial infections including campylobacteriosis has emerged and led to the increase of animal and human health care costs. The use of antimicrobials in cattle for therapy in both cattle and humans and for growth promotion in exerts selective pressure on bacterial pathogens, which may result in the emergence of antimicrobial resistant *Campylobacter* spp. strains which can be transferred from animals to humans along the food chain or through contact between animals and humans. In South Africa, studies on the occurrence and antimicrobial resistance profiles of *Campylobacter* spp. of public health importance are lacking. The main objectives of this study were to: 1) investigate the occurrence of *Campylobacter* spp. in beef cattle on cow-calf operations in Gauteng and North West Provinces and 2) determine the antimicrobial resistance profiles of *Campylobacter* spp. isolates. The overall aim of the study was to contribute to monitoring and surveillance of *Campylobacter* spp. of public health importance in South Africa.

Methodology: A total of 537 fresh faecal samples from beef cattle consisting of 453 from adult cows and 102 from calves were collected on 5 cow-calf operations in Gauteng and North West provinces. The samples were screened for *Campylobacter* spp., including *C. jejuni subsp. jejuni, C. coli* and *C. upsaliensis* by culture and the polymerase chain reaction (PCR). Furthermore, 86 *Campylobacter* spp. isolates consisting of 46 *C. jejuni subs. jejuni*, 24 *C. coli* and 16 *C. upsaliensis* were tested for antimicrobial resistance against a panel of nine antimicrobial agents including azithromycin, ciprofloxacin, erythromycin, gentamicin, tetracycline, florfenicol, nalidixic acid, telithromycin and clindamycin by the broth microdilution method.

Results: Out the 537 cattle faecal samples tested in this study, PCR revealed that 29.4% (158/537) [16.23%-42.57%] 95%CI of cattle carried *Campylobacter* spp. Among the 158 *Campylobacter* spp. positive cattle, 62.6% (99/158) carried *C. jejuni* subsp. *jejuni*, 25.3% (40/158) *C. coli*, 10.1% (16/158) *C. upsaliensis* and 3.1% (5/158) cows that had mixed infections. Three cows harbored both *C. jejuni* and *C. coli*, one cow carried *C. jejuni* and *C. upsaliensis* and one cow carried both *C. coli* and *C. upsaliensis*. Further antimicrobial resistance profiling of 86 *Campylobacter* spp. isolates (46 *C. jejuni* isolates, 24 *C. coli* and 16 *C. upsaliensis*) by the broth microdilution method revealed that the highest resistance rates for clindamycin (36%), nalidixic acid (19.7%), tetracycline (18.6%) and erythromycin (17.4%). However, lower resistance rates against florfenicol (3.4%), gentamicin (4.6%), telithromycin and ciprofloxacin (5.8%) were observed. The isolates were multidrug resistant against tetracycline/clindamycin, erythromycin/tetracycline/clindamycin, and nalidixic acid/clindamycin.

Conclusion: Little is known about the occurrence rates of *Campylobacter* spp. in beef cattle in South Africa. The prevalence of *Campylobacter* recorded in this study was consistent with various studies that have reported *Campylobacter* spp. prevalence rates within the same range in cattle in a number of countries with *C. jejuni subsp. jejuni* as the most predominant species. *Campylobacter* spp. isolates were mainly resistant to clindamycin, nalidixic acid and tetracycline. Findings from this study highlight the importance of beef cattle as a reservoir and a potential source of clinically relevant and antimicrobial resistant *Campylobacter* spp. isolates in South Africa.

CHAPTER 1: GENERAL INTRODUCTION

Cattle farming has contributed to the livelihood of humans around the world for millennia (Randolph *et al.*, 2007; Thornton, 2010). Cattle farming has allowed South Africa to meet the growing demand in animal proteins as the country's population increases (56.62 million) (Statistics South Africa, 2017; Stroebel *et al*., 2008). Currently, cattle provide the largest quantity of animal proteins in South Africa in comparison to other ruminant species (Stroebel *et al*., 2008). However, consumption of cattle products has long been associated with zoonotic foodborne diseases including campylobacteriosis (Sheppard *et al.*, 2009).

Campylobacter is the leading foodborne pathogen implicated in human bacterial gastroenteritis worldwide (World Health Organization, 2013). A number of studies have shown that cattle are an important reservoir of *Campylobacter* spp. (Hakkinen, 2010; Thépault *et al*., 2018). Contaminated cattle products including undercooked meat, unpasteurized milk and various dairy products can be an important source of *Campylobacter* spp. infections for humans (Fernandes *et al*., 2015). Furthermore, direct contact with contaminated sources in the dairy farm environment and drinking contaminated water (Vanselow, *et al*., 2006) are also important sources of *Campylobacter* spp. for humans (Oliver, Jayarao & Almeida, 2005).

Campylobacter is commonly recovered from the faeces of healthy cattle (Inglis, Kalischuk, 2003; Hakkinen, 2010; Thépault *et al*, 2018). The most frequently reported foodborne *Campylobacter* spp. in humans *C. jejuni, C. coli*, *C. lari*, and *C. upsaliensis* **(**Garrity *et al***.,** 2005**).** *Campylobacter jejuni* and *C. coli* are the most common species implicated in cattle-associated human infections (Cha *et al.*, 2017). Typical human cases of zoonotic foodborne campylobacteriosis are characterized by diarrhea, fever and abdominal cramps (Blaser, Engberg, 2008). The disease may also lead to more serious medical complications including Guillain-Barre-Syndrome, a debilitating ascending bilateral paralysis or its variant the Miller Fisher Syndrome which affects facial and cranial nerves and is characterized by abnormal muscle coordination, paralysis of the eye muscles, and absence of tendon reflexes in humans (Takahashi *et al.*, 2005). Furthermore, other syndromes including reactive arthritis, inflammatory bowel syndrome, Barrett's esophagus, and colorectal cancer have been associated with *Campylobacter* spp. in humans (Man, 2011). In a small number of human patients, *Campylobacter* spp. have been incriminated in lung infections, brain

abscesses, meningitis, and bacteremia in humans (Man, 2011). In South Africa, *C. jejuni, C. coli,* and *C. upsaliensis* have been incriminated in human disease (Shobo *et al*., 2016; Lastovica and Le Roux, 2001; Lastovica, 2006).

In addition to foodborne disease, *Campylobacter* species that are resistant to antimicrobials are also emerging (Shen *et al*., 2018; Yao *et al*., 2016; Sproston *et al*., 2018). Although, *Campylobacter* spp. have been previously isolated in South Africa from water (Diergaardt *et al*., 2004), milk (Mabote, Mbewe & Ateba, 2011), and animals faeces (Uaboi-Egbenni *et al.,* 2012), data on the prevalence and antimicrobial resistance patterns of *Campylobacter* spp.in cattle remains scarce.

1.1. Aims and Objectives

The overall aim of this study was to investigate the occurrence and antimicrobial resistance patterns of *Campylobacter* spp. in beef cattle on cow calf operations in South Africa.

Specific objectives were to:

1) Determine the prevalence of *Campylobacter* spp. including *C. jejuni*, *C. coli* and *C. upsaliensis* in healthy beef cattle on cow-calf operations in Gauteng and North West provinces, South Africa.
2) Determine antimicrobial resistance profiles of *Campylobacter* spp. isolates recovered from cattle.

The ultimate aim was to contribute to *Campylobacter* spp. surveillance in South Africa.

CHAPTER 2: LITERATURE REVIEW

2.1. Background

Campylobacter belong to the family of Campylobacteraceae and the *Campylobacter* genus is composed of 26 species and 9 subspecies (Kaakoush *et al.*, 2015). Most members of the *Campylobacter* genus are typically Gram-negative, non-spore forming s-shaped (spiral) bacteria (0,2 - 0,8 µm wide and 0,5 - 5µm in size); with a single polar flagellum, bipolar flagella, or without flagellum (Man, 2011). When stressed *Campylobacter* spp. cells typically become spherical in shape. *Campylobacter* spp. grows optimally from 37˚C to 42˚C in a micro-aerophilic atmosphere containing 5-10% oxygen. To replicate in the host, *Campylobacter* spp. require temperatures ranging from 32˚C to 45˚C; but can also survive at lower temperatures (Murphy, Carroll & Jordan, 2006). A hydrogen-enriched atmosphere has also been used to stimulate *Campylobacter* spp. growth (Kaakoush *et al.*, 2015). Some *Campylobacter* spp. including *C. jejuni, C. coli*, *C. lari*, and *C. upsaliensis* **(**Garrity *et al***.,** 2005**)** are thermotolerant (i.e. thrive at relatively high temperatures of 42°C). Furthermore, *Campylobacter jejuni* can survive in the environment at 4°C for up to 21 days in feces, close to 28 days in water, and 35 days in urine (Blaser, Engberg, 2008, Silva *et al*., 2011). *Campylobacter jejuni* and *C. coli* can be differentiated based on the capacity to hydrolyze sodium hippurate (Nakari, Puhakka & Siitonen, 2008). Most *Campylobacter jejuni* hydrolyse sodium hippurate while *Campylobacter coli* cannot (Park, 2002). However, some *C. jejuni* strains can be hyppurase negative (Rönner *et al*., 2004). In warm-blooded animals (mammals and birds), *Campylobacter* spp. are commensals in the intestinal tract (duodenum, jejunum, small and large intestines caeca).

2.2. Foodborne *Campylobacter* infections

It is estimated that foodborne *Campylobacter* spp. may be responsible of 400 to 500 million cases of human bacterial gastroenteritis worldwide, annually (Friedman, 2000). In the United States, *Campylobacter* accounts for about 9.4 million cases with 56,000 hospitalizations and 1,351 deaths every year (Hu, L. *et al*., 2018). In the European Union (EU), *Campylobacter* remains the most frequent cause of bacterial foodborne gastroenteritis and previous estimates have shown that the bacteria may be responsible for 9 millions of human infections per year; with over 200,000 laboratory confirmed cases; and an estimated financial cost of 2,4 million Euros which are

allocated to *Campylobacter* surveillance per annum (EFSA, 2014). Data from the United Kingdom has shown that *Campylobacter* remains the most frequent bacterial pathogen with an estimated incidence of 9.3 cases per 1000 persons annually (EFSA, 2014).

Human campylobacteriosis is characterized by acute watery or bloody diarrhea, fever, weight loss and abdominal cramps; these symptoms last for 6 days on average (Blaser, Engberg, 2008). *Campylobacter jejuni* is the most frequent species in human disease causing more than 90% cases of gastroenteritis infections followed by *C. coli* to a lesser extent (Zilbauer *et al.*, 2008). Human campylobacteriosis is an important disease in developed countries, especially in young children (Platts-Mills, Kosek, 2014), where a higher rate of asymptomatic carriage and milder clinical symptoms of watery, non-inflammatory diarrhea are frequently observed in young children under 7 years (Grzybowska *et al.*, 2013). Zoonotic cattle-associated human *Campylobacter* spp*.* infections occur mainly as a result of ingesting contaminated raw milk and undercooked beef or beef products (Fernandes *et al.*, 2015).

2.3. *Campylobacter* Epidemiology

2.3.1. *Campylobacter* in humans

In the last decade, of foodborne *Campylobacteriosis* has increased and become a public health concern in both developed and developing countries (World Health Organization, 2013). Although, campylobacteriosis is a self-limiting disease, human infections due to *Campylobacter* have a significant impact on productivity and are a financial burden to any public health system due to extended hospital stays in hospitals (health cost), long recovery periods, clinic visits, expensive medication and loss in man-hours for the labour market (Devleesschauwer *et al*., 2017; Havelaar *et al*., 2015).

In developing countries such as South Africa the epidemiology of *Campylobacter* is poorly understood to a large extent due to lack of national *Campylobacter* surveillance programs (Olson *et al.*, 2008; World Health Organization, 2013). *Campylobacter*-associated diarrhea and bacteremia occurs mainly in the young, the elderly and immunocompromised humans worldwide including HIV/AIDS and tuberculosis patients (Snijders *et al.*, 1997). South Africa remains among the countries with the

largest number of immunocompromised persons in the world due to a high HIV and TB infected population. This vulnerable section of the population may develop serious *Campylobacter* complications when exposed to *Campylobacter*.

Human campylobacteriosis is endemic in developing countries, particularly in the younger population in the earlier phase of life (Platts-Mills, Kosek, 2014; Kaakoush *et al.*, 2015). Reports from a number of African countries have estimated the incidence of *Campylobacter* spp*.* infections in children less than 5 years to vary between 40.000 and 60.000 cases/100.000 population each year (Coker *et al.*, 2002, Oberhelman, Taylor, 2000). In South Africa, previous estimates showed that the prevalence of *Campylobacter* spp*.* was 21% in children less than 5 years of age in Durban (Mackenjee *et al.*, 1984), while in the Vhembe district, the *Campylobacter* isolation rate was 30.4% in children aged between 0-2 years old, with *C. jejuni* as the most prevalent species (85%) followed by *C. coli* (15%) (Samie *et al.*, 2007b). In addition, Samie *et al.* (2007) reported that 10.2% and 6.5% of diarrheal cases in school children in Venda (Limpompo province) were associated with *C. jejuni* and *C. coli*, respectively (Samie *et al.*, 2007a). Furthermore, Lastovica *et al.* (1996) revealed that in the blood cultures of pediatric patients with bacteraemia in South Africa, *Campylobacter* spp. isolates accounted for 0.18% while adults' blood culture specimens showed relatively higher rates during different seasons of the year: 36% in autumn (March-May), 24% in summer (Dec-Feb), 21% in winter (Jun-Aug), and 19% in spring (September-November) from 1977 to April 1995 (Lastovica, 1996).

Studies that have reported on the prevalence of *Campylobacter* in other African countries have shown that the prevalence of *Campylobacter* spp. was 21% in children (median age, 11 months) who were hospitalized in Blantyre, Malawi, between 1997 and 2007, with *C. jejuni* as the predominant species (85%) (Mason *et al.,* 2013*)*. In Madagascar, *Campylobacter* spp*.* was detected in 8.95% of pediatric patients (Randremanana *et al.*, 2014). A survey conducted in Kenya revealed that *Campylobacter* was present in 3.1% of diarrheic patients (Turkson, Lindqvist & Kapperud, 1988). In another study in Uganda, *Campylobacter* was recovered from 9.3% of diarrheic children with *C. jejuni* accounting for 80.9%, *C. coli* for 4.5%, and coinfections with both *C. jejuni and C. coli* for 4.5% (Mshana *et al.*, 2009). In Liberia, *Campylobacter* spp*.* infections were confirmed in 44.8%, and 22.4% of children under 5 years from rural and urban communities, respectively (Mølbak, Højlyng & Gaarslev, 1988). In Ethiopia, *C. coli* were associated with 17.6% of human *Campylobacter*

infections; which was a high rate of infection when compared to worldwide statistics that have mostly shown that *C. jejuni* is more frequent than *C. coli* in humans (Asrat, Hathaway & Ekwall, 1999). A study on diarrheic and non-diarrheic children under 5 years from Tanzania reported *Campylobacter* isolation rates of 18% and 12% respectively (Lindblom *et al.*, 1995). Data from diarrheic children aged from 0-2 years revealed *Campylobacter* spp. isolation rates of 22%, and 11% among non-diarrheic children (Lindblom *et al.*, 1995). In the same study, *Campylobacter* was detected in 2% of diarrheic children aged between 3-5 years, and 15% of non-diarrheic children while in both diarrheic and non-diarrheic adults *Campylobacter* was present in only 1% of cases (Lindblom *et al*., 1995). In a study conducted from 2008-2009, the annual incidence of campylobacteriosis in developed countries ranged from 4.4 to 9.3 per 1000 population (World Health Organization, 2013). *Campylobacter* was the most frequent cause of bacterial gastroenteritis in humans from 2004 to 2009 in the USA, accounting for 41.7% cases of foodborne disease in humans (Kendall *et al*., 2012).

From 2005-2009, *Campylobacter* spp. infections in European Union countries accounted for 9.2 million notified cases (Havelaar *et al*., 2013). In the United Kingdom and Wales, human *Campylobacter* gastroenteritis cases amounted to 57,674 cases in 2000 (Kwan *et al*., 2008a). However, due to the implementation of foodborne disease surveillance and control programmes, human *Campylobacter* spp. infections decreased significantly to 46,236 cases in 2006 (Kwan *et al*., 2008b). In Australia, *Campylobacter* was the most common cause of foodborne gastroenteritis accounting for 16,968 of reported human cases in 2010 (OzFoodNet Working Group, 2012). In New Zealand, the incidence of *Campylobacter* in 2008 was 161.5 cases per 100.000 persons (Gilpin *et al.*, 2008b). Furthermore, in New Zealand, out of 364 human cases of *Campylobacter* spp., 47% were ascribed to ingestion of contaminated animal products; 27.7% to physical contact with animals; 6.9% to foreign travel; 3.3% to consumption of contaminated water; and 11% to an unknown source (Gilpin *et al*., 2008a).

2.3.2. *Campylobacter* Epidemiology in Cattle

Cattle are an important reservoir of *Campylobacter* spp. (Stanley *et al*., 2003; Besser *et al*., 2005; McAuley et al., 2014; Ju *et al*., 2018; Thépault et al., 2018). A number of studies have linked cattle products including meat and dairy products with *Campylobacter* in humans (Hakkinen, 2010; El-Zamkan, Hameed, 2016; Fernandes *et*

al., 2015; Thépault, 2018). *Campylobacter* spp. have been isolated from both healthy and diarrheic cattle and calves (Thépault *et al.*, 2018; Klein *et al.*, 2013). *Campylobacter jejuni* and *C. coli* remain the most common cattle associated species with human disease (Bae *et al.*, 2005), and the most incriminated in foodborne gastroenteritis (Silva et al., 2011). However, *C. upsaliensis*, *C. hyointestinalis* and *C. lari* may also cause human infections but less frequently (Silva *et al*., 2011).

In cattle, *Campylobacter* infections are asymptomatic (Hoar *et al*., 2001) and in most instances, cattle become carriers (Minihan *et al*., 2004). Based on several studies, up to 70% of apparently healthy cattle may harbor *Campylobacter* (Sanad *et al*., 2013). Newborn calves may acquire the organisms from the farm environment or via horizontal transmission from cow to calf within 4 days of their life (Klein, *et al.*, 2013). Calves from first-calf heifers are more susceptible to *Campylobacter* spp. than calves from multiparous cows (Rebhun, Guard & Richards, 1995). Furthermore, calves and young cattle may shed very high numbers of *Campylobacter* spp*.* in their feces (Klein, *et al.*, 2013)**.** Cattle acquire *Campylobacter* spp. by ingestion of food or water which is contaminated with faecal matter or from various sources in the farm environment including direct contact with bedding or litter (Hannon, *et al*., 2009)**.**

Case-control studies have highlighted that beef and dairy products can be an important source of human campylobacteriosis (El-Zamkan and Hameed, 2016, Fernandes *et al*., 2015). Beef products have been incriminated less frequently (0 to 5%) as a source of human *Campylobacter* spp. infections when compared to dairy products (Kwan *et al*., 2008b; Schildt, Savolainen & Hänninen, 2006), Wilson *et al.* (2008) reported that cattle food products were major sources of *Campylobacter* spp*.* infections in humans in 35% of cases in England (Wilson *et al.*, 2008). In Ireland, *Campylobacter* spp. was not isolated from beef carcass although 54% of rectal faecal samples from the same animals were positive for *Campylobacter* spp. (Whyte *et al.*, 2004). In Sweden, food products of cattle origin were incriminated in human campylobacteriosis in more than 94% of cases (Møller Nielsen, Engberg & Madsen, 1997). In Finland, most human *Campylobacter* spp. infections were attributed to cattle and poultry consumption (De Haan *et al*., 2010). In Pakistan, a study conducted from 2002-2004, incriminated raw beef (10.9%) and cattle raw bulk milk (10.2%) as sources of human campylobacteriosis (Hussain *et al.*, 2007). Taylor at al., (2013), reported that dairy products were implicated in 29% of *Campylobacter* spp. outbreaks in the USA (Taylor *et al*., 2013).

In South Africa, *Campylobacter* spp. was found in 87.5 % of cow milk samples, which were obtained from seven local markets in the North-West province in 2010 (Mabote, Mbewe & Ateba, 2011). Similar studies conducted in Ethiopia have implicated cattle food products as a source of *Campylobacter* infections in 12.7% cases of human campylobacteriosis (Kassa, Gebre-selassie & Asrat, 2005). Recently, EL-Zamkan and Hameed, (2016), observed that *Campylobacter* spp. was present in raw cow milk and dairy products (cheese and yoghurt) in Egypt with *C. jejuni* as the most predominant species. In Nigeria, in the region of Sokoto, a study Salihu *et al.*, (2010) reported that that *Campylobacter* spp. was present in 4.8% of samples milk samples collected from lactating cows. Data from Nairobi (Kenya) showed that *Campylobacter* spp. was present in 5.8% of healthy cattle (Turkson, Lindqvist & Kapperud, 1988). A report from Tanzania, detected 13.4% of *Campylobacter* in raw milk with the predominance of *C. jejuni* (58.1%) followed by *C. coli* (30.7%) (Kashoma *et al.*, 2016). *Campylobacter* spp*.* has been isolated from farmed animals and carcasses of slaughtered animals in Tanzania (Nonga, Sells & Karimuribo, 2010). In Tanzania, animal carcasses were contaminated by faecal material during slaughter (Nonga, Sells & Karimuribo, 2010). In another investigation in Tanzania, on dairy and beef cattle faecal samples, Kashoma, *et al.* (2015) reported that 35.4% of dairy cattle were *Campylobacter* spp. positive, while the overall prevalence in beef cattle was 19.6%. *Campylobacter coli* was the most common species (19.3%) followed by *C. jejuni* (8, 8%) (Kashoma et al., 2015).

Cattle farms are generally considered a major source and reservoir of *Campylobacter* spp*.* (Hakkinen, 2010). Stanley *et al*. (1998) suggested that the prevalence of *Campylobacter* in dairy cattle may vary between 5 to 53% (Stanley *et al*., 1998). In the USA, some reports have estimated that up to 80% of cattle herds and 40-60% of individual animals may carry *Campylobacter* spp. (Wesley *et al*., 2000; Besser *et al*., 2005). Several studies in various countries around the world have reported faecal *Campylobacter* spp. shedding rates in cattle ranging from 0,8% to 46,7% (Wesley *et al.,* 2000; Inglis, Kalischuk & Busz, 2003*)*. However, *Campylobacter* spp. isolation rates from cattle or cattle products are very variable, and some studies have revealed relatively low levels of *Campylobacter* spp. on cattle carcasses or meat and milk samples (Rahimi, Alipoor-Amroabadi & Khamesipour, 2017; Wieczorek, Osek, 2013).

A number of researchers have observed variable rates of *Campylobacter* carriage in cattle herds in European countries. The prevalence of *Campylobacter* in cattle in Portugal was 19.5% (Cabrita *et al*., 1992) and 23% in Denmark (Nielsen, 2002). A study on *Campylobacter* in Norway indicated that *Campylobacter* carriage among cattle was higher in calves (46%) than in adult cattle (29%) with *C. jejuni* the most frequent species (Inglis, Kalischuk & Busz, 2004). In France, the overall isolation rate of *Campylobacter* spp*.* in young beef cattle, calves, and culled cows was 16.5%, with *C. jejuni* as the most common species (12.8%) followed by *C. coli* (3.7%) (Châtre *et al*., 2010). In the United Kingdom, a higher prevalence of *Campylobacter* species (89%) (Stanley, Jones, 2003) was reported on cattle farms and *Campylobacter jejuni* was the most frequent species in more than 90% cases of cattle gastroenteritis followed by *C. coli* (Stanley, Jones, 2003).

In the USA, prevalence of *Campylobacter* in cattle was estimated to be up to 68% depending on on the cattle production system: whether cattle were fed in feedlots or or grazed on pasture (Beach *et al*., 2002; Horrocks *et al*., 2009). *C. jejuni* was the most predominant species followed by *C. coli* (Englen *et al*., 2007). However, Bae *et al*., (2005) reported that the rates of both *C. jejuni* (20%) and *C. coli* (23.8%) were closely similar in calf-rearing operations. In Canada, a higher prevalence of 90% has been reported in cattle (Inglis, Kalischuk & Busz, 2004).

Studies from Oceania and Asia have also documented variable rates of *Campylobacter* spp*.* in adult cattle and calves and have shown that young calves are the most frequent carriers of *Campylobacter* because of their weak immunity system in comparison to adult cattle (Indikova, *et al*., 2015). In New Zealand, *Campylobacter* spp. was isolated from 66% of cattle with 59% of infected dairy cattle and 75% of calves (Gilpin *et al.,* 2008b*)*, *C. jejuni* was the most common species followed by *C. coli* in calves (Gilpin *et al*., 2008b). In Japan, Giacoboni *et al.,* (1993) observed that 97.1% and 46.7% % of healthy calves and adult cattle carried *Campylobacter* spp. respectively (Giacoboni *et al*., 1993).

Campylobacter spp. can also be found in the natural or farm environment including contaminated water and slurries on the farm, cattle feeders, and abattoir effluents (Hannon *et al*., 2009, Minihan *et al.*, 2004). However, *Campylobacter* transmission pathways from cattle to humans may not necessarily include environmental routes (Kwan *et al*., 2008a). Several factors including herd type and size, season, geographical site, sampling technique and frequency, sample type (faecal or organs),

age and isolation methods (direct plating or enrichment) influence *Campylobacter* isolation rates (Ramonaitė *et al.,* 2013*)*. In addition, husbandry practices, climatic factors and diet have also been shown to play a determinant role in *Campylobacter* carriages rates in cattle (Ramonaitė *et al.,* 2013). *Campylobacter jejuni* incidence was higher in feedlot cattle compared to grazing cattle (Stanley *et al*., 1995). Stanley *et al*., (1998) suggested that the prevalence of *Campylobacter* in cattle might also depend on seasonal trends with peak shedding in summer or spring (Stanley *et al.*, 1998). Winter-housed cattle had a higher prevalence of *Campylobacter* spp. than summer-grazing cattle (Hänninen, Niskanen & Korhonen, 1998). Physiological factors such as stress may influence *Campylobacter* shedding in livestock (Whyte *et al.*, 2001). Asymptomatic excretion of *Campylobacter* spp. in dairy cows with mastitis has also been documented (Stanley, Jones, 2003). The level of *Campylobacter* colonization in cattle plays also an important role in the frequency of contamination of milk, and meat after slaughter (Linton *et al.*, 1997).

2.4*. Campylobacter* Pathogenesis and Virulence factors

A small number of *Campylobacter* cells may be required to initiate *Campylobacter* disease in humans (Dasti *et al*., 2010). The infective dose of *Campylobacter* spp*.* for humans has been estimated to range from 50-10 000 bacterial cells (Hunt *et al*., 2001). After ingestion by the host, *Campylobacter* spp. bacterial cells pass through the stomach environment whereby stomach acids and bile salts naturally eliminate most *Campylobacter* spp. cells during infection (Fouts *et al.*, 2005). However, under favourable conditions *Campylobacter* spp. bacterial cells may evade natural defense mechanisms and colonize the mucosa of the small intestine and the colon (Fouts *et al*., 2005). Various virulence determinants are involved in *Campylobacter* pathogenesis infection including: (1) motility and chemotaxis, (2) adherence to, translocation, and invasion of intestinal epithelial cells, (3) toxin production, (4) survival in the epithelial cells, and (5) immune responses and inflammation of the intestinal epithelium (konkel *et al*., 2001).

Flagella motility and corkscrew motion, enables *Campylobacter* bacteria to penetrate and inhabit the viscous intestinal environment (Masanta, *et al*., 2013). *Campylobacter* spp*.* invades the intestinal barrier and attaches to enterocytes in the intestinal crypts (Gaynor *et al*., 2005). Flagella play an important role in the attachment, internalization and transfer of *Campylobacter* spp. to host enterocytes (Chaban, *et al.*, 2015). After

internalization into enterocytes, *Campylobacter jejuni* is capable of surviving in the intracellular environment and subverting the host immunity (Backert *et al*., 2013). *Campylobacter* spp. translocation across the intestinal barrier and invasion of enterocytes and proliferation in the lamina propria may cause intestinal inflammation and result in tissue damage, and spread to mesenteric lymph nodes and blood stream (Backert *et al.,* 2013*)*. Enterotoxin production (enteritis) may be observed in patients with acute watery diarrhea due to *C. jejuni* (Man, S., 2011). Manifestations such as bloody diarrhea and the presence of inflammatory cells in human feces showing *Campylobacter*-associated enteritis are major clinical evidence of *Campylobacter* spp. enterotoxin secretion in the colon and terminal ileum (Camilleri, M., 2015).

The molecular virulence mechanisms of *Campylobacter* spp. are not fully understood. Environmental conditions in the host gastro-digestive tract can stimulate *C. jejuni* to synthesize virulence-associated factors that are involved in the development of campylobacteriosis (Malik-Kale, 2008). A number of candidate genes have been proposed as *Campylobacter* spp. virulence factors and markers in human disease (Biswas *et al*., 2011). These virulence factors play an important role in motility, adherence and colonization, and invasion of host cells in the intestinal epithelium during *Campylobacter* pathogenesis (Dasti *et al*., 2010). Adhesion is a prerequisite to host cell invasion whereby the bacteria is protected against cellular and immunological responses and is able to initiate and produce disease (Isberg, Van Nhieu, 1994).

A number of proteins have been associated with survival and passage of *Campylobacter* spp. through the adverse environment of the stomach to allow the bacteria to colonize the gastrointestinal tract (Reid, *et al*., 2008). In the stomach, *Campylobacter* spp. produce chemoattractants which aid in the sensing of mucin, metabolic substrates (L-glutamate) and chemotaxis proteins (*cheA, cheB, cheR, cheW, cheY*, and *cheZ* genes) (Zautner *et al*., 2012). *Campylobacter* motility is mainly coordinated by flagellin (*flaA, flaB* and *flaC)*; and flagellar type III secretion genes (*flhA*, *flhB*) (Guerry, 2007). Tripartite complexes of cytolethal distending toxin-encoding genes contribute to *Campylobacter* pathogenesis through binding to host cells (Purdy *et al*., 2000). The c*adF* gene (*Campylobacter* adhesion to fibronectin) encodes a protein that interacts with fibronectin, a host extracellular matrix protein (Ziprin *et al*., 2000). The virulence genes linked with *Campylobacter* invasions and adherences are the invasion-associated markers such as *iam, capA* and *cst-II* gene (Ashgar *et al*., 2007).

2.5. Guillain-Barré Syndrome (GBS)

Patients affected by *Campylobacter* spp. may rarely develop postinfectious complications including Guillain Barré Syndrome (GBS) or its variant the Miller Fisher's syndrome (MFS), reactive arthritis (Reiter's syndrome), postinfectious irritable bowel syndrome, and potentially immunoproliferative small intestinal disease (Dasti *et al*., 2010). Guillain Barré Syndrome (GBS) is a rare complication of campylobacteriosis which affects the peripheral nervous system and is characterized by ascending acute flaccid paralysis which may lead to respiratory paralysis and death; while Miller Fisher Syndrome is a descending neural paralysis characterized by facial and cranial nerve involvement including ataxia, areflexia, and ophtalmoplegia (Takahashi *et al*., 2005).

C. jejuni is the most incriminated species in GBS and Miller-Fisher's syndrome (MFS) (Nyati *et al*., 2013). According to the World Health Organisation, (2013), GBS case fatality rates range between 3-10% in developed countries where the disease has been studied comprehensively. Both GBS and MFS develop when autoimmune antibodies against *C. jejuni* lipo-oligosaccharide (LOS) are induced by infecting bacteria and produced by host cells (Heikema, 2013). Autoimmune antibodies are induced by terminal sugars of *C. jejuni's* LOS and attack human gangliosides (Heikema, 2013). Particular *C. jejuni* serotypes including O:19, O:41 have been associated with the development of Guillain-Barre Syndrome (GBS) (Chatzipanagiotou *et al.*, 2003). Serotype O:2 has been mainly associated with Miller Fischer syndrome (MFS) (Godschalk *et al.*, 2007). Other serotypes that have been linked to GBS sporadically are: O:1, O:2, O:4, O:4 complex, O:5, O:10, O:16, O:23, O:37, O:44, and O:64 (Koga *et al*., 2006).

2.6. Antimicrobial resistance in *Campylobacter* species

2.6.1. *Campylobacter* resistance in humans

Food animal production depends on the appropriate use of antimicrobials for prevention and control of animal diseases. When antimicrobials are misused or abused, favorable conditions for the spread and persistence of resistant *Campylobacter* spp*.* may occur (Aarestrup, Wegener, 1999). In developing countries such South Africa, the absence of antimicrobial resistance monitoring and surveillance programs have significantly contributed to the rise in antimicrobials resistance among

bacteria including *Campylobacter* spp. (Moore *et al.*, 2006). Furthermore, the use of antimicrobials as growth promoters in food animals has increased antimicrobial resistance among enteric bacteria in humans (Alfredson, Korolik, 2007). Some reports have suggested that there is a link between human *Campylobacter* strains that are resistant, and the use of antimicrobials in animal production (van den Bogaard, Stobberingh, 2000).

Currently, *Campylobacter* spp. resistance to antimicrobials (AMR) has become a public health concern (Tang *et al*., 2017). *Campylobacter jejuni* and *C. coli* strains resistance to fluoroquinolones (Engberg *et al.*, 2004), aminoglycosides (Alfredson, Korolik, 2007) and macrolides has emerged as one of the current clinical challenges in developed and developing countries worldwide (Gibreel, Taylor, 2006). Specifically, resistance to macrolides and fluoroquinolones is considered a major public health concern because these compounds are frontline antimicrobials for treatment of human *Campylobacter* spp. gastroenteritis infections (Wise *et al*., 1998).

A report from Venda district (Limpopo province) showed an increase in resistance rates to ciprofloxacin (8% to 13%), gentamicin (8% to 17.3%), while a steady rate of resistance to tetracycline (27%) was also observed in *Campylobacter* spp. isolates from human diarrheal stools (Samie *et al.*, 2007b). *C. jejuni* isolates that were multiresistant to three classes of antimicrobials (ciprofloxacin (fluoroquinolones), erythromycin (macrolides), nalidixic acid (quinolone) and ceftriaxone (cephalosporin) were observed in South Africa for the first time in 2005 (Moore *et al.*, 2006).

Antimicrobial resistance in *Campylobacter* spp. isolates has also been observed in other African countries. In Egypt, Putnam *et al.,* (2003) reported decreased susceptibility to ciprofloxacin in *Campylobacter* spp. in isolates which were recovered from pediatric patients between 1995 - 2000 (Putnam *et al*., 2003). Another report by Wasfy *et al.* (2000) revealed that *C. jejuni* and *C. coli* were resistant to cephalothin, aztreonam, and streptomycin (Wasfy *et al.*, 2000). Data from Senegal indicated that 34% of *Campylobacter jejuni* and *C. coli* isolates were resistant to fluoroquinolones (Cardinale, 2003). In Bulgaria 31% of *Campylobacter* spp. isolates were resistant to macrolides (Gibreel, Taylor, 2006)**.** Vlieghe *et al. (*2008) observed resistance to fluoroquinolones and macrolides in *Campylobacter* isolates from travelers returning from Asia, Latin America, and Africa between 1994-2006, with resistance rates of 70.5%, 30.6%, and 60.6% respectively to norfloxacin; while resistance to erythromycin

was 2.7% for Asia, 8.6% for Africa, and 7.5% for Latin America (Vlieghe *et al*., 2008). Multi-resistant *C. jejuni* and *C. coli* isolates were recovered from 0.8% of international travelers in Antwerp, Belgium (Vlieghe *et al*., 2008)

A study from Thailand indicated that 84% of *Campylobacter* isolates from diarrheal patients were highly resistant to ciprofloxacin while resistance to azithromycin was observed in 7-15% of patients (Hoge *et al.*, 1998)**.** In Northern India, high resistance rates of *Campylobacter* spp. to fluoroquinolones (71.4%) were also reported (Prasad *et al.,* 1994). In Singapore, moderate to high resistance rates of *Campylobacter* spp. to macrolides were also reported in 31% and 51% of *C. jejuni* and *C. coli* isolates respectively (Gibreel, Taylor, 2006). A survey carried out in Kuwait, between 2000-2003 observed that of the 64 *Campylobacter* isolates tested, 5% were resistant to erythromycin, and 53% to ciprofloxacin (Albert *et al.*, 2005).

In Australia, Sharma *et al*., (2003) investigated resistance among 180 *C. jejuni* isolates from humans and observed that 3.4% of isolates were resistant to erythromycin, 2.9% to ciprofloxacin, 48% to roxithromycin, 11% to tetracycline, 6.4% to ampicillin, and 3.4% to nalidixic acids. In New Zealand, Goodchild *et al*., (2001) documented that *C. jejuni* isolates from diarrheal patient from a community hospital were resistant to erythromycin (3%), and to ciprofloxacin (4%) when compared to other *Campylobacter* species.

2.6.2. Antimicrobial resistance of *Campylobacter* in cattle

Campylobacter spp. isolates from cattle have shown resistance to most antimicrobials of choice that are used in clinical veterinary medicine to treat animal diseases worldwide. For example, a survey conducted in Nigeria found that *C. coli* isolates from beef cattle were resistant to ciprofloxacin, erythromycin, ofloxacin and ceftriazone (Okunlade *et al*., 2015). Data from Tanzania, showed that *C. jejuni* isolates from dressed beef carcasses and raw milk were resistant to ampicillin (94.1%) tylosin (90%), streptomycin (88.2%), erythromycin (70.6%), tetracycline (17.7%), ciprofloxacin (11.8%), and gentamicin (11.8%) (Kashoma *et al*., 2016). In another study from Tanzania, Kashoma *et al*., (2015) noted resistance to ampicillin (75.7%) and ciprofloxacin (7.2%) in *Campylobacter* isolates from dairy and beef cattle (Kashoma *et al*., 2015).

In South Africa, resistance to fluoroquinolones, macrolides and multiresistance to two or more antimicrobials in some instances were observed in *Campylobacter* spp. isolates that were recovered from various food-producing animals including pigs, poultry and cattle (Jonker, Picard, 2010; Bester, Essack 2008; Uaboi-Egbenni *et al*., 2013). *Campylobacter* resistance to erythromycin (25% to 53%), nalidixic acid (5.7% to 41%), and ceftriaxone (3.6% to 24.6%) was reported in poultry (Bester, Essack, 2008). However, Jonker *et al.,* (2010) observed low levels (<1%) of resistance to aminoglycosides including gentamicin, neomycin and spectinomycin (Jonker, Picard, 2010) in *Campylobacter* spp. isolates from pigs.

In European countries, antimicrobial resistance among cattle *Campylobacter* isolates has also been on the rise in the past years (Alakomi *et al*., 2016). Data from Spain has shown that *Campylobacter* isolates from animals and foods were highly resistant to fluoroquinolones (72%) but had low resistance to macrolides (11%) (Saenz *et al.*, 2000). A report from Denmark indicated that only 10% of *Campylobacter jejuni* isolates from cattle were susceptible to streptomycin (Aarestrup *et al*., 1997). In an investigation conducted in France among cattle, Châtre *et al*., (2010) observed increasing resistance to tetracycline in 88.1% and 52.8% of *C. coli* and *C. jejuni,* respectively. Higher resistance rates were observed for nalidixic acid (70.4%) and fluoroquinolones (70.4%) in *Campylobacter* spp. isolates from cattle between 2002 and 2006 in France (Châtre *et al*., 2010).

In the USA, an increase was observed in the frequency of resistance to frontline antimicrobials including macrolides and fluoroquinolones antimicrobials that are used in *Campylobacter* spp. enteritis control (Bae et al., 2005)**.** The most common resistance was observed against doxycycline (42.3% of 350 isolates) in *Campylobacter* spp. isolates from cattle calf rearer facilities (Bae *et al.*, 2005). Resistance against quinolones and erythromycin and multiresistance was more frequent in *C. coli* isolates in comparison to *C. jejuni* (Tang *et al.*, 2017). Another report from the USA, observed that 50.5% cattle *C. jejuni* and 69·5% of *C. coli* isolates were resistant to azithromycin, ciprofloxacin, chloramphenicol, clindamycin, erythromycin, gentamicin, tetracycline and nalidixic acids with multiresistance in 20.3% of *Campylobacter coli* (Englen *et al.*, 2007). Data from Iran showed that cattle *Campylobacter* strains were resistant to nalidixic acid (up to 75%), ciprofloxacin (69.4%), tetracycline (45.8%), amoxicillin (11.1%), streptomycin (4.2%), and chloramphenicol (2.8%), and

multiresistance was observed in 75% of *Campylobacter* spp. isolates (Taremi *et al.*, 2006).

Five common mechanisms by which bacteria resist to antimicrobials agents include: (i) enzymatic inactivation or modification of antimicrobials; (ii) impermeability of the bacterial cell wall or membrane; (iii) active expulsion of the drug by cell efflux pump; (iv) alteration of target receptors; (v) and drug trapping or titration (Alfredson, Korolik, 2007). A summary of bacterial mode of action and mechanisms of resistance to antimicrobial can be found in Table 1.

Table 1: Antimicrobial mode of action and mechanisms of resistance to bacteria (Morar, Wright, 2010).

Antimicrobial class	Antimicrobial agents	Target	Mechanism of resistance
Aminoglycosides	Gentamicin, Streptomacin, Spectinomycin and Kanamycin	Translation	Phosphorylation, acetylation, nucleotidylation, efflux, altered target
β-Lactams	Penicillins (ampicillin), cephalosporins (cephamycin), penems (meropenem), monobactams (aztreonam).	Peptidoglycan biosynthesis	Hydrolysis, efflux, altered target
Cationic peptides	Colistin	Cell membrane	Altered-target and efflux
Lincosamides	Clindamycin	Translation	Nucleotidylation, efflux, altered target
Macrolides	Erythromycin and Azithromycin and Telithromycin	Translation	Hydrolysis, glycosylation, Phosphorylation, efflux, altered target
Amphenicols	Chloramphenicol and florfenicol	Translation	Acetylation, efflux, altered target.
Pyrimidines	Trimethoprim	C1 Metabolism	Efflux, altered target
Tetracyclines	Tetracycline, Minocylcine and Tigecycline	Translation	Monooxygenation, efflux, altered target
Sulfonamides	Sulfamethoxazole	C1 Metabolism	Efflux, altered target
Quinolones	Ciprofloxacin, nalidixic acid	DNA replication	Acetylation, efflux, altered target.

2.6.3. Antimicrobial resistance surveillance trends in South Africa

In South Africa, thermophilic *Campylobacter* spp. from farmed animals have long been reported to be resistant to the most commonly recommended antimicrobial agents, fluoroquinolone, macrolide and tetracycline (Uaboi-Egbenni *et al.*, 2012; Jonker, Picard, 2010; Lammie, Hughes, 2016). The actual increasing trend of multidrug resistant *Campylobacter* spp. has significantly limited the use of antimicrobials for prophylaxis and animal growth promotion in the South African farming systems (Brink *et al*., 2014). In human bacterial infections, the increasing number of antimicrobial resistant *Campylobacter* strains outbreaks to ciprofloxacin, erythromycin, azithromycin (macrolide) and tetracycline has also been reported in South Africa (Lammie,

Hughes, 2016; Shobo *et al*., 2016). However, antimicrobial resistance surveillance data on clinical *Campylobacter* spp*.* infections remains scarce in South Africa (Shobo *et al*., 2016).

Currently, South Africa has adopted the national antimicrobial resistance strategy framework in response to concerns regarding the occurrence of antimicrobial resistance in various bacterial infections and antimicrobial treatment failure (Brink *et al.*, 2014). After analysis of the antimicrobial resistance situation, South Africa created the Global Antibiotic Resistance Partnership-South Africa (GARP–SA) (Winters, Gelband, 2011). Related organizations have also been created including the National Veterinary Surveillance and Monitoring Program for resistance to Antimicrobial drugs (SANVAD), and the South African Antibiotic Stewarship Program (SAASP) under the Federation of Infectious Diseases Societies of Southern Africa (FIDSSA) (Brink *et al*., 2014). SAASP includes public and private health sectors members (epidemiologists, veterinarians, physicians, pediatricians, microbiologists, intensivists, quality improvement experts, surgeons, IPC practitioners, pharmacists, and pharmacologists) contributing with essential skills in bacterial and infectious diseases. The main focus of the SAASP is the implementation of the Antimicrobial Stewardship System (AMS) to strengthen national antimicrobial resistance surveillance programs and improve prevention and control strategies; formulate recommendations on AMR program, initiate awareness and educational campaigns to preclude the misuse of antimicrobials and reduce the misuse and abuse of antimicrobials in human and animal health (Brink , 2014).

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CHAPTER 3: OCCURRENCE AND ANTIMICROBIAL RESISTANCE OF *CAMPYLOBACTER* SPP. ISOLATES IN BEEF CATTLE ON COW-CALF OPERATIONS IN SOUTH AFRICA

Abstract

This study investigated the occurrence and antimicrobial resistance profiles of *Campylobacter* spp. isolates from beef cattle on five cow-calf operations in South Africa. A total of 537 samples consisting of fresh faeces from adult beef cattle (n=435) and rectal swabs from calves (n=102) were screened for *Campylobacter jejuni*, *C. coli* and *C. upsaliensis* by culture and PCR. Furthermore, 86 *Campylobacter* spp. isolates including 46 C*. jejuni*, 24 C. coli and 16 *C. upsaliensis* were tested for antimicrobial resistance profiles using the broth microdilution method against a panel of nine antimicrobials. *Campylobacter* spp. was detected in 29.7% (158/537) of cattle. Among the 158 cattle which were positive for *Campylobacter* spp., 61.8% (99/160) carried *C. jejuni*, 25% (40/160) *C. coli*, and 10% (16/160) *C. upsaliensis*. Five cows (3.1%) had mixed infections: three cows carried both *C. jejuni* and *C. coli* concurrently, one cow had *C. jejuni* and *C. upsaliensis*, and one cow harboured *C. coli* and *C. upsaliensis*. Antimicrobial resistance profiling revealed that 52.3% (45/86) of isolates were resistant to one or more antimicrobials including 46.7% (21/45) of *C. jejuni*, 35.6% (16/45) of *C. coli* and 17.8% (8/45) of *C. upsaliensis*. Thirty-six percent (31/86) of isolates were resistant to clindamycin, 19.7% (17/86) to nalidixic acid, 16/86 (18.6%) to tetracycline and 17.4% (15/86) to erythromycin. Lower resistance rates were recorded for florfenicol, 3.4%; gentamicin, 4.8%; telithromycin and ciprofloxacin, 5.8%. Multidrug resistance (MDR) was observed in 32.5% (28/86) of isolates. Significantly more multidrug resistant *C. jejuni*, 36.9% (17/86) and *C. coli*, 33.3% (8/24) isolates were detected compared to *C. upsaliensis*, 18.7% (3/16). Multidrug resistance was mainly observed among *C. jejuni*. Two main multiresistance patterns were detected: nalidixic acid/clindamycin, 17.8% (5/86) and tetracycline/clindamycin, 14.2% (5/86). This study showed that beef cattle on the cow-calf operations in South Africa are an important reservoir and a potential source of clinically relevant and antimicrobial resistant strains of *Campylobacter* spp.

Keywords: Campylobacter spp., antimicrobial resistance, beef cattle.

3.1. Introduction

Campylobacter spp. is the leading cause of human bacterial enteritis globally (World Health Organization, 2013). *Campylobacter* spp. may be associated with 400 to 500 million of human gastroenteritis cases annually, worldwide (World Health Organization, 2013; EFSA, 2014). Human Campylobacteriosis is sporadic in developed countries with an incidence ranging from 4.4 to 9.3 per 1000 population per year (World Health Organization, 2013). *Campylobacter jejuni* and *C. coli* are the most common and important species of public health importance. In addition, *C. upsaliensis* has also emerged as an important species in humans (Lynch et al., 2011; Couturier, et al., 2012). Typical manifestations of gastrointestinal campylobacteriosis include self-limiting mild diarrhea to severe bloody diarrhea and neuropathic complications such as Guillain-Barré Syndrome or its variant the Miller Fisher Syndrome is a small number of patients (Epps et al., 2013). A smaller proportion (5 to 20%) of humans infected with campylobacteriosis is likely to develop reactive arthritis, brain abscesses, bacteremia, lung infections and irritable bowel syndrome (Man, 2011).

Cattle are considered a reservoir and a potential source of *Campylobacter* spp. for humans (Besser et al., 2005; Hakkinen, 2010; McAuley et al., 2014; Ju et al., 2018; Thépault et al., 2018). *Campylobacter* spp. are commensals in the gastrointestinal tracts (Epps et al., 2013) of healthy cattle (Thépault et al., 2018; Guévremont et al., 2014), diarrheic and non-diarrheic calves (Klein et al., 2013; Izzo et al., 2011). Calves are naturally free of *Campylobacter* spp. at birth but become infected through the farm environment via horizontal transmission within the first 4 days of life (Klein et al., 2013). Due to their weak immune system, calves may show clinical *Campylobacter* enteritis (Klein et al., 2013).

Transmission of *Campylobacter* spp. to humans occurs through contact with faeces, consumption of contaminated food or water. Ingestion of contaminated cattle food products has been associated with foodborne campylobacteriosis in humans (Boysen et al., 2014). A number of cattle products including raw milk, cheese and minced meat have been associated with foodborne *Campylobacter* spp. infections in humans worldwide (Fernandes et al., 2015; Wieczorek, Osek, 2017; El-Zamkan, Hameed, 2016). In Africa, a few reports have incriminated raw cow milk and dairy products as sources of foodborne campylobacteriosis in humans (El-Zamkan, Hameed, 2016; Kassa, Gebre-selassie & Asrat, 2005; Kashoma et al., 2016; Salihu et al., 2010).

However, data on the prevalence of *Campylobacter* spp. in both animals and humans in most developing countries remains scanty (Platts-Mills, Kosek, 2014).

Antimicrobials are prescribed for the treatment of *Campylobacter* infections in humans (Johnson, Shank & Johnson, 2017). Furthermore, a number of antimicrocrobials belonging to different classes are used for growth promotion and therapy in cattle (Eagar et al., 2008). These antimicrobials include quinolones, sulphonamides, cephalosporins, macrolides lincosamides, tetracyclines, aminoglycosides, and amphenicols, (Eagar et al., 2008). The misuse and abuse of antimicrobials in animal husbandry and clinical medicine exerts selective pressure on pathogenic bacteria including *Campylobacter* spp. Selective pressure may lead to the development of antimicrobial resistant *Campylobacter* spp. strains that have the potential to spread from animals to humans or vice versa through the food chain or other routes (Ganan et al., 2012, Chang et al., 2015). Consequently, antimicrobial resistant *Campylobacter* spp. strains have emerged worldwide which has led to treatment failure in humans affected with *Campylobacter* infections (Johnson, Shank & Johnson, 2017; Moore et al., 2006). Particularly, resistance to macrolides (erythromycin) and fluoroquinolones (ciprofloxacin) which are considered antimicrobials of choice in the treatment of human Campylobacteriosis has emerged among *Campylobacter* isolates from animals and humans (Gonzalez-Hein et al., 2013; Webb et al., 2018).

Campylobacter spp. that are commonly associated with human disease have been recovered from cattle faecal samples in a number of countries (Inglis, Kalischuk & Busz, 2003; Bae et al., 2005; Gilpin et al., 2008). Furthermore, a number of studies have reported the occurrence of antimicrobial resistant *Campylobacter* strains in cattle (Haruna et al., 2013; Châtre et al., 2010; Premarathne et al., 2017; Tang et al., 2017). However, in South Africa, studies on the prevalence of *Campylobacter* spp. in cattle are lacking. Furthermore, studies that have investigated antimicrobial resistance patterns in *Campylobacter* spp. isolates from cattle are scanty. The objectives of this study were to investigate the occurrence and antimicrobial resistance profiles of *C. jejuni*, *C. coli* and *C. upsaliensis* in beef cattle from cow-calf operations in South Africa. The overall aim of the study was to contribute to *Campylobacter* spp. monitoring and surveillance in South Africa.

3.2. Materials and Methods

3.2.1. Sample Source

This study was conducted on five cow-calf operations in the Gauteng and North-West provinces, South Africa, from July 2015 to April 2016. The cow-calf-operations supply calves to feedlots and are routinely serviced by the Onderstepoort Veterinary Animal Hospital (OVAH). Only cow-calf operations consisting of more than 20 cows/heifers and on which animals were maintained on grazing pasture all year were considered for the study. This study was approved by the Animal Ethics Committee of the University of Pretoria (V090-17).

3.2.2. Sample Collection

A total of 537 fresh faecal samples including 453 from adult cows and 102 from calves were collected. Fresh rectal faecal samples were obtained during routine pregnancy diagnosis checks from adult cows and heifers using a new plastic examination glove for each animal. Rectal swabs were used to collect faecal samples from calves. The sampling period was from June 2015 to March 2016. Samples were placed in sterile specimen bottles, transported on ice to the laboratory and stored at 4°C and processed in the next 24 hours. Each herd was visited once. Cattle herds were designated using alphabetical letters as shown in (Table 2): farm A (n= 65), farm B (n = 102 calves), farm C (n = 76), D (n = 181), and farm E (n = 113) from Gauteng and North West provinces in South Africa.

Table 2: **Herds/farms, animal type and sample number**

Herds	Animal type	Nature of sample	Number of faecal Sample (N = 537)
A	Adult cattle	Faeces	65
B	Adult cattle	Faeces	76
C	Calves	Rectal swabs	102
D	Adult cattle	Faeces	181
E	Adult cattle	Faeces	113
Total			537

3.2.3. Culture and isolation of *Campylobacter* spp.

For culture and isolation of *Campylobacter* spp., faecal swabs were spread-plated on Campy CVA agar (Brucella Agar containing 5% defibrinated sheep blood and

20mg cefoperazone, 10mg vancomycin, and 2mg amphotericin B) (Becton Dickinson and Company, MD, USA). The inoculated plates were incubated at 37°C for 48-72 hours in anaerobic jars containing GasPakTM EZ Campy System sachets (Becton Dickinson Microbiology Systems, Sparks, MD, USA) to generate a microaerophilic atmosphere (approximately 6-16% oxygen, and 2-10% carbon dioxide).

3.2.4. DNA extraction

Briefly, a sterile swab was used to harvest colony sweeps from all Campy CVA plates that showed growth after 48-72 hrs. The colony sweeps were suspended in 1.5 ml Eppendorf tubes containing 1ml of FA buffer (Becton Dickinson and Company Sparks, MD, USA). Bacterial suspensions were washed using a vortex mixer, followed by centrifugation for 5 minutes. After the first wash and centrifugation cycles, the supernatant was discarded and the bacterial pellet was resuspended in FA buffer (Becton Dickinson and Company Sparks, MD, USA). Two additional washes and centrifugation cycles were performed, after which the pellet was resuspended in 500 µl of sterile water and mixed. The homogeneous suspension was boiled to 100°C for 15 min, thawed on ice, centrifuged and the supernatant was stored at -20°C until further processing.

3.2.5. *Campylobacter* spp. screening by PCR

A multiplex PCR protocol was performed to screen samples for *C. jejuni*, *C. coli* and *C. upsaliensis*. Oligonucleotide primers which were used in PCR reactions are described in **Table 3**.

Table 3. **Oligonucleotide primers used in this study.**

Primers	**Sequence (5'-3')**
lpxAC.*jejuni* (Klena et al., 2004)	ACAACTTGGTGACGATGTTGTA
lpxAC.*coli* (Forbes,Horne, 2009)	GATAGTAGACAAATAAGAGAGAATMAG
lpxAC.*upsaliensis* (Klena et al., 2004)	AAGTCGTATATTTTCYTACGCTTGTGTG
lpxAR1(Forbes,Horne, 2009)	CAATCATGTGCGATATGACAATAYGCCAT
lpxAR2 (Forbes,Horne, 2009)	CAATCATGAGCAATATGACAATAAGCCAT
lpxARKK2m (Klena et al., 2004)	CAATCATGDGCDATATGASAATAHGCCAT

PCR was carried out in a 25-µl reaction mixture containing 2.5µL of 10X Thermopol reaction buffer, 2.0µl of 2.5mM dNTPs, 0.5 µM of each forward primer and reverse primer and 0.25µl of 1U of Taq DNA Polymerase and 5µl of DNA template (supernatant). All PCR reagents were purchased from New England BioLabs® Inc. (USA) except the primers which were purchased from Inqaba Biotec (South Africa) or IDT (USA). Thermal cycling conditions consisted of 2 min denaturation at 95 ºC, followed by 35 cycles of denaturation at 94ºC for 15s, annealing at 55ºC for 30s, extension at 72ºC for 30s, and a final extension at 72ºC for 5 min. PCR reactions were performed in a C100 TouchTM (Bio-Rad, USA) or a Veriti™ (Applied Biosystems®, USA) thermal cycler. *Campylobacter* jejuni ATCC 33560 and *Campylobacter* coli ATCC 33559 (Microbiologics, USA) and an in-house *C. upsaliensis* dog isolate (Dr. M. Karama's collection, Veterinary Public Health Laboratory, Faculty of Veterinary Science, University of Pretoria) were used as positive PCR controls. Sterile water was used as a negative PCR control. Amplicons were electrophoresed through a 2.5% agarose gel in 1XTAE (Tris-acetate-ethylenediamine tetra-acetic acid) buffer. To visualize PCR products, gels were stained with ethidium bromide. Gel images were captured under Ultraviolet light in a Gel Doc system (Bio-Rad, CA, USA).

3.2.6. *Campylobacter* species differentiation

Colony sweeps from Campy CVA Agar plates which were *Campylobacter* spp. positive on initial PCR screening were streaked on horse blood agar plates and incubated at 37°C for 48-72h to obtain single colonies. Three suspect *Campylobacter* spp. colonies were obtained from each horse blood agar plate, spread-plated separately on horse blood agar and incubated at 37°C for 48-72 hours to purify and multiply the single colonies. After incubation, pure colony sweeps were harvested from agar plates using a sterile plastic loop or a swab and suspended in FA buffer. Once gain, DNA was extracted from bacterial sweeps of pure single colonies by the boiling method and again, the aforementioned multiplex PCR was carried out to differentiate isolates into *C. jejuni*, *C. coli* and *C. upsaliensis* (Klena et al., 2004; Forbes, Horne, 2009). Confirmed *C. jejuni*, *C. coli* and *C. upsaliensis* isolates were stored at -80°C in cryovials containing a sterile freezing mixture (700 µl of Brucella broth and 300 µl of 30% glycerol) for further processing.

3.2.7. Antimicrobial susceptibility testing by broth microdilution

A total of 86 *Campylobacter* spp. isolates (one isolate per animal) were tested for resistance against a panel of 9 antimicrobials by the broth microdilution method, using Sensititre™ *Campylobacter* MIC plates (Sensititre, TREK Diagnostic Systems Ltd, OH, USA). The 86 isolates comprised 46 *C. jejuni*, 24 *C. coli* and 16 *C*. *upsaliensis*. The following dilution ranges were used to test the isolates for antimicrobial resistance: azithromycin (AZI; 0.015 to 64 µg/ml), ciprofloxacin (CIP; 0.015–64 µg/ml), erythromycin (ERY; 0.03 to 64 µg/ml), gentamicin (GEN; 0.12 to 32 µg/ml), tetracycline (TET; 0.06 to 64 µg/ml), florfenicol (FFN; 0.03 to 64 µg/ml), nalidixic acid (NAL; 4 to 64 µg/ml), telithromycin (TEL; 0.015 to 8 µg/ml) and clindamycin (CLI; 0.03 to 16 µg/ml).

To test the isolates for antimicrobial resistance, purified *Campylobacter* spp. isolates were streaked separately on horse blood agar and incubated for 48 hours at 42 °C in a microaerophilic atmosphere to obtain single colonies. Several colonies were picked from horse agar plates and seeded into tubes containing a solution of 5ml of Mueller-Hinton II broth (cation-adjusted) (Becton Dickinson and Company, MD, USA) and 5ml of TES buffer (10 mM Tris pH 7.5, 1 mM EDTA, 100 mM NaCl). The bacterial suspension was adjusted to 0.5 McFarland standard using a MicroScan® Turbidity Meter (Siemens Health Diagnostic, CA, USA) to make an estimate 5 to 6 log CFU/ml and was supplemented within 1ml of 50 % horse blood to make a total volume of 11 ml. One hundred microliters (100µl) of the 11 ml suspension were transferred into each 96-well Sensititre™*Campylobacter* MIC plate using a multichannel pipette. Each well was supplemented with 5µl of 50% horse blood. Inoculated plates were sealed with perforated gas-permeable covers (Sensititre, TREK Diagnostic Systems Ltd, OH, USA) and incubated for 24 hours at 42°C under microaerophilic conditions. *Campylobacter jejuni* ATCC 33560 and *C. coli* ATCC 33559 were used as quality control strains. After incubation, minimum inhibitory concentrations (MICs) were read visually using a VetMIC-Reading Mirror (Statens Serum Institute, Denmark). MIC values were defined as the lowest concentration of an antimicrobial agent that produced no visible bacterial growth in the wells of Sensititre™ *Campylobacter* MIC plates. MICs breakpoints for azithromycin, ciprofloxacin, erythromycin, gentamicin, tetracycline and nalidixic acid were evaluated according to the Clinical and Laboratory Standard Institute interpretive criteria (Clinical and Laboratory Standards Institute,

2012). However, florfenicol, telithromycin and clindamycin MICs breakpoints were interpreted using the National Antimicrobial Resistance Monitoring System (NARMS) interpretive criteria for Enteric Bacteria (NARMS, 2006) because CLSI does not have *Campylobacter* interpretative criteria for these antimicrobials. The CLSI and NARMS *Campylobacter* spp. breakpoints interpretive criteria are indicated in **Table 4**. Initially, each isolate was assigned to the susceptible (S), intermediate or resistant category. However, in the final interpretation of antimicrobial susceptibility results, intermediate readings were assigned to the resistant (R) category. Multi-drug resistance (MDR) was defined as resistance to two or more classes of antimicrobials

Table 4: Guidelines used to determine the antimicrobial resistance breakpoints using the CLSI (2012) and NARMS (2006) standards.

Resistance testing						
		Test ranges (µg/ml)		**MIC breakpoints(µg/ml)**		
Antimicrobial agents	**Interpretive criteria used**	**Minimum**	**Maximum**	**S**	**I**	**R**
Azithromycin	CLSI, 2012	0.015	64	≤2	4	≥8
Ciprofloxacin	CLSI, 2012	0.015	64	≤1	2	≥4
Erythromycin	CLSI, 2012	0.03	64	≤8	16	≥32
Gentamicin	CLSI, 2012	0.12	32	≤2	4	≥10
Tetracycline	CLSI, 2012	0.06	64	≤4	8	≥16
Florfenicol	NARMS, 2006	0.03	64	≤4	N/A	N/A
Nalidixic acid	CLSI, 2012	4	64	≤16	32	≥64
Telithromycin	NARMS, 2006	0.015	8	≤4	8	≥16
Clindamycin	NARMS, 2006	0.03	16	≤2	4	≥8

3.2.8. Statistical Analysis

Data was summarized in Microsoft Excel spreadsheets. Descriptive analysis was carried out using percentages. The Fisher's exact test was used to determine whether there were statistical differences between the prevalence rates of *Campylobacter* spp. of the two provinces surveyed. Furthermore, the 95% confidence interval was calculated by taking into account the cluster size and assuming an intraclass correlation coefficient of 0.1 using the formulas below (Dohoo, Martin, Stryn, 2003). Tables, bar graphs and pie charts were used to present data.

1. Correction of sample size

$$m = \frac{n'}{1 + \rho(m - 1)}$$

2. Absolute Precision (AP)

$$AP = Z\alpha\sqrt{\frac{\rho(1-\rho)}{n}}$$

m is the herd size

p is the correlation

n is the corrected sample size

n' is the sample size used

$Z\alpha$ = is the (1-α/2) percentile of a standard normal distribution

3.3. Results

3.3.1. Occurrence of *Campylobacter* spp. in cow-calf operations from Gauteng and North West provinces, South Africa

Out of the 537 cattle faecal samples tested, PCR revealed that 29.4% (158/537) [16.23%-42.57%]$_{95\%CI}$ of cattle carried *Campylobacter* spp., of which 62.6% (99/158) were identified as *C. jejuni subsp. jejuni*, 25.3% (40/158) as *C. coli*, and 10.1% (16/158) as *C. upsaliensis* (**Fig. 1**). Co-infections (mixed infections) were observed in 3.1% (5/158) of cattle including three cows which carried both *C. jejuni* and *C. coli,* one cow had *C. jejuni* and *C. upsaliensis,* and another cow carried *C. coli* and *C. upsaliensis* (**Fig. 1**, **Appendix 1**). *Campylobacter* spp. occurrence rates on individual farms are presented in **Table 5**.

3.3.2. Distribution of *Campylobacter* spp. by cattle age group

Adult cattle: Out of the 435 adult cows, 33.8% (147/435) harboured *Campylobacter* spp. Among the 147 adult cattle which were positive for *Campylobacter* spp., 61.2% (90/147) carried *C. jejuni subsp. jejuni*, 27.2% (40/147) *C. coli*, and 9.5% (14/147) *C. upsaliensis***. Calves**: Of the 102 calves tested, 10.8% (11/102) carried *Campylobacter* spp. including 81.8% (9/11) which had *C. jejuni* and 18.1% (2/11) with *C. upsaliensis*. *Campylobacter coli* was not detected in calves (**Table 5**).

3.3.3. Distribution of *Campylobacter* spp. by farm.

The overall prevalence of *Campylobacter* spp. in farm **A** cattle was 32.3% (21/65)**.** Within farm **A**, *C. jejuni subsp. jejuni,* 52.3% (11/21) was the most frequent *Campylobacter* species, followed by *C. upsaliensis,* 19% (4/21) and *C. coli,* 14.3% (3/21). Two cows (9.5%) were co-infected by *C. jejuni* and *C. coli*, and one (1) cow (4.8%) by *C. jejuni* and *C. upsaliensis*. On farm **B,** *Campylobacter* spp. was detected

in 10.8% (11/102) of calves, with 81.8% (9/11) carrying *C. jejuni* and 18.1% (2/11) *C. upsaliensis*. On Farm **C,** *Campylobacter* spp. was detected in 53.9% (41/76) of cattle, with *C. jejuni* in 51.2% (21/41), *C. coli* in 39% (16/41), and *C. upsaliensis* in 9.7% (4/41).

On Farm D, 35.9% (65/181) of cattle were positive *Campylobacter* spp., of which 70.7% (46/63) carried *C. jejuni*; 26.1% (17/63) *C. coli* and 1.5% (1/63) *C. upsaliensis*. One animal (1.6%) was co-infected with *C. jejuni* and *C. coli* (Appendix 1). On farm **E,** 19.5% (22/113) of cattle were contaminated with *Campylobacter* spp., including 54.5% (12/22) which carried *C. jejuni*, 22.7% (5/22) *C. upsaliensis* and 18.1% (4/22) *C. coli.* One animal (1.6%) was co-infected by *C. coli* and *C. upsaliensis*. The difference between the prevalence of *Campylobacter* spp. in Gauteng and North West provinces was not statistically significant (p value= 0.7763).

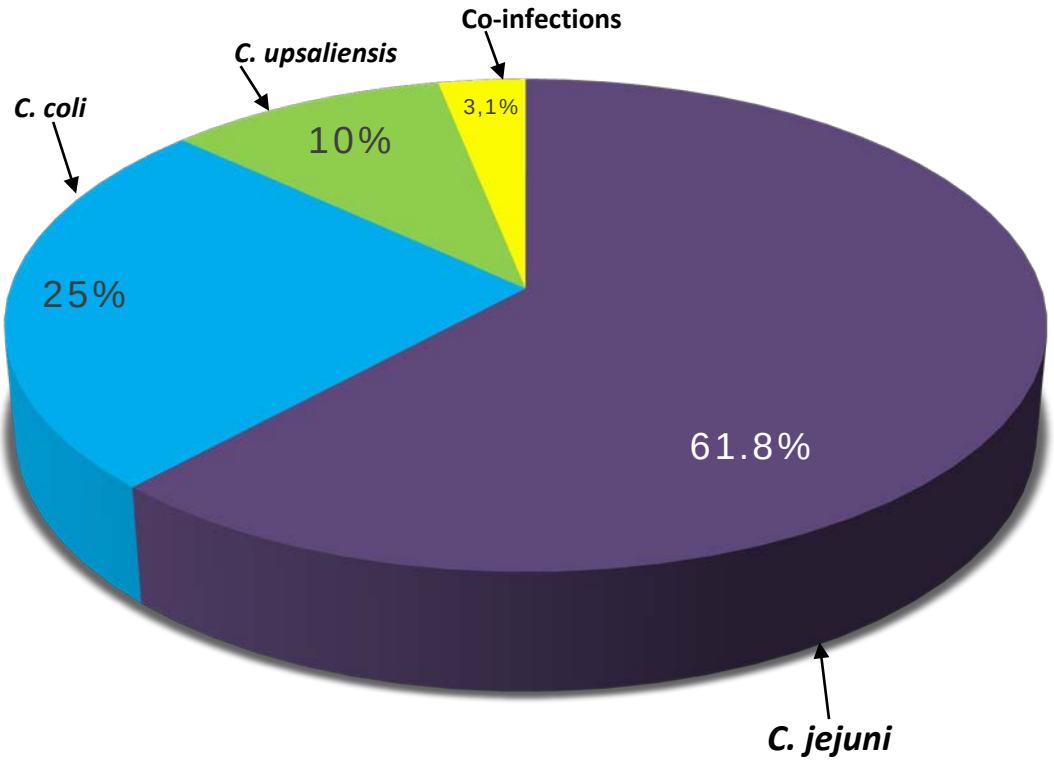

Fig. 1. Distribution of different *Campylobacter* spp. in positive cattle.

Table 5: Occurrence of *Campylobacter* species in cattle from Gauteng and North West provinces

	Distribution of *Campylobacter* species among 537 cattle					
Provinces	**Cow-calf operations**	**Positive cattle (%)**	***C. jejuni* (%)**	***C. coli* (%)**	***C. upsaliensis* (%)**	**Co-infections (%)**
Gauteng	***A*** *n=65*	21(32.3)	11(52.3)	3(14.3)	4(19.0)	3(14.3)
	B *n*=102 (calves)	11(10.8)	9(81.8)	0(0)	2(18.1)	0(0)
	C *n*=76	41(53.9)	21(51.2)	16(39.0)	4(9.7)	0(0)
North West	***D*** *n*=181	65(35.9)	46(70.7)	17(26.1)	1(1.5)	1(1.5)
	E *n*=113	22(19.5)	12(54.5)	4(18.1)	5(22.7)	1(4.5)
Total	***N*= 537**	**160 (29.7)**	**99/160(61.8)**	**40(25)**	**16(10)**	**5(3.1)**

3.3.4. Antimicrobial resistance profiles of *Campylobacter* isolates

Broth microdilution was carried out to determine the antimicrobial resistance profiles of 86 confirmed *Campylobacter* isolates including 46 *C. jejuni subsp. jejuni*, 24 *C. coli* and 16 *C. upsaliensis*. Overall, 52.3% (45/86) of the tested *Campylobacter* spp. isolates were resistant to one or more antimicrobials. Among the 45 isolates that showed resistance to one or more antimicrobials, 46.7% (21/45) were *C. jejuni*, 35.6% (16/45) as *C. coli* and 17.8% (8/45) as *C. upsaliensis*. Thirty-six percent of isolates (31/86) were resistant to clindamycin, 19.7% (17/86) to nadixic acid, 18.6% (16/86) to tetracycline and 17.4% (15/86) to erythromycin (**Table 6**). There were 8.1% (7/86) isolates which were resistant to azithromycin, 5.8% (5/86) to both ciprofloxacin and telithromycin, 4.6% (4/86) to gentamicin and 3.4% (3/86) to florfenicol (**Table 6**).

Of the 46 *C. jejuni* isolates which were resistant to one or more antimicrobials, 32.6% (15/46) were resistant to clindamycin, 19.5% (9/46) to nalidixic acid, 17.3% (8/46) to tetracycline and 15.2% (7/46) to erythromycin. Three *C. jejuni* isolates (6.5%) were resistant to azithromycin and ciprofloxacin while two *C. jejuni* isolates (4.3%) showed resistance to telithromycin. Two *C. jejuni* isolates were each resistant to gentamicin and florfenicol respectively (**Table 6**).

Among the 24 *C. coli* isolates which were resistant to one or more antimicrobial classes, 50% (12/24) were resistant to clindamycin, 25% (6/24) to nalidixic acid and

tetracycline, 20.8% (5/24) to erythromycin, and 12.5% (3/24) to azithromycin and gentamicin. Resistance to ciprofloxacin, florfenicol and telithromycin was low among the *C. coli* isolates (two isolates for each antimicrobial) (**Table 6**).

Among the 16 cattle *C. upsaliensis* isolates tested, 25% (4/16) were resistant to clindamycin, 18.7% (3/16) to erythromycin, and 12.5% (2/16) to tetracycline and nalidixic acid. Lower resistance levels were observed against azithromycin and telithromycin (one isolate per antimicrobial). None of the *C. upsaliensis* isolates were resistant to ciprofloxacin, gentamicin and florfenicol (**Table 6**).

Multidrug resistance (MDR) was recorded in 32.5% (28/86) of isolates. Multiple drug resistance was more frequent among *C. jejuni* which were mainly resistant against erythromycin, tetracycline, nalidixic acid and clindamycin. Of the 46 *C. jejuni* isolates tested, 36.9% (17/46) were multiresistant (resistant to two or more antimicrobials classes), 19.5% (9/46) were resistant to three antimicrobials, 13.0% (6/46) were resistant to four antimicrobials. One *C. jejuni* isolate (1/46; 2.1%) to four antimicrobials and another one to six antimicrobials including azithromycin, erythromycin, tetracycline, nalidixic acid, telithromycin and clindamycin (**Table 6**).

The antibiogram of antimicrobial resistant *Campylobacter* isolates by species to individual antimicrobials is presented in **Table 6**. *Campylobacter jejuni* isolates were mainly multi-resistant against nalidixic acid and clindamycin (**Table 7; Appendix 3**). Among the 24 *C. coli* isolates tested, 33.3% (8/24) were multiresistant, 12.5% (5/24) were resistant to two antimicrobials, one was resistant to four antimicrobials and two isolates (8.3%) were resistant to all nine antimicrobials tested. *C. coli* isolates (50%;4/8) were mainly multi-resistant against clindamycin, tetracycline and nalidixic acid (**Table 7 or Appendix 3**). Among the 16 *C. upsaliensis* isolates tested, only three isolates, 18.7% (3/16) displayed multidrug resistance to three antimicrobial agents each (**Table 7; Appendix 3).**

Table 6: Antimicrobial resistance in *Campylobacter jejuni* (*n*=46), *C. coli* (*n*=24), and *C. upsaliensis* (*n*=16)

Antimicrobial agents	*C. jejuni* *n*=46 (%)	*C. coli* *n*=24 (%)	*C. upsaliensis* *n*=16 (%)	*Campylobacter* spp. Total *N*=86 (%)
Azithromycin	3(6.5)	3(12.5)	1(6.2)	7(8.1)
Ciprofloxacin	3(6.5)	2(8.3)	0(0)	5(5.8)
Erythromycin	7(15.2)	5(20.8)	3(18.7)	15(17.4)
Gentamicin	1(2.1)	3(12.5)	0(0)	4(4.6)
Tetracycline	8(17.3)	6(25)	2(12.5)	16(18.6)
Florfenicol	1(2.1)	2(8.3)	0(0)	3(3.4)
Nalidixic acid	9(19.5)	6(25)	2(12.5)	17(19.7)
Telithromycin	2(4.3)	2(8.3)	1(6.2)	5(5.8)
Clindamycin	15(32.6)	12(50)	4(25)	31(36.0)

Table 7: Multi-drug resistance among 86 *Campylobacter* spp. isolates from cattle.

Resistance patterns		Number of multi-drug resistant *Campylobacter* isolates by species			
No. of antimicrobials	Antimicrobial agents	*C. jejuni* (*n*=46)	*C. coli* (*n*=24)	*C. upsaliensis* (*n*=16)	Total (*N*=86)
2	AZI, NAL	1	0	0	1
2	CIP,NAL	2	0	0	2
2	ERY,CLI	1	1	0	2
2	TET,CLI	1	3	0	4
2	NAL,CLI	4	1	0	5
3	AZI,ERY,CLI	0	0	1	1
3	AZI,ERY,TEL	1	0	0	1
3	ERY,TET,CLI	2	0	1	3
3	CIP,NAL,CLI	1	0	0	1
3	GEN,TET,CLI	1	0	0	1
3	NAL,TEL,CLI	0	0	1	1
3	TET,FFN,CLI	1	0	0	1
4	ERY,TET,NAL,CLI	1	0	0	1
4	AZI,GEN,NAL,CLI	0	1	0	1
6	AZI,ERY,TET,NAL,TEL,CLI	1	0	0	1
9	AZI,CIP,ERY,GEN,TET,FFN,NAL,TEL,CLI	0	2	0	2
Total		**17**	**8**	**3**	**28**

Table: 8.Multi-drug resistance patterns among *C. jejuni* (*n*=46), *C. coli* (*n*=24) and *C. upsaliensis* (*n*=16). **AZI**, Azithromycin; **CIP**, Ciprofloxacin; **ERY**, Erythromycin; **GEN**, Gentamicin; **TET**, Tetracycline; **NAL**, Nalidixic acid; **TEL**, Telithromycin; **CLI**, Clindamycin.

3.4. Discussion

Campylobacter spp. have become a public health concern as they are frequently implicated in human gastroenteritis outbreaks worldwide. Beef and other cattle products contaminated with *Campylobacter* spp. are commonly implicated in *Campylobacter* spp human outbreaks. However, current data on the prevalence of *Campylobacter* spp. in cattle in South Africa is nonexistent. This study revealed that the overall prevalence of *Campylobacter* spp. in beef cattle on the five cow-calf operations surveyed was 29.7% (**Table 4**). *Campylobacter* spp. occurrence rates in healthy cattle have been reported to range from 5.3% to 78.5% in different countries (Wieczorek, Osek, 2017, Rahimi, Alipoor-Amroabadi & Khamesipour, 2017, Smith *et al*., 2018, Ramonaitė *et al.*, 2013). The prevalence of *Campylobacter* spp. in this study was comparable to reports on dairy cattle in Ohio state, USA (36.6%) (Sanad *et al*., 2013), beef cattle fecal samples (33%) in Selangor, Malaysia (Premarathne *et al*., 2017), beef cattle in Chile (35.9%) (Fernández and Hitschfeld, 2009**)** and dairy cattle (35.4%) in Tanzania (Kashoma *et al*., 2015). However, much lower rates of *Campylobacter* have been reported in beef cattle in Ghana (13.2%) (Karikari *et al.*, 2017), Cambodia (5,3%) (Osbjer *et al.*, 2016), and Iran (5.3%) (Rahimi, Alipoor-Amroabadi & Khamesipour, 2017). Previously, variations in *Campylobacter* spp. occurrence rates in different countries have been ascribed to a number of factors including farm management, sampling methods, isolation media, culture conditions, incubation temperature and atmosphere, antimicrobial supplements used in enrichment or isolation media, and identification methods (PCR vs biochemical) that are used for *Campylobacter* spp. recovery.

Beef is the second most consumed meat in South Africa after poultry (DAFF, 2017) and the presence of *Campylobacter* spp. in cattle from the cow-calf operations surveyed is public health concern. This study showed that the cattle farms under study represent an important reservoir and a potential source of *Campylobacter* spp. for humans who might consume contaminated meat products from these herds. *Campylobacter jejuni*, was the most frequent (62.6%) species in the cattle population surveyed; followed by *C. coli* (25.3%), and *C. upsaliensis* (10.1%). The predominance of *C. jejuni* over *C. coli* and *C. upsaliensis* in cattle is consistent with previous studies which were carried out on cattle faecal samples in South Africa and other countries including the USA (Cha *et al.*, 2017), Canada (Viswanathan *et al*., 2017), France (Thépault *et al*., 2018) and Finland (Hakkinen, Heiska & Hanninen, 2007). However,

some reports from the United States of America (USA) and Ghana have reported *C. coli* as the most frequent species in cattle samples (Smith *et al.*, 2018, Sanad et al., 2011, Karikari *et al.*, 2017).

Of particular interest in this study, was the recovery of *C. upsaliensis* in 10.1% of cattle. Similar studies that have searched for *C. upsaliensis* in Ghana and Lithuania could not detect *C. upsaliensis* in cattle samples (Ramonaitė *et al*., 2013, Karikari *et al*., 2017). The recovery of *C. upsaliensis* may have been facilitated by the use of CVA agar which for *Campylobacter* spp. culture and isolation. CVA agar is recommended for *Campylobacter* spp. isolation when a single medium is to be used (Fitzgerald, Nachamkin, 2007). In this study, CVA Agar was a reliable medium for isolation of *Campylobacter* spp. of public health importance including *C. jejuni*, *C. coli* and *C. upsaliensis*. As an emerging and clinically important diarrheal pathogen worldwide, *C. upsaliensis* has been previously incriminated in bacteremia in debilitated and immunocompromised patients, extra-intestinal infections, spontaneous human abortion, hemolytic uremic syndrome and Guillain-Barre Syndrome (Bourke, Chan & Sherman, 1998). This finding suggests that *C. upsaliensis* colonizes cattle and further investigations are needed to evaluate to what extent livestock constitute a reservoir of less common but clinically relevant emerging *Campylobacter* species such as *C. upsaliensis*. The occurrence of *C. upsaliensis* in the cattle under study may point towards the presence of a dog reservoir (Chaban et al., 2010) which may be acting as a source of *Campylobacter* spp for cattle on the farms which were investigated. However, further studies will be needed to investigate and pinpoint the exact source of *C. upsaliensis* in these herds.

The prevalence rates of *Campylobacter* spp. on individual farms were lower compared to similar studies in which the prevalence of *Campylobacter* spp. ranged from 75-83.3% and from 58.6-83.8% in Lithuania and in mid-Michigan (USA) respectively (Ramonaitė et al., 2013; Cha *et al*., 2017). However, much lower rates have been previously observed in South Africa (Uaboi-Egbenni *et al.*, 2012).

Campylobacter spp. occurrence in calves was low (6.9%), in contrast to studies that have reported higher *Campylobacter* prevalence rates in calves in France (99.4%) and Lithuania (86.5%) (Thépault *et al.*, 2018, Ramonaitė *et al.*, 2013). However, the low rate of *Campylobacter* spp. in calves observed in this study is in agreement with similar reports from Austria (14.9%) and Algeria (14.0%) (Klein *et al*., 2013, Guessoum *et al.*, 2016). It should be noted that calves were tested on only one farm.

Furthermore, the sample size of calves tested in our study was smaller and not representative to conclusively explain the difference in *Campylobacter* proportions between our study and a number of reports from other countries. Previously, two studies in France and Lithuania have reported higher prevalences of *Campylobacter* in calves (Thépault *et al.*, 2018, Ramonaitė *et al.*, 2013) and the expectation is that calves should have higher *Campylobacter* spp. contamination rates, as they more susceptible to *Campylobacter* spp. colonization because of a weak immune system. However, the low prevalence of *Campylobacter* spp in calves in this study shows that the prevalence of *Campylobacter* spp. in calves may be dependent upon other factors that still remain to be identified.

Overall, antimicrobial resistance profiling revealed that 52.3% (45/86) of the *Campylobacter* isolates tested were resistant to one or more antimicrobial agents. The levels of antimicrobial resistance detected in this study were low in comparison to *Campylobacter* spp. resistance rates that have been reported in beef cattle in the USA (83.7%), France (64.6%) and Poland (65.4%) respectively (Châtre *et al.*, 2010, Cha *et al*., 2017, Wieczorek, Osek, 2013). Higher resistance rates were observed in *C. jejuni* isolates in comparison to *C. coli* or *C. upsaliensis,* consistent with research on cattle *Campylobacter* spp, isolates in the USA, France and South Africa (Châtre *et al.*, 2010, Cha et al., 2017, Uaboi-Egbenni *et al.*, 2012). However, previously, a number of studies have detected higher rates of antimicrobial resistance in *Campylobacter coli* isolates in comparison to *Campylobacter jejuni* isolates elsewhere (Bae *et al.*, 2005; Englen *et al*., 2007; Tang *et al.*, 2017).

Among the nine antimicrobials tested, resistance to clindamycin (36%) was the highest. However, this was in contrast to most studies, from Canada and the United States of America (USA) which have reported much lower proportions of antimicrobial resistant *Campylobacter* spp. isolates to clindamycin (Webb *et al.*, 2018; Tang *et al.*, 2017; Cha *et al*., 2017). Resistance to clindamycin in this study was mostly observed in *C. coli* (50%) followed by *C. jejuni* (32.6%) and *C. upsaliensis* (25%). The higher resistance rate to clindamycin among *C. coli* isolates may be due to the intrinsic ability of *C. coli* to develop resistance to this antimicrobial (Chen *et al*., 2010). Currently, although there is limited information on resistance to clindamycin in *Campylobacter* spp. isolates from cattle, a report on *Campylobacter* spp. isolates from Chinese pigs revealed also significant antimicrobial resistance to clindamycin of up to 43.2% (Qin *et al*., 2011). Resistance to clindamycin is of public significance as clindamycin has

been shown to display good *in vitro* activity against *C. jejuni* and is considered a therapeutic alternative for the treatment of *Campylobacter* spp. infections in humans (Gomez-Garces, Cogollos & Alos, 1995; Varela et al., 2007). Previously, a combination of clindamycin and amoxicillin has also been successfully used to treat human adults with femoral infections in which *Campylobacter* fetus was implicated (Breda *et al.*, 2016).

Next to clindamycin, lower resistance levels were recorded for nalidixic acid (19.7%), tetracycline (18.6%) and erythromycin (17.4%) in *C. coli* isolates mainly, consistent with previous reports from the United States of America (USA), Malaysia, Iran and Japan (Haruna *et al*., 2013, Premarathne *et al.,* 2017, Tang *et al*., 2017, Rahimi, Alipoor-Amroabadi & Khamesipour, 2017, Cha *et al*., 2017). However, Châtre *et al*., (2010), Wieczorek *et al.,* (2013) and Karikari *et al*., (2017), recorded much higher resistance rates to nalidixic acid (70.4%), tetracycline (51.4%) and erythromycin (up to 97%), mostly among cattle *C. coli* isolates in France, Poland and Ghana respectively (Châtre *et al.*, 2010, Karikari *et al*., 2017, Wieczorek, Osek, 2013). Resistance to nalidixic acid, tetracycline and erythromycin is intriguing as these antimicrobials are supposed to be minimally used on cow-calf operations and for only therapeutic purposes. Higher resistance rates against these antimicrobials maybe due to the indiscriminate use and abuse of these antimicrobials or closely related compounds on the cow-calf operations surveyed. The indiscriminate use and abuse of these antimicrobials or closely related compounds may be exerting selective pressure leading to subsequent cross resistance or development of resistance to these antimicrobials in *Campylobacter* spp. isolates (Luangtonkun *et al*., 2009).

Of clinical interest, was resistance to erythromycin which is considered an antimicrobial of choice for *Campylobacter* spp. in the treatment of *Campylobacter*-associated gastroenteritis. Erythromycin is considered a safe antimicrobial for children and pregnant women showing *Campylobacter*-associated gastroenteritis (Platts-Mills, Kosek, 2014). Much lower resistance levels were recorded for azithromycin, ciprofloxacin gentamycin, florfenicol and telithromycin (**Table 6**). However, *Campylobacter* resistance to azithromycin (8.1%) in this study was higher when compared to results obtained by Tang *et al.,* (2017) (0.3%) previously, in which none of their *C. coli* isolates tested were resistant to azithromycin. Resistance to azithromycin (macrolide), although low is worrisome, and has to be monitored because this antimicrobial is also an antimicrobial of choice against *Campylobacter*

spp. infections in humans (Mukherjee, Dutta & Mukhopadhyay, 2017, Gilbert *et al.*, 2007). Azithromycin is also recommended for use in pregnant women and children affected by *Campylobacter* spp. (Bardon *et al*., 2009).

The rate of *Campylobacter* resistance to ciprofloxacin (6%) in this study was comparable to that reported by Webb, *et al*., (2018) in beef cattle isolates in Southern Alberta, Canada (Webb *et al*., 2018). In South Africa, higher *Campylobacter* resistance rates to ciprofloxacin were previously reported in *C. jejuni* (33.3%) and *C. coli* (56.3%) isolates from dairy cattle from Limpopo province (Uaboi-Egbenni *et al.*, 2012). The use of fluoroquinolones in food animals has led to the development of fluoroquinolone-resistant *Campylobacter* spp. strains globally (Price *et al.*, 2005). Resistance to ciprofloxacin is worrisome as ciprofloxacin (fluoroquinolone) is often used as last resort antimicrobial in the treatment of many bacterial infections including *Campylobacter* spp. (Tang *et al*., 2017).

Much lower resistance rates to gentamicin (5%) and florfenicol (3.4%) were observed among *C. coli* isolates (12.5% and 8.3%), consistent with previous studies which have been reported similar results in *Campylobacter* isolates recovered cattle (Tang *et al.,* 2017, Cha *et al*., 2017, Sanad *et al.*, 2011). However, Uaboi-Egbenni, e*t al*. (2012) reported higher resistance rates of *Campylobacter* spp. to gentamicin mostly among *C. coli* isolates (62.5%). The very low levels of gentamicin and florfenicol resistance observed in this study is in agreement with a previous study (Tang *et al.*, 2017), and may be ascribed to the rare use of these antimicrobials in animal prophylaxis, metaphylaxis, and growth promotion (Saenz *et al.*, 2000).

Our findings revealed that 35.2% (28/86) of cattle *Campylobacter* isolates were multi-resistant (two or more antimicrobials classes). Similar proportions of multiresistant isolates were observed among cattle *Campylobacter* spp. isolates recovered from faecal samples (Premarathne *et al.*, 2017, Tang *et al.*, 2017, Cha *et al.*, 2017, Noormohamed, Fakhr, 2014). Multiresistance to nalidixic acid/clindamycin (17.8%) and tetracycline/clindamycin (14.3%) were the most common pattern of multidrug resistance. However, Cha, *et al*., (2017) and Tang, Yizhi *et al*., (2017) reported much lower resistance rates to these antimicrobials in cattle *Campylobacter* isolates (Tang *et al*., 2017, Cha *et al*., 2017). Furthermore, multidrug resistance was mostly (60.7%) observed among *C. jejuni* isolates in contrast to other studies that have recorded much higher multi-drug resistance in *C. coli* isolates from cattle (Wieczorek, Osek, 2013, Okunlade *et al*., 2015). While factors governing resistance among

Campylobacter spp. are not fully understood, previous studies have reported a number of mechanisms that regulate resistance against important antimicrobials in *Campylobacter* spp. Mutations in particular genes encoding GyrA (fluoroquinolones) (Han *et al.*, 2012), 23S rRNA (macrolides) (Payot *et al*. 2006), and multidrug efflux pump CmeABC (Lin *et al*., 2002) are among some of the mechanisms involved in regulating resistance in *Campylobacter* spp. Resistance to tetracycline is acquired through lateral gene transfer *tet(O)* and *aphA-3*) (Crespo *et al*., 2016). Furthermore, the ribosomal RNA methylase gene *erm(B)* in *C. jejuni* and *C. coli* has been associated with resistance to macrolides (Qin *et al*., 2013; Wang *et al.*, 2014).

Little is known about the prevalence of *Campylobacter* spp. in healthy beef cattle on cow-calf operations in South Africa. To our knowledge, this is the first study which has investigated the occurrence of *Campylobacter* spp. of public health significance in beef cattle on cow-calf operations in South Africa. This study demonstrated that beef cattle from cow-calf operations in South Africa are a reservoir of clinically relevant *Campylobacter* spp. including *C. jejuni*, *C. coli* and *C. upsaliensis*. The results of this investigation provided evidence that antimicrobial resistance is common among *Campylobacter* spp. isolates from beef cattle in South Africa and cattle are potential source of antimicrobial resistant *Campylobacter* spp. strains. Resistance against a number of antimicrobials which are commonly used in the treatment of bacterial infections including *Campylobacter* spp disease in humans is a public health concern. Adequate *Campylobacter* spp. surveillance, education and training of cattle farmers on the prudent use of antimicrobials and application of good veterinary practices in clinical practice are needed to raise awareness about zoonotic *Campylobacter* spp. and prevent the spread of resistant *Campylobacter* spp. strains to humans.

3.5. Acknowledgements

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3.6. References

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CHAPTER 4: GENERAL DISCUSSION

Cattle are considered an important reservoir of foodborne *Campylobacter* spp. infections and a potential source of antimicrobial resistance strains for humans (Hakkinen, 2010; Stanley, Jones, 2003). A number of studies have implicated *Campylobacter* spp. in human gastroenteritis after ingestion of contaminated undercooked beef meat, unpasteurized milk or contact with cattle (Fernandes *et al.*, 2015, El-Zamkan, Hameed, 2016). In South Africa, beef is the second most consumed meat after poultry (Department of Agriculture, Forestry and Fisheries, 2017). However, only a few studies have reported on the prevalence and antimicrobial resistance patterns of *Campylobacter* spp. isolates from beef cattle in South Africa (Uaboi-Egbenni *et al*., 2012, Thobela, 2017, Mabote, Mbewe & Ateba, 2011). The aim of this study was to investigate the occurrence of *Campylobacter* spp. in beef cattle and determine the resistance patterns of *Campylobacter* spp. isolates. We collected faecal samples from 537 apparently healthy beef cattle on five cow-calf operations and searched for *Campylobacter* species of public health importance including *C. jejuni, C. coli* and *C. upsaliensis* using culture and PCR.

Findings from this study revealed that *Campylobacter* spp. were present in 29.7% (160/537) of the beef cattle surveyed, of which 62.6% (99/158) were *C. jejuni subsp. jejuni*, 25.3% were *C. coli* and 10.1% (16/158) *C. upsaliensis*. The high prevalence of *C. jejuni* over other species of *Campylobacter* observed in this study was not surprising. Previous studies have also reported that *C. jejuni* is the most prevalent *Campylobacter* spp. in cattle (Thépault *et al.*, 2018, Premarathne *et al.*, 2017, Kashoma *et al.*, 2015). However, some studies have reported higher recovery rates of *C. coli* (Smith *et al.*, 2018, Sanad *et al*., 2011). The recovery of *C. upsaliensis* in 10.1% of healthy beef cattle in this study was of particular interest, as this clinically important and emerging *Campylobacter* species is not common in cattle but mainly found in dogs (Westgarth *et al*., 2009).

Our results also demonstrated that beef cattle on cow-calf operations in South Africa harbour *Campylobacter* species which have been incriminated in *Campylobacter* spp. gastroenteritis in humans previously. However, of note, was the low recovery rate of *Campylobacter* spp. from calves (6.9%), in contrast to previous studies which have recorded much higher recovery rates of *Campylobacter* spp. among calves (Thépault *et al.*, 2018, Klein *et al*., 2013). The low rate of *Campylobacter* spp. in calves was not

expected because it has been suggested that calves carry high loads of *Campylobacter* spp. due to an immature immune system (Klein *et al*., 2013)**.**

Furthermore, antimicrobial resistance profiling (phenotypic) using broth microdilution method on 86 confirmed isolates including 46 *C. jejuni*, 24 *C. coli* and 16 *C. upsaliensis* revealed that, 52.3% (45/86) of the *Campylobacter* isolates tested were resistant to one or more antimicrobial agents. Some of the antimicrobial agents to which *Campylobacter* spp. were resistant are front-line drugs commonly used for treatment of *Campylobacter* infection in humans (Prescott, Dowling, 2013). Antimicrobial resistance was more common among *C. jejuni,* (60.7%) compared to *C. coli* and *C. upsaliensis*. This finding was consistent with earlier studies carried on cattle in Limpopo and Western Cape provinces, South Africa (Uaboi-Egbenni *et al.*, 2012, Thobela, 2017). However, Englen, *et al.* (2007) indicated that in *Campylobacter* species from the same environment, *C. coli* species tend to develop more antimicrobial resistance than *C. jejuni* (Englen *et al.*, 2007).

Campylobacter isolates were mostly resistant to clindamycin (36%). Currently, there is lack of information on antimicrobial resistance to clindamycin among *Campylobacter* isolates from cattle in South Africa. Next to clindamycin, a number of isolates were resistant to nalidixic acid (quinolone), tetracycline and erythromycin (macrolide) and azithromycin (macrolide). This is a public health concern since macrolides and quinolones are frontline antimicrobials used in the treatment of bacterial infection including *Campylobacter* spp. gastroenteritis in humans. *Campylobacter* spp were mostly multidrug resistant to nalidixic acid/clindamycin and tetracycline/clindamycin. Multidrug resistance constitutes a significant threat as it may limit antiomicrobial treatment use against bacterial infections in both humans and beef cattle in South Africa.

To our knowledge, this is the first study reporting on *Campylobacter* occurrence and antimicrobial resistance of beef cattle from cow-calf operations in Gauteng and North West provinces, South Africa. The limitation of this study was that the 537 beef cattle fecal samples analyzed were not representative of the cattle population in South Africa. Therefore, further investigations on larger and representative populations of cattle in various provinces of South Africa are needed to fully understand the epidemiology of *Campylobacter* spp. in cattle operations in South Africa. In addition, surveillance studies focusing on antimicrobial resistance in *Campylobacter* spp.

isolates recovered from livestock (cattle, pigs, sheep, goats and poultry) on commercial and communal farms will also be needed to formulate evidence-based policies aimed at mitigating the occurrence antimicrobial resistantce isolates in cattle in South Africa.

4.2. References

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Appendix 1. Confirmed *Campylobacter* spp. isolates from cattle (n = 158)

Provinces	Farms	ID	*C. jejuni*	*C. coli*	*C. upsaliensis*	*C. jejuni & C. coli*	*C. coli & C. upsaliensis*	*C. jejuni & C. upsaliensis*
Gauteng	A	AK1531	*C.jejuni*	*0*	*0*	0	0	0
Gauteng	A	AK1139	C.jejuni	0	0	0	0	0
Gauteng	A	A292K	*0*	*0*	*C. upsaliensis*	0	0	0
Gauteng	A	Ak11/14	*0*	*0*	*0*	*C. jejuni & C. coli*	0	0
Gauteng	A	AK1136	*C.jejuni*	*0*	*0*	*0*	0	0
Gauteng	A	AK0625	*C.jejuni*	*0*	*0*	*0*	0	0
Gauteng	A	AK094	*0*	*0*	*0*	*C. jejuni & C. coli*	0	0
Gauteng	A	AK30/13	*C.jejuni*	*0*	*0*	0	0	0
Gauteng	A	AK1096	*0*	*0*	*C. upsaliensis*	0	0	0
Gauteng	A	AK22/13	*C.jejuni*	*0*	*0*	0	0	0

4.1. Appendix 1: Confirmed *Campylobacter* isolates from cattle (n = 158)

Gauteng	A	AK23/13	*0*	*C. coli*	*0*	0	0	0
Gauteng	A	AK18/13	*0*	*C. coli*	*0*	0	0	0
Gauteng	A	AK1075	*C.jejuni*	*0*	*0*	0	0	0
Gauteng	A	AK4320	*C.jejuni*	*0*	*0*	0	0	0
Gauteng	A	AK2B11-2	*C.jejuni*	*0*	*0*	0	0	0
Gauteng	A	AK37/13	*C.jejuni*	*0*	*0*	0	0	0
Gauteng	A	AKO919	*C.jejuni*	*0*	*0*	0	0	0
Gauteng	A	AK0956	*0*	*0*	*C. upsaliensis*	0	0	0
Gauteng	A	ABMXI	*0*	*0*	*C. upsaliensis*	0	0	0
Gauteng	A	AK69-2/1	*0*	*C.coli*	*0*	0	0	0
Gauteng	A	**A**K1026,	*0*	*0*	*0*	0	0	*C.jejuni & C. upsaliensis*
		Total Positive: 32.3% (21/65)	***52.3% (11/21)***	***14.2% (3/21)***	***19% (4/21)***	**9.5% (2/1)**	**(0/21)**	**4.7% (1/21)**
Gauteng	B	BK8	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	B	BK11	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	B	BK24	*0*	*0*	*0*	0	0	0
Gauteng	B	BK35	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	B	BK36	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	B	BK38	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	B	BK44	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	B	BK49	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	B	BK50	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	B	BK79	*0*	*0*	*C. upsaliensis*	0	0	0
Gauteng	B	BK98	*0*	*0*	*C. upsaliensis*	0	0	0
		Total Positive: 10.8% (11/102)	**81.8% (9/11)**	***0 (0/11)***	***18.2% (2/11)***	**(0.11)**	**(0.11)**	**(0.11)**
Gauteng	C	C0935	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C1257	*0*	*C. coli*	*0*	0	0	0
Gauteng	C	C0641	*0*	*C. coli*	*0*	0	0	0
Gauteng	C	C5	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C1436	*0*	*C. coli*	*0*	0	0	0
Gauteng	C	C14-3	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C10-64	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C1218	*0*	*C. coli*	*0*	0	0	0
Gauteng	C	C0925	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C0936	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C1213	*0*	*0*	*C. upsaliensis*	0	0	0
Gauteng	C	C1340	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C1246	*0*	*C. coli*	*0*	0	0	0
Gauteng	C	C10-28	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C0958	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C14-2	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C0847	*0*	*C. coli*	*0*	0	0	0
Gauteng	C	C0611	*0*	*0*	*C. upsaliensis*	0	0	0
Gauteng	C	C31	*0*	*0*	*C. upsaliensis*	0	0	0

Gauteng	C	C33	*0*	*C. coli*	*0*	0	0	0
Gauteng	C	C0533	*0*	*C. coli*	*0*	0	0	0
Gauteng	C	C089	*0*	*C. coli*	*0*		0	0
Gauteng	C	C40	*0*	*C. coli*	*0*	0	0	0
Gauteng	C	C0726	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C2063/10	*0*	*C. coli*	*0*	0	0	0
Gauteng	C	C1323	*0*	*C. coli*	*0*	0	0	0
Gauteng	C	C1190	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C1128	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C0713	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C60	*0*	*0*	*C.upsaliensis*	0	0	0
Gauteng	C	C1529	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C1536	*0*	*C. coli*	*0*	0	0	0
Gauteng	C	C1533	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C1526	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C1521	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C1519	*0*	*C. coli*	*0*	0	0	0
Gauteng	C	C70	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C1537	*0*	*C. coli*	*0*	0	0	0
Gauteng	C	C1535	*C. jejuni*	*0*	*0*	0	0	0
Gauteng	C	C1523	*0*	*C.Coli*	*0*	0	0	0
Gauteng	C	C1527	*C.jejuni*	*0*	*0*	0	0	0
		Total Positive: 53.9% (41/76)	**51.2% (21/41)**	**39% (16/41)**	**9.7 % (4/41)**	**(0/41)**	**(0/41)**	**(0/41)**
North West	D	D11-1/13	*C.jejuni*	*0*	*0*	0	0	0
North West	D	D08/12-3	*0*	*C.Coli*	*0*	0	0	0
North West	D	DCC2-1	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D34-3/10	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D34/13	*C. jejuni*	*0*	*0*	0	0	0
North West	D	DCC5	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D138-09	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D5-07	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D4903	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D108/10	*0*	*C. coli*	*0*	0	0	0
North West	D	D37/13	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D116/13	*0*	*0*	*C. upsaliensis*	0	0	0
North West	D	D43/13	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D4/13	*C. jejuni*	*.*	*0*	0	0	0
North West	D	D100/13	*0*	*C. coli*	*0*	0	0	0
North West	D	D51/09	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D17/02-1	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D31/12-2	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D127/13	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D63/03	*0*	*C. coli*	*0*	0	0	0
North West	D	DBRAX1	*0*	*C. coli*	*0*	0	0	0

North West	D	D108/13	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D303/10	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D83/13	*0*	*C. coli*	*0*	0	0	0
North West	D	D0/13	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D12/10	*0*	*C. coli*	*0*	0	0	0
North West	D	D104/13	*C.jejuni*	*0*	*0*	0	0	0
North West	D	D80/13	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D23-3	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D79--10	*0*	*C. coli*	*0*	0	0	0
North West	D	D77/13	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D24/12	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D8/11-1	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D174-3/12	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D36-2-10	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D147/12	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D58/12	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D133-1	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D82/13	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D56-1	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D45-3	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D67/13-3	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D119/13	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D148/13	*0*	*0*	*0*	*C. jejuni & C. coli*	0	0
North West	D	D51-02	*0*	*C. coli*	*0*	0	0	0
North West	D	D73/13	*0*	*C. coli*	*0*	0	0	0
North West	D	D87/13	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D156/13-1	*0*	*C. coli*	*0*	0	0	0
North West	D	D108/09	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D60/08	*0*	*C. coli*	*0*	0	0	0
North West	D	D153-1/09	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D27-10	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D74-13	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D6/13	*0*	*C. coli*	*0*	0	0	0
North West	D	D92/13	*0*	*C. coli*	*0*	0	0	0
North West	D	D126/13	*0*	*C. coli*	*0*	0	0	0
North West	D	D72/13-2	*0*	*C. coli*	*0*	0	0	0
North West	D	D42-13	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D178-13	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D176/13-1	*0*	*C. coli*	*0*	0	0	0
North West	D	D85-3	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D68/12-1	*C. jejuni*	*0*	*0*	0	0	0
North West	D	D1-1	*C. jejuni*	*0*	*0*	0	0	0
		Total Positive (%): 34.8(63/181)	**73 (46/63)**	**26.9 (17/63)**	**1.6 (1/63)**	**1.6 (1/63)**	**(0/63)**	**(0/63)**
North West	E	EJ031	*C. jejuni*	*0*	*0*	0	0	0

North West	E	EA83	*C. jejuni*	*0*	*0*	0	0	0
North West	E	E0292A	*0*	*C. coli*	*0*	0	0	0
North West	E	EA25	*C. jejuni*	*0*	*0*	0	0	0
North West	E	EA1096B	*C. jejuni*	*0*	*0*	0	0	0
North West	E	EJ19A	*0*	*0*	*C. upsaliensis*	0	0	0
North West	E	E1701	*0*	*0*	*C. upsaliensis*	0	0	0
North West	E	EA19	*C. jejuni*	*0*	*0*	0	0	0
North West	E	E207	*0*	*0*	*C. upsaliensis*	0	0	0
North West	E	EMFT68	*C. jejuni*	*0*	*0*	0	0	0
North West	E	EO31-1	*0*	*0*	*C. upsaliensis*	0	0	0
North West	E	E1818	*0*	*C. coli*	*0*	0	0	0
North West	E	E035-2	*0*	*C. coli*	*0*	0	0	0
North West	E	E203	*0*	*C. coli*	*0*	0	0	0
North West	E	E210-1	*0*	*0*	*C. upsaliensis*	0	0	0
North West	E	E0001	*C. jejuni*	*0*	*0*	0	0	0
North West	E	E5109	*C. jejuni*	*0*	*0*	0	0	0
North West	E	E536	*C. jejuni*	*0*	*0*	0	0	0
North West	E	EA71	*C. jejuni*	*0*	*0*	0	0	0
North West	E	E269-2	*C. jejuni*	*0*	*0*	0	0	0
North West	E	ETSS24	*C. jejuni*	*0*	*0*	0	0	0
North West	E	E2063/10	*0*	*0*	*0*	0	*C. coli & C. upsaliensis*	*0*
		Total Positive (%): 19.5% (22/113)	**54.5% (12/22)**	**18.2% (4/22)**	**22.7% (5/22)**	**(0/22)**	**4.5% (1/22)**	**(0/22)**
		160	**99**	**40**	**16**	**3**	**1**	**1**

Appendix 2: Distribution of confirmed *Campylobacter spp*. in farms (A, B, C, D and E)

Distribution of *Campylobacter spp*. on farms (A,B,C,D and **E)**			
Farm	**Cattle ID**	**Farm Prevalence**	**No. of cattle (+) by species *n*=158**
A	**A**K1531, **A**K1139, **A**K292, **A**K11/14, **A**K1136, **A**K0625, **A**K094, **A**K30/13, **A**K1096, **A**K22/13, AK1075, **A**K23/13, **A**K2B112, **A**K18/13, **A**K37/13, **A**K0919, **A**K0956, **A**KBMXI, **A**K69-2/1, **A**K1026, **A**K4320	**32.3%** (21/65)	*C. jejuni* 7% (11/158) *C. coli* 2% (3/158) *C. upsaliensis* 2.5% (4/158) Co-infection 2% (3/158)
B	**B**K8, **B**K11, **B**K24, **B**K35, **B**K36, **B**K38, **B**K44, **B**K49, **B**K50, **B**K79, **B**K98	**10.8%** (11/102)	*C. jejuni* 5.7% (9/158) *C. coli* 0% (0/158) *C. upsaliensis* 1.3% (2/158) Co-infection 0% (0/158)
C	**C**0935, **C**1257, **C**0641, **C**5, **C**1436, **C**14-3, **C**10-64, **C**1218, **C**0925, **C**0936, **C**1213, **C**1340, **C**1246, **C**10-28, **C**0958, **C**14-2, **C**0847, **C**0611, **C**31, **C**33, **C**0533, **C**89, **C**40, **C**0726, C2063/10, **C**1323, **C**1190, **C**1128, **C**60, **C**0713, **C**1529, **C**1536, **C**1533, **C**1526, **C**1521, C1519, **C**70, **C**1537, **C**1535, **C**1523, **C**1527	**54%** (41/76)	*C. jejuni* 13.2% (21/158) *C. coli* 10.1% (16/158) *C. upsaliensis* 3.8% (6/158) Co-infection 0.6% (1/158)
D	**D**11-1/13, **D**08/12-3, **D**CC2-1, **D**34-3/10, **D**34/13, **D**CC5, **D**138-09, **D**5-07, **D**4903, **D**108/10, **D**37/13, **D**116/13, **D**43/13, **D**44/13, **D**100/13, **D**51/09, **D**17/02-1, **D**31/12-2, **D**127/13, **D**63/03, **D**BRAXI, **D**108/13, **D**303/10, **D**83/13, **D**30/13, **D**12/10, **D**104/13, **D**80/13, **D**23-3, **D**79-10, **D**77/13, **D**24/12, **D**811-1, **D**174-3/12, **D**36-2-10, **D**147/12, **D**58/12, **D**133-1, **D**82/13, **D**56-1, **D**45-3, **D**67/13-3, **D**119/13, **D**148/13, **D**51-02, **D**73/13, **D**87/13, **D**156/13-1, **D**108/09, **D**60/08, **D**153-1/09, **D**27-10, **D**74-13, **D**6/13, **D**92/13, **D**126/13, **D**72/13-2, **D**42-13, **D**178-13, **D**176/13-1, **D**85-3BULL, **D**68/12-1	**34.8%** (63/181)	*C. jejuni* 29.1% (63/158) *C. coli* 10.7% (17/158) *C. upsaliensis* 0.6% (1/158) Co-infection 0.6% (1/158)
E	**E**J031, **E**A83, **E**0292A, **E**A25, **E**A1096B, **E**J19A, **E**1701, **E**A19, **E**207, **E**MFT68, **E**O31-1, **E**1818, **E**35-2, **E**J2296, **E**210-1, **E**0001, **E**5109, **E**536, **E**A71, **E**269-2, **E**TSS24, **E**2063/10	**19.5%** (22/113)	*C. jejuni* 7.5% (12/158) *C. coli* 2.5% (4/158) *C. upsaliensis* 3.2% (5/158) Co-infection 0.6% (1/158)

4.3. Appendix 3: Antimicrobial resistant *Campylobacter* isolates for 86 cattle

	AN. ID	SPP.	AZI (mg/L)	CIP (mg/L)	ERY (mg/l)	GEN (mg/l)	TET (mg/L)	FFN (mg/L)	NAL (mg/L)	TEL (mg/L)	CLI (mg/L)
1	**C**S1213	*C.U*	S ≥0.5	S≥0.06	S≥0.25	S≥0.12	S≥0.12	S≥2	S≥16	S≥0.06	S≥0.25
2	**E**1701 (**MDR**)	*C.U*	**R ≥16**	S≥0.25	S≥0.25	**R≥16**	S≥1	S≥4	**R6≥4**	S≥1	**R≥64**
3	**E**MFT68	*C.J*	S ≥0.5	S≥0.12	**R≥64**	S≥0.5	S≥0.5	S≥2	S≥16	S≥2	S≥2
4	**B**K36-1	*C.C*	S ≥2	s≥0.015	S≥4	S≥1	**R≥64**	S≥2	S≥4	S≥2	S≥4
5	**D**56-1 (**MDR**)	*C.J*	S ≥0.05	S≥0.015	S≥0.012	S≥2	**I≥16**	**R≥16**	S≥4	S≥2	**I≥4**
6	**D**148/13 (**MDR**)	*C.J*	S ≥2	S≥0.06	**R≥32**	S≥0.5	**R≥32**	S≥2	S≥4	S≥2	**R≥16**
7	**C**S1527 (**MDR**)	*C.J*	S ≥2	S≥1	**I≥16**	S≥1	**R≥32**	S≥1	S≥4	S≥4	**I≥4**
8	**E**035	*C.C*	S ≥0.015	S≥0.15	S≥0.03	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.015	S≥0.03
9	**A**K10-96-4	*C.U*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	**R≥32**	S≥0.03	S≥4	S≥0.015	S≥0.03
10	**C**S60	*C.U*	S ≥0.015	S≥0.015	**I≥32**	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.015	S≥0.03
11	**C**S31	*C.U*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.015	S≥0.03
12	**E**120-2	*C.C*	S ≥0.5	S≥0.015	**I≥32**	S≥0.25	S≥012	S≥1	S≥4	S≥4	S≥1
13	**A**292K (**MDR**)	*C.U*	S ≥0.015	S≥0.015	**I≥32**	S≥0.12	**R≥32**	S ≥0.3	S≥4	S≥0.03	**I≥4**
14	**D**17/02-1 (**MDR**)	*C.J*	S ≥0.06	S≥0.12	S≥2	S≥0.12	S≥4	S≥0.25	**I≥32**	S≥0.5	**R≥8**
15	**E**1818 (**MDR**)	*C.C*	S ≥012	S≥0.015	S≥1	S≥0.12	**R≥16**	S≥0.5	S≥4	S≥1	**I≥4**
16	**C**S1218 (**MDR**)	*C.C*	S ≥0.5	S≥0.015	S≥2	S≥0.12	**R≥64**	S≥0.6	S4	S≥1	**R≥8**
17	D73/13-2 (**MDR**)	*C.C*	S ≥0.5	S≥0.015	S≥4	S≥0.12	**R≥32**	S≥1	S≥4	S≥1	**I≥4**
18	**D**116/13	*C.U*	S ≥0.25	S≥0.5	S≥0.5	S≥1	S≥0.12	S≥4	**I≥32**	S≥0.5	S≥2
19	**D**70-1	*C.J*	S ≥1	S≥0.015	S≥0.5	S≥1	**I≥8**	S≥2	S≥4	S≥1	S≥1
20	**D**99-2 (**MDR**)	*C.J*	S ≥0.5	S≥0.25	S≥04	S≥0.5	**R≥32**	S ≥0.5	S≥4	S≥1	**I≥4**
21	**B**K49-3	*C.J*	S ≥0.5	S≥0.015	S≥2	S≥0.25	S≥0.12	S≥1	S≥4	S≥0.5	S≥2
22	**D**85-3	*C.C*	S ≥0.5	S≥0.015	**I≥16**	S≥0.5	S≥0.12	S≥2	S≥4	S≥2	S≥2
23	**D**153-1	*C.J*	S ≥0.25	s≥0.015	S≥2	S≥0.5	S≥0.06	S≥4	S≥4	S≥1	**R≥8**
24	**B**K50-3 (**MDR**)	*C.J*	I ≥4	S≥0.015	**R≥32**	S≥0.5	**R≥64**	S≥4	**I≥16**	**I≥8**	**R≥8**
25	**D**178-3 (**MDR**)	*C.J*	I ≥4	S≥0.015	**R≥32**	S≥0.5	S≥1	S≥4	S≥4	**I≥8**	S≥2
26	**C**S1537	*C.C*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.06	S≥4	S≥0.015	S≥0.03
27	**B**K98-1 (**MDR**)	*C.U*	**R ≥8**	S≥0.015	**I≥16**	S≥0.5	S≥1	S≥4	S≥4	S≥4	**I≥4**
28	**C**S0726	*C.J*	S ≥0.015	S ≥0.015	S ≥ 0.015	S ≥0.12	S≥0.06	S≥0.06	S≥4	S≥0.015	S≥0.03
29	**A**K10-26	*C.J*	S ≥2	S≥0.5	S≥8	S≥0.5	S≥0.12	S≥2	S≥4	S≥4	**I ≥4**
30	**A**k35-3	*C.C*	S ≥0.015	s≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.06	S≥4	S≥0.015	**I ≥4**
31	**E**124-2 (**MDR**)	*C.J*	S ≥2	S≥0.06	**I≥16**	S≥2	**R≥64**	S≥2	**I≥16**	S≥4	**R≥16**
32	**C**S1320	*C.J*	**R ≥16**	S≥0.015	S≥0.012	S≥0.06	S≥0.03	S≥0.06	S≥4	S≥0.015	S≥0.03
33	**E**J19A	*C.U*	S≥0.015	S≥0.015	S≥8	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.015	S≥0.03
34	**E**210-1	*C.U*	S ≥0.015	S≥0.-015	S≥0.03	S≥0.12	S≥0.06	S≥0.06	S≥4	S≥0.015	S≥0.03
35	**E**207	*C.U*	S ≥0.5	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.06	S≥4	S≥0.015	S≥0.03
36	**D**42-3 (**MDR**)	*C.J*	S≤0.015	**R≥64**	S≥0.03	S≥0.12	S≥0.25	S≥0.25	**I≥32**	S≥0.015	**I ≥4**
37	**D**133-1 (**MDR**)	*C.J*	S ≥1	**R≥16**	S≥0.25	S≥0.12	S≥0.12	S≥4	**I ≥32**	S≥0.06	S≥0.12
38	**D**2063/10	*C.C*	S ≥0.5	S≥0.015	S≥2	S≥0.25	S≥0.25	S≥1	**I≥16**	S≥1	S≥0.5
39	**C**S14-36	*C.C*	S ≥0.25	S≥0.015	S≥1	S≥0.5	S≥0.06	S≥0.06	S≥4	S≥0.25	S≥1
40	**D**156/13-1	*C.C*	S ≥0.25	S≥0.03	S≥4	S≥0.12	S≥0.25	S≥1	S≥4	S≥1	**I≥4**
41	**B**K8-4 (**MDR**)	*C.J*	S ≥1	S≥0.03	**I≥16**	S≥0.5	S≥0.12	S≥2	S≥4	S≥2	**R≥8**
42	**E**67/13-3	*C.J*	S ≥0.25	S≥0.03	S≥2	S≥2	S≥0.25	S≥2	S≥4	S≥0.5	S≥2
43	**A**k11/14 (**MDR**)	*C.C*	S ≥2	S≥2	**I≥16**	S≥0.5	S≥0.5	S≥4	S≥4	S≥0.5	**R≥8**
44	**C**S0925	*C.C*	S ≥4	S≥0.3	S≥8	S≥0.5	S≥1	S≥4	S≥4	S≥4	**R≥8**
45	**D**36-2-1 (**MDR**)	*C.J*	S ≥1	S≥2	S≥4	S≥0.5	S≥0.25	S≥0.5	**R≥64**	S≥0.5	**I≥4**
46	**C**S70 **(MDR**)	*C.J*	S ≥0.5	S≥0.015	S≥8	S≥2	S≥4	S≥2	**I≥16**	S≥2	**R≥8**
47	**A**K24-1	*C.C*	S ≥0.5	S≥0.5	S≥0.5	S≥0.5	S≥4	S≥1	S≥4	S≥0.12	S≥0.5
48	**D**92/13	*C.C*	S ≥0.25	S≥0.03	S≥4	S≥0.12	S≥0.25	S≥1	S≥4	S≥1	I≥4
49	**C**S1529 (**MDR**)	*C.C*	**R ≥64**	**R≥64**	**R≥64**	**R≥32**	**R≥64**	**R≥4**	**R≥64**	**R≥16**	**R≥8**
50	**B**K79-1	*C.U*	S ≥4	S≥0.12	S≥8	S≥1	S≥8	S≥2	S≥4	S≥0.015	**R≥8**

51	**B**K44-3	*C.J*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.06	S≥4	S≥0.015	S≥0.03
52	**E**269-2	*C.U*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.06	S≥4	S≥0.015	S≥0.03
53	**C**S123 (**MDR**)	*C.J*	S ≥ 0.12	**I ≥2**	S≥2	S≥0.12	S≥0.06	S≥0.06	**R≥64**	S≥0.5	S≥0.03
54	**D**83/13	*C.J*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.06	S≥4	S≥0.015	S≥0.03
55	**D**68/12-1 (**MDR**)	*C.C*	S ≥0.25	S≥2	S≥0.5	S≥1	S≥1	S≥0.5	**R≥32**	S≥0.06	**I ≥4**
56	**D**59-1	*C.J*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.06	S≥4	S≥0.015	S≥0.03
57	**D**43/13	*C.J*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.015	S≥0.03
58	**D**37/13-2	*C.J*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.015	S≥0.03
59	**D**174-3	*C.J*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.015	S≥0.03
60	**D**1-1	*C.J*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.06	S≥4	S≥0.015	S≥0.03
61	**A**K0956	*C.U*	S ≥0.015	S≥0.015	S≥0.03	S≥0.06	S≥0.03	S≥0.03	S≥4	S≥0.015	S≥0.03
62	**D**72/13-2 **(MDR)**	*C.C*	**R ≥64**	**R≥64**	**R≥64**	**R≥32**	**R≥64**	**R≥64**	**R≥64**	**R≥8**	**R≥16**
63	**D**23-3	*C.J*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.06	S≥4	S≥0.015	S≥0.03
64	**D**31/12-1	*C.J*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.06	S≥4	S≥0.015	S≥0.03
65	**D**85-3	*C.J*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.015	S≥0.03
66	**D**45-3	*C.J*	S ≥0.5	S≥0.015	S≥2	S≥0.12	S≥0.06	S≥0.5	S≥4	S≥0.5	S≥0.5
67	**C**S0925	*C.J*	S ≥0.25	S≥0.06	S≥8	S≥0.25	S≥0.25	S≥1	S≥4	S≥1	S≥2
68	**C**S0935	*C.C*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.25	S≥0.03	S≥4	S≥0.015	S≥0.03
69	**C**S10-28	*C.C*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.015	S≥0.03
70	**C**S0847	*C.J*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.015	S≥0.03
71	**D**184-3	*C.C*	S ≥ 1	S≥1	S≥0.12	S≥1	S≥1	S≥1	**I≥16**	S≥0.06	S≥0.25
72	**D**176/13-1	*C.C*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.015	S≥0.03
73	**C**S0835	*C.J*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.015	S≥0.03
74	**C**S0611	*C.U*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.015	S≥0.03
75	**C**S1533 (**MDR**)	*C.J*	S ≥ 2	S≥0.015	S≥4	**I ≥4**	**R≥64**	S≥4	S≥4	S≥4	**R≥16**
76	**D**53-3 (**MDR**)	*C.J*	S ≥0.5	S≥0.25	S≥2	S≥0.12	S≥0.25	S≥0.5	**R ≥64**	S≥0.5	**I ≥4**
77	**D**34-3	*C.J*	S ≥0.06	S≥0.015	S≥0.03	S≥0.12	S≥0.06	R≥032	S≥4	S≥0.5	S≥1
78	**D**172-2	*C.J*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.015	S≥0.03
79	**E**A19-3	*C.J*	S ≥0.5	S≥0.015	S≥0.5	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.25	S≥2
80	**C**S10-64	*C.J*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.015	S≥0.03
81	**E**A83	*C.J*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.015	S≥0.03
82	**E**BMXI	*C.J*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.06	S≥0.03	S≥4	S≥0.015	S≥0.03
83	**D**69-2-1 (**MDR**)	*C.U*	S ≥0.5	S≥0.5	S≥2	S≥1	S≥0.12	S≥4	**I ≥16**	**I ≥8**	**I ≥4**
84	**D**27-1	*C.J*	S ≥0.015	S≥0.015	S≥0.03	S≥0.12	S≥0.12	S≥0.03	S≥16	S≥0.015	S≥0.03
85	**D**125-2	*C.J*	S ≥0.5	S≥0.015	S≥0.5	S≥0.12	S≥0.06	S≥0.06	S≥4	S≥0.5	S≥1
86	**D**6-13	*C.C*	S ≥0.015	S ≥0.015	S ≥0.03	S ≥0.12	S ≥0.06	S ≥0.06	S ≥4	S ≥0.015	S ≥0.03
TOTAL			86	86	86	86	86	86	86	86	86
RESISTANT (%)			8.1 (86)	5.8 (5/86)	17.4 (15/86)	4.6 (4/86)	18.6(16/86)	3.5(3/86)	19.8 (17/86)	5.8 (5/86)	36(31/86)

AZI, Azithromycin; **CIP**, Ciprofloxacin; **ERY**, Erythromycin; **GEN**, Gentamicin; **TET**, Tetracycline; **FFN**, Florfenicol; **NAL**, Nalidixic acid; **TEL**, Telithromycin; **CLI**, Clindamycin.

APPENDIX 4: Copy of the ethical approval letter with Reference number.