# Antimicrobial susceptibility in thermophilic *Campylobacter* species isolated from pigs and chickens in South Africa

by

**Annelize Jonker**

**Submitted in partial fulfilment of the requirements for the degree MSc (Bacteriology) in the Faculty of Veterinary Science, University of Pretoria**

**2009**

# ABSTRACT

## Antimicrobial susceptibility in thermophilic *Campylobacter* species isolated from pigs and chickens in South Africa

By

Annelize Jonker

Supervisor : Dr Jackie Picard

Department of Veterinary Tropical Diseases
Faculty of Veterinary Science
University of Pretoria

Submitted in partial fulfilment of the requirements for the degree
**MSc(Veterinary Tropical Diseases)**

The thermophilic *Campylobacters*, *Campylobacter jejuni* and *Campylobacter coli* are found as commensals in the intestinal tract of healthy mammals and birds. *Campylobacter jejuni* is one of the leading causes of sporadic food-borne bacterial disease in humans which is predominantly contracted from poultry products. Although the vast majority of these infections are mild, life-threatening complications should be treated with antimicrobials. Patients are usually treated with either macrolides of fluoroquinolones. However, globally there is an increased trend in the development of resistance to these antibiotics. This trend has also been observed in infection of poultry and pigs.

The aim of this investigation was to determine antimicrobial sensitivity of thermophilic *Campylobacter*s isolated from pigs and poultry by broth microdilution minimum inhibitory concentration testing.

A total of 482 samples of the small intestinal content from poultry and pigs from the Western Cape and Gauteng Provinces were collected and analysed. Thirty-eight

*Campylobacter* isolates were obtained. Statistical analyses included percentage resistance, minimum inhibitory concentrations ($MIC_{50}$ and $MIC_{90}$) as well as the distribution percentages of the MICs. The non-parametric Mann-Whitney U test was used to establish any significant differences at an interspecies, interhost and interprovincial level.

Analyses of the data obtained revealed indications of decreasing susceptibility to several antibiotic groups including the tetracyclines, macrolides, erythromycin and tylosin, as well as the lincoasamides, and fluoroquinolones. It was found that isolates from the Western Cape were more likely to be resistant to the fluoroquinolones ($p$ = 0.0392), macrolides ($p$= 0.0262), and lincoasmides ($p$ = 0.0001) and, as well as to a certain extent the pleuromutulins ($p$= 0.0985), whereas isolates from Gauteng were more resistant to the tetracycyclines ($p$ = <.0001). Poultry *Campylobacter* spp. were more prone to be resistant to enrofloxacin ($p$= 0.0021). *Campylobacter jejuni*, mainly isolated from poultry, was more liable to be resistant to the tetracyclines (chlrotetracycline $p$= 0.0307), whereas *C. coli*, predominatly isolated from pigs was more likely to be resistant to the macrolides (tylosin $p$= 0.063). Four of the bacteria isolated from the Western Cape were resistant to three or more antibiotic classes, namely; tetracyclines, macrolides, lincosamides, pleuromutulins and fluoroquinolones. No multi-resistant *Campylobacter* spp. were isolated from the flocks in Gauteng. With the exception of tiamulin, the bacterial populations could clearly be divided into resistant and susceptible populations.

As consequence of the increased resistance to the antimicrobial classes used for human therapy and the geographical differences in antimicrobial susceptibility, it is recommended that an antimicrobial resistance monitoring system for the thermophilic *Campylobacter* spp. be initiated in the South Africa National Veterinary Surveillance and Monitoring Programme for Resistance to Antimicorbial Drugs (SANVAD).

**Keywords:** thermophilic, *Campylobacter jejuni*, *Campylobacter coli*, antimicrobial susceptibility, broth microdilution, minimum inhibitory concentration, MIC, Western Cape, Gauteng, pigs, poultry

## ACKNOWLEDGEMENTS

The author thanks County Fair Laboratory and Winelands Pork Abattoir for assisting with the collection of samples.

## ABBREVIATIONS

| | |
|---|---|
| AMR | Antimicrobial resistance |
| BCA | Columbia agar containing 5% defibrinated sheep blood |
| *C. coli* | *Campylobacter coli* |
| CLSI | Clinical Laboratory Standards Insitute |
| *C. jejuni* | *Campylobacter jejuni* |
| CAMHB | Cation-adjusted Mueller-Hinton Broth |
| CampyGen | Campylobacter gas |
| cfu | Colony forming units |
| CLSI | Clinical and laboratory standards institute |
| FDA | Food and Drug Administration |
| $H_2$ | Hydrogen |
| MIC | Minimum inhibitory concentration |
| SVA | National Veterinary Institute (Sweden) |
| SA | Skirrow's agar |
| spp. | Species |
| USA | United States of America |

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# CHAPTER 1

## INTRODUCTION

*Campylobacter jejuni* and *Campylobacter coli* are thermophilic campylobacters that occur worldwide as commensals in the digestive tracts of healthy animals, especially birds.

Some strains, however, are pathogenic and have the ability to cause potentially serious diarrhoeal illness in humans and other animals such as sheep, cattle, pigs and dogs. In fact, campylobacteriosis is considered to be one of the most common causes of sporadic food-borne bacterial illnesses worldwide (Jay 2000; Acha & Szyfres 2003; Gormley, MacRae, Forbes, Ogden, Dallas & Strachan 2008). Animal-derived foods, especially poultry products, are thought to be the major sources of *Campylobacter* infections in humans (Acha & Szyfres 2003; Cui, Ge, Zheng & Meng 2005; Songer & Post 2005).

Evidence that populations of *Campylobacter jejuni* in animals and humans overlap partially, has been documented (Manning, Dowson, Bagnall, Ahmed, West & Newell 2003).

In addition, *C. jejuni* and *C. coli* of food animal origin resistant to one or more antimicrobials have been documented in several countries (van der Walt 2004; Cui *et al*. 2005; Luangtongkum, Morishita, Ison, Huang, Mcdermott & Zhang 2006; Moore, Barton, Blair, Corcoran, Dooley, Fanning, Kempf, Lastovica, Lowery, Matsuda, McDowell, McMahon, Millar, Rao, Rooney, Seal, Snelling & Tolba 2006). Therefore regular monitoring for antimicrobial resistance (AMR) in these bacteria is essential so that measures can be taken in animals to avoid the infection of humans with resistant bacteria as well as limit the transfer of resistance to the enteric microfloral species of humans.

## LITERATURE REVIEW

### Aetiology

The thermophilic campylobacters, *Campylobacter jejuni* and *Campylobacter coli* belong to the Family: Campylobacteraceae, Order: Campylobacterales, Class: Epsilonproteobacteria, Subphylum: Delta/ Epsilon Subdivisions, Phylum: Proteobacteria and Superkingdom: Bacteria (NCBI Taxonomy Browser 2008).

Campylobacters are micro-aerophilic, motile, helical to vibroid Gram-negative rods 0.2-0.5 µm x 1.5-5 µm in size. Their appearance varies from curved to spiral or gull wing-shaped. Gull wing shapes are formed when daughter cells do not separate (Quinn, Markey, Carter, Donnelly & Leonard 2002). They are motile by means of polar flagellae at one or both ends and move in straight lines with a cork-screw motion (Prescott 1990; Acha & Szyfres 2003; van der Walt 2004).

Colonies take between two to five days to grow and are non-haemolytic, flat, grey and mucoid (Prescott 1990). On moist plates they may resemble water droplets that spread along streak lines. Young cultures yield short, irregularly curved rods 0.25-0.30 µm x 0.95-2.8 µm in size. Cultures older than 48 hours or that have been exposed to air, may yield coccoid, filamentous or spiral forms (Prescott 1990; van der Walt 2004).

Although campylobacters have a typical morphology and are catalase and oxidase positive, they are difficult to identify to species level as they are non-fermentative and non-reactive on many biochemical tests (Prescott 1990; Quinn *et al* 2002; van der Walt 2004). *Campylobacter jeuni* and *C. coli* are characterized by the fact that they grow at 42℃ but not at 25℃. Furthermore they are susceptible to nalidixic acid but not cephalothin. *Campylobacter jejuni* and *C. coli* are distinguished from each other by their ability to hydrolyse sodium hippurate (*C. coli* does not hydrolyse sodium hippurate whereas *C. jejuni* does) (Prescott 1990).

**Epidemiology**

Thermophilic campylobacters have a worldwide distribution and are found as commensals in large numbers of up to $10^7$ colony forming units per gram (cfu/g) in the intestinal tract of healthy mammals and birds (Songer & Post 2005). They occur not only in birds to which they are believed to be specially adapted (Manning *et al.* 2003) but also in experimental animals, farm animals, pets and humans (van der Walt 2004). *Campylobacter jejuni* is isolated most commonly from broilers, and *C. coli* most commonly from pigs (Engberg, Aarestrup, Taylor, Gerner-Smidt & Nachamkin 2001; Zhang 2008).

Colonisation of birds by *C. jejuni* occurs asymptomatically where the region, season, population density and diet seem to have an influence on the rate of colonisation (Zhang 2008). The ability to colonise the intestines of chickens, tropism, pathogenicity and the extent of invasion appear to be strain related (Stern, Bailey, Blankenship, Cox & McHan 1988; van der Walt 2004; Songer & Post 2005, Moore *et al.* 2006). Colonisation peaks in

summer and *Campylobacter* is more common among organic and free-range poultry than in conventionally managed poultry managed for commercial purposes, the reason is that these chickens are exposed to a greater variety of environmental conditions and are older when they are slaughtered (Zhang 2008).

Once a flock is infected most chickens are colonised in a short time span (Zhang 2008). In a Russian study conducted in 2004, almost all broilers were found to be colonised by 30-45 days after hatching. This trend was also noticed in the United States (Moore *et al.* 2006). *Campylobacter* is not often detected in birds younger than 3 weeks of age; its prevalence increasing as birds grow and reaches a peak at slaughter age. Carriage rate appears to be higher in commercial poultry than in wild birds. This could be the result of high population densities in commercial poultry houses (Zhang 2008).

*Campylobacter* isolates from poultry are genetically diverse and colonisation by more than one genotype is possible (Zhang 2008). A genetic study using multilocus sequence typing conducted on 266 veterinary and human *C. jejuni* isolates in the United Kingdom revealed a degree of overlapping between veterinary and human isolates with sequence types being shared between humanand animal isolates, suggesting that animals and birds may be potential reservoirs for pathogenic *Campylobacter* species. Manning *et al.* (2003) found that besides, there being *Campylobacter* strains that are common to both chickens and humans, there are also strains infectious to humans that do not colonise chickens and strains that colonise chickens, but do not infect humans. Therefore, in outbreaks of diarrhoea in humans it may be advisable to genetically “fingerprint” the strains to determine their origin.

Among pigs, the carrier status of *Campylobacter* spp. varies between the species and its origins (van der Walt 2004). Piglets raised by the sow tend to be colonised by *Campylobacter* more often than piglets reared in isolation units (Thomson 2006). There does not appear to be a difference in numbers of *Campylobacter* found in healthy and in diarrhoeic pigs (van der Walt 2004).

*Campylobacter jejuni* may survive for several weeks in cold (4℃), moist environments, but dies quickly in those at ambient temperature. It is susceptible to desiccation, freezing, heating and most disinfectants. In milk, it does not survive pasteurisation and is inactivated on the cow’s teats by iodine containing udder washes within eight minutes (van der Walt 2004).

**Pathogenesis**

Despite the progress made, many aspects of the pathogenesis of campylobacteriosis are still not completely understood (Jay 2000).

In poultry, infection occurs via the faeco-oral route, and the caeca and cloacal crypts are colonised. *Campylobacter* may on occasion also be cultured from the blood and several internal organs such as the spleen, liver, and gallbladder. In the case of poultry, *Campylobacter* does not adhere directly to the intestinal wall; instead it colonises the mucous layer of the crypts. Invasion of the intestinal epithelium is rare with pathology of the intestines and organs being even more unusual (Zhang 2008).

Ingestion of a pathogenic strain of thermophilic *Campylobacter*, such as *C. jejuni*, by a non-avian species is followed by attachment to, and penetration of the mucosal layer of the intestine. The ability to attach to the mucosa prevents it from being eliminated from the body via peristalsis and is thought to play a part in the development of enteritis (Jay 2000; Songer & Post 2005). Attachment is mediated by attachment factors, e.g. fibronectin binding protein (CadF), lipoprotein (JipA) and possibly flagellin, pilus protein and lipopolysaccharide (Songer & Post 2005).

Invasion of enterocytes takes place by means of directed endocytosis where the bacterium remains membrane-bound when it has entered the cell (Songer & Post 2005). Colonisation and invasion of the intestinal wall leads to severe oedema and an inflammatory reaction characterised by neutrophil infiltration, the development of mucosal ulcers and focal goblet cell hyperplasia. During the initial acute inflammatory stage, lymphocytes and macrophages are the most numerous cells. Bacteria surrounding blood vessels in the *lamina propria* cause endothelial hypertrophy and thickening of the basal lamina which possibly contributes to the formation of oedema (van der Walt 2004, Zhang 2008). The suspicion exists that the enteritis is not only caused by the bacterial invasion of cells, but also by an enterotoxin (van der Walt 2004) and a cytolethal toxin produced by the bacteria (Zhang 2008).

During a short bacteraemic phase of 72 hours duration, dissemination to the mesenteric lymph nodes, gastro-intestinal tract, gallbladder and spleen may occur. After 72 hours, disappearance of the bacteraemia coincides with the appearance of circulating antibodies (van der Walt 2004).

**Campylobacteriosis in humans**

Campylobacteriosis is one of the leading causes of sporadic food borne bacterial illnesses worldwide. It is even more common than salmonellosis (Jay 2000; Acha & Szyfres 2003; Gormley, Macrae, Forbes, Ogden, Dallas & Strachan 2008). *Campylobacter jejuni* predominates in human infections in whom it accounts for approximately 95 % cases of the disease. The disease appears to be more common in the more affluent sectors of society in the developed world, whereas in South Africa it seems to occur more commonly among the less affluent sectors (van der Walt 2004).

The major sources of human *Campylobacter* infections are animal-derived foods, especially poultry products (Cui *et al.* 2005; Songer & Post 2005). Untreated surface water and unpasteurised milk may also be sources of infection as a result of faecal contamination (Acha & Szyfres 2003; Clark, Price, Ahmed, Woodward, Melito, Rodgers, Jamieson, Ciebin, Li & Ellis 2003; van der Walt 2004). A study by Evans, Ribeiro & Salmon (2003) suggests that contaminated salad vegetables and bottled water may also be added to the list of sources of infection.

The infective dose of *Campylobacter* is low (Herenda & Franco 1996). The 50 % infective dose for humans appears to be approximately 900 cells. Therefore, the risk for infection of humans by *Campylobacter* in contaminated food or water is high (Gormley *et al.* 2008).

The incubation period of campylobacteriosis is 24-72 hours or longer. The disease in humans is acute and characterised by severe diarrhoea, fever, vomiting and abdominal pain. It tends to be self-limiting, but serious complications such as Guillain-Barré syndrome may develop in a small number of patients (Avrain, Humbert, L'Hospitalier, Sanders, Vernozy-Rozand & Kempf 2003; Songer & Post 2005).

**Campylobacteriosis in animals**

Thermophilic campylobacters may also cause disease in mammals and birds. Intestinal disease tends to be mild: *C. jejuni* can cause diarrhoea in young animals such as puppies, lambs and calves; and more rarely *C. coli* has been implicated in cases of mild diarrhoea in pigs (Acha & Szyfres 2003; Songer & Post 2005).

Extraintestinal infections by *C. jejuni* have been reported in sheep, goats, cattle, pigs poultry and ratites. In ruminants, *C. jejuni* has been implicated in cases of abortion and mastitis, and *C. coli* has been been the cause of abortion sheep and pigs (Acha & Szyfres 2003; Songer & Post 2005).

In poultry and ratites *C. jejuni* has been implicated in 'avian vibrionic hepatitis'. It is, however, suspected that there may be some other another primary cause, and that *C. jejuni* plays a secondary role. Clinical signs in poultry may include depression; poor weight gain; dry, scaly combs; an increase in culled birds; anaemia; jaundice and diarrhoea. Macroscopic lesions include intestinal haemorrhage and distention, mucoid or watery intestinal contents, necrotic lesions and haemorrhage in the liver, swelling of the kidneys and spleen, and loss of muscle mass (Herenda & Franco 1996; Songer & Post 2005).

**Diagnosis**

*Culture of thermophilic campylobacters*

Isolation of *Campylobacter* by means of microbiological culture is the confirmatory method for determining the presence of the bacterium in a sample (van der Walt 2004). However, difficulties associated with the poor viability of campylobacters in samples as well as the fact that they are easily overgrown by contaminants has lead to the use of polymerase chain reactions to amplify species-specific nucleotides which are easily detected by gel electrophoresis (Avrain *et al*. 2003). These methods, however, cannot currently be used to monitor for antimicrobial resistance (AMR), because the genes or mutations governing the resistance must be known (Moore *et al*. 2006). Furthermore, several genes usually interact to produce a particular resistance phenotype.

Specimens of choice from poultry are the contents of jejenum, caecum and cloaca (Herenda & Franco 1996), in living pigs faeces and from pig carcasses intestinal content (Prescott 1990).

Diluted faecal wet preparations may be used for direct examination by dark field or phase contrast microscopy. However, these are very difficult to interpret and require large numbers of viable campylobacters. In the case of abortions, smears may be made from foetal stomach contents and stained by the Gram's method. Bile (diluted 1:1 with saline) may also be used for the making of smear stained by the Gram's method or for wet preparations, especially in cases of chronic infection (Prescott 1990).

Specimens should be plated out as soon as possible as putrefactive organisms rapidly eliminate campylobacters. Transport media such as Stuart's or Amies' may be used to improve the chances of recovery after long in transit periods, but this is not usually effective with faecal specimens. The best is to tie off a section of intestine as this keeps moist and free from oxygen. Samples must be transported and stored at 4 ℃ (Prescott 1990). For the culture of *Campylobacter* species, especially those originating from the intestine, selective procedures or selective media should be employed to improve the chances of recovery of organisms. A filter with pore size 0.65 µm may be used to filter the sample onto the growth media (van der Walt 2004). Several selective media exist, for example, Blaser's Campy-BAP medium, Bolton and Hutchinson's charcoal-cefoperazone-deoxycholate agar, Karmal's charcoal based medium (Prescott 1990) and Skirrow's medium (van der Walt 2004). However, irrespective of the media used, the presence of antibiotics in them prevents the isolation of all strains. Bolton and Hutchinson's charcoal-cefoperazone-deoxycholate agar and Karmali's charcoal-based medium have been found to give the highest sensitivity for the thermophilic campylobacters (Prescott 1990).

Use of a selective pre-enrichment broth has proven to improve the recovery of campylobacters in faecal, environmental and milk samples that contain low numbers of organisms (Prescott 1990).

As are other members of this genus, thermophilic campylobacters are fastidious in their growth requirements. They are microaerophilic and require a lower oxygen tension (± 6 % $O_2$); therefore a microaerophilic environment consisting of 6 % $O_2$, 10 % $CO_2$, 84 % N has to be created for them for culturing purposes (van der Walt 2004). According to some authors, hydrogen can be added as it stimulates the growth of both species (Prescott 1990; Lastovica 2006). Skirrow *et al*. 1991, cited by Corry, Post, Colin & Laisney (2002), proposed that the concentration of hydrogen should not be less than 7%. When culturing thermophilic campylobacters, plates are incubated at 42 ℃ for up to 72 hours and examined for growth at 48 and 72 hours (Prescott 1990).

*Identification of thermophilic campylobacters*

The differential characteristics of *C. jejuni* and *C. coli* are: growth at 42 ℃, but not at 25 ℃; better growth at 42 ℃ than at 37 ℃; sensitivity to nalidixic acid; resistance to cephalothin and hydrolysis of sodium hippurate. It should be noted that*C. coli* does not hydrolyse sodium hippurate but that *C. jejuni* does (Prescott 1990).

Use of nalidixic acid disks as an identification aid is becoming less useful as *Campylobacter* spp. are increasingly being encountered which are of intermediate sensitivity or resistant to this antibiotic (Lastovica 2006). Cephalothin sensitive *C. coli* have been described (Prescott 1990).

Serotyping by the use of one of two schemes, may differentiate strains. The Penner scheme employes heat stable somatic antigens. It includes 60 serotypes and identification is done by passive haemagglutination. The Lior scheme utilises a heat labile flagellar antigen and includes 90 serotypes. Identification is done by slide plate agglutination (Prescott 1990; Acha & Szyfres 2003). Other means of differentiating strains include phage typing, restriction endonuclease digestion of DNA (van der Walt 2004) and multilocus sequence typing (Manning *et al.* 2003).

In tissue sections, the presence of *Campylobacter* may be confirmed by immunofluorescent or immunoperoxidase methods (van der Walt 2004).

Where tests of a high sensitivity are required, with no requirements for bacterial viability the modified multiplex PCR (Avrain *et al.* 2003), or real-time quantitative PCR (Inglis, Kalischuk & Busz 2004) can be used on samples. Note that intestinal tract samples must be processed in such a way as to remove any PCR inhibitors.

*Immunology*

Due to the difficulties in culturing campylobacters immunological methods such as a latex slide agglutination test or ELISA tests have been developed for use in human diarrhoea cases (Prescott 1990; van der Walt 2004).

**Antimicrobial treatment and resistance**

Antimicrobials suggested for treatment of animals are penicillin, streptomycin, tetracyclines (Songer & Post 2005), nitrofurans, gentamicin, chloramphenicol and erythromycin (van der Walt 2004).

According to Acha & Szyfres (2003), erythromycin is the antibiotic of choice in human cases that require medication. Other macrolides that are effective are azithromycin and clarithromycin, but they are more expensive than erythromycin (Allos 2001). Fluoroquinolones and erythromycin are often prescribed in the USA for human cases of

campylobacteriosis (Gupta, Nelson, Barrett, Tauxe, Rossiter, Friedman, Joyce, Smith, Jones, Hawkins, Shiferaw, Beebe, Vugia, Rabatsky-Ehr, Benson, Root & Angulo 2004).

In the 1980s and early 1990s, campylobacters were recorded as being only resistant to the tetracyclines. Resistance to quinolones was first recorded in the early 1990s in Asia, UK, USA and European countries such as Sweden, The Netherlands, Finland and Spain. Since then the number of *Campylobacter* strains found to be resistant to fluoroquinolones has increased rapidly worldwide (Allos 2001). In many cases resistance was found more frequently among *C. coli* isolates than among *C jejuni* isolates (Moore *et al.* 2006; Shin *et al.* 2007). Several studies mention the correlation between the introduction of fluoroquinolones for veterinary use and increased resistance (Sáenz, Zarazaga, Lantero, Gastañares, Baquero & Torres 2000, Allos 2001; Frediani-Wolf & Stephan 2003, Moore *et al.* 2006).

In comparison, the level of AMR to erythromycin remains relatively low and for this reason it has become the antibiotic of choice in several countries (Taylor & Courvalin 1988; Allos 2001). Occurence of resistance to erythromycin tends to be higher in *C. coli* than in *C. jejuni* (Taylor & Courvalin 1988; Engberg *et al*. 2001; Luangtongkum *et al.* 2006).

Since 1998, increasing resistance to erythromycin, nalidixic acid and ciprofloxacin was reported in *C. jejuni* isolates from South Africa (Moore *et al.* 2006). Now resistance to one or more antimicrobials has been documented in several countries (Avrain, Humbert, L'Hospitalier, Sanders, Vernozy-Rozand & Kempf 2003; Frediani-Wolf & Stephan 2003; van der Walt 2004; Cui, *et al*. 2005; Luangtongkum *et al.* 2006; Shin & Lee 2007).

In general, *C. coli* and *C. jejuni* are susceptible to aminoglycosides, chloramphenicol, clindamycin, nitrofurans and imipenem. High rates of resistance have been recorded to tetracyclines, amoxicillin, ampicillin, metronidazole and cephalosporins. Intrinsic resistance exists to vancomycin, rifampin, trimethoprim (Allos 2001), bacitracin and novobiocin (Taylor & Courvalin 1988).

**Testing for resistance using a Minimum Inhibitory Concentration (MIC) method**

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that completely inhibits visible growth (CLSI 2008). The MIC obtained provides an indication of the concentration of antimicrobial necessary at the site of infection to inhibit the microorganism tested, but is not an absolute value as the 'true' MIC lies between the result and the next lower concentration (CLSI 2008). These results can then be interpreted by the use of "breakpoint" values. If available, breakpoints may be used to describe isolates as sensitive, intermediate or resistant to an antimicrobial. Breakpoints may be defined as 'discriminatory antimicrobial concentrations used in the interpretation of results of susceptibility testing'. Clinical, pharmacological, microbiological and pharmacodynamic data are considered when setting breakpoints (MacGowan & Wise 2005).

Four methods have been employed in studies to determine susceptibility of campylobacters to antibiotics: broth microdilution, agar dilution; agar disk diffusion; and the epsilometer test (E-test). Broth microdilution, agar dilution and the E- test are minimum inhibitory concentration test methods (Moore *et al.* 2006). Both broth microdilution and agar dilution are recommended by the Clinical and Laboratory Standards Institute (CLSI) as preferred methods when testing *Campylobacter* (CLSI 2008). Broth microdilution is the preferred method for larger collections of isolates (Moore *et al.* 2006).

A number of studies have been done to investigate the correlation between methods. In general good correlation was found between agar disk diffusion, E-test and microdilution methods when separating isolates into susceptible and resistant groups (Sáenz *et al* 2000, Wittwer, Keller, Wassenaar, Stephan, Howald, Regula & Bissig-Choisat 2005; Moore *et al.* 2006). However, when comparing values E-test results tend to be slightly lower than broth microdilution results (Engberg, Aarestrup, Taylor, Gerner-Schmidt & Nachamkin, 2001; Frediani-Wolf & Stephan 2003). Agar dilution and E-test results agree when used on a small number of isolates (Moore *et al*. 2006).

Although the agar disk diffusion method (Kirby-Bauer method) has not been validated for testing campylobacters by the CLSI, it is seen as a reliable tool to monitor prevalence of resistant strains. For surveillance of changes in susceptiblitiy concentration levels, however, a minimum inhibitory concentration method should be used (Moore *et al*. 2006)

## STUDY AIM

The aim of this investigation was to determine the antimicrobial susceptibility of thermophilic *Campylobacters* isolated from the intestines of healthy pigs and poultry in mainly the Western Cape Province and compare them to some farms in Gauteng Province by broth dilution minimum inhibitory concentration testing.

# CHAPTER TWO

## MATERIALS AND METHODS

### Introduction

Two hundred and twenty-six samples of chicken caeca and 256 samples of porcine colon were collected and cultured on Columbia agar containing 5% defibrinated sheep blood and Skirrow's medium in a microaerophilic atmosphere. The thermophilic *Campylobacter* species thus isolated were identified to species level by means of biochemical analyses.

Susceptibility of isolates to a selection of commonly used veterinary antimicrobial drugs in poultry and pigs was determined by the microbroth dilution minimum inhibitory concentration as recommended by the CLSI (2008). The selected antimicrobial drugs were chlortetracycline, doxycycline, enrofloxacin, erythromycin, fosfomycin, lincomycin, norfloxacin, tiamulin and tylosin.

Quality control procedures were followed with each batch of tests. The goal of quality control was to monitor the precision and accuracy of a test as well as the performance of reagents, viability of organisms and the performance of persons carrying out tests and interpreting results (NCCLS 2002).

### Statistical determination of sample size

Since the study was aimed at finding out the current antimicrobial resistance trends within poultry and pigs in the Western Cape and to compare the results to another area, in this case an abattoir in Gauteng Province, it was necessary to determine the number of samples required to obtain results that could be statistically analysed. For this reason the following equation was used, which is generally used for biological specimens with a large population size (Fosgate 2009).

n (sample size) = $Z^2P(1-P)/ d^2$

where:

Z is the statistic for a level confidence (at 95% or 1.96)

P is the expected prevalence of 4% or 0.04, and

d is the precision

Estimating the expected isolation rate (prevalence) of *Campylobacter* species to be 4 % (0.04) and utilising a confidence interval of 95 % (1.96) and precision of P/2 i.e. 0,0025 a minimum sample size of 238.49 (238) was calculated.

**Collection, culture and preliminary identification of *Campylobacter* spp.**

During the period November 2007 to June 2008, ileum and/ or colon samples were collected from pig carcasses and caeca from chicken carcasses at necropsy and abattoirs. One complete chicken caecum or ± 7cm of pig colon from each carcass was tied off with string, separated from the intestinal tract by means of sterile scissors and placed in sterile containers. The samples were transported on ice and cultured within three hours of collection.

To improve the sensitivity of isolation two methods were used. In the first method the intestinal mucosa was rubbed with a cotton tipped swab. A plate of Skirrow's agar (SA)[1] was inoculated with the swab. In the second method a cellulose nitrate filter with pore size 0.65 µm[2] was placed on a plate of Columbia agar containing 5% defribrinated sheep blood (BCA)[3] and a generous sample of intestinal content placed on it (Prescott 1990).

[1] CM0935 & SR 0069. Oxoid Limited, Wade Road, Basingstoke, Hampshire, RG24 8PW, United Kingdom
[2] Sartorius Stedim Biotech
[3] CM0331, Oxoid Limited, Wade Road, Basingstoke, Hampshire, RG24 8PW, United Kingdom

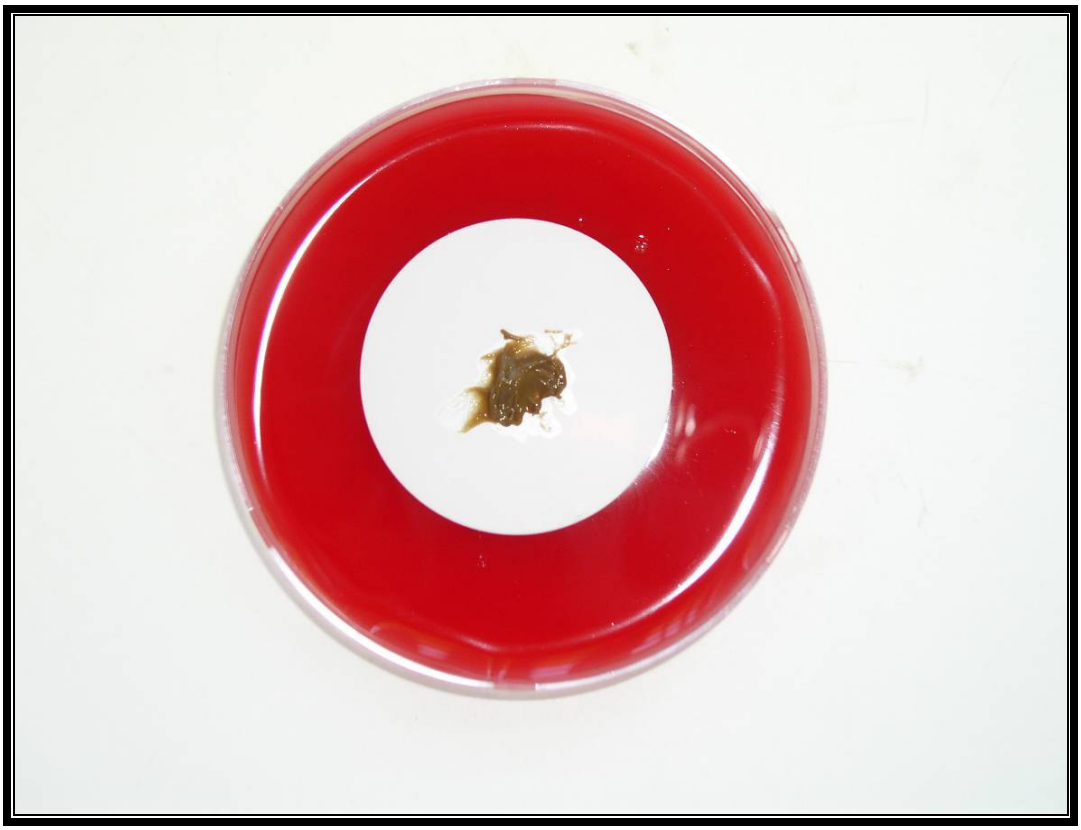


Figure 1. BCA with filter and sample

Inoculated plates were incubated in *Campylobacter* gas (CampyGen)[4] at 42 ℃ for 48 to 72 hours. After incubation of 24 hours the filter on the BCA was removed and the inoculum streaked out for single colonies and re-incubated under the same conditions as previously. After 48 to 72 hours of incubation, any dew-drop like colonies were streaked out on a SA and BCA plate for purification. The plates were placed in CampyGen and incubated for 48 hours at 42 ℃.

At the same time a colony was mixed with water and placed under a coverslip to examine for motility and another colony was smeared on a glass slide and a Gram's stain performed. The wet preparation was viewed by means of phase contrast microscopy and the stained smear by normal light microscopy. Typical colony morphology, microscopic appearance and motility was taken as confirmation of a *Campylobacter* species.

[4] CN0225, Oxoid Limited, Wade Road, Basingstoke, Hampshire, RG24 8PW, United Kingdom

**Biochemical characterization of thermophilic *Campylobacter* spp.**

Catalase[5] and oxidase[6] tests were done on purified isolates. Catalase and oxidase-positive isolates were further identified.

Three BCA plates were inoculated with the purified, 48 hour culture for testing of growth at different temperatures namely 42 ℃, 37 ℃ and 25 ℃, all under microaerophilic conditions.

Three to five colonies were picked off a plate after 48 hours incubation and suspended in a tube of saline to a turbidity equal to 0.5 McFarlane Standard. The inoculum was spread on two plates of Mueller-Hinton agar[7] to form a lawn of growth. A nalidixic acid impregnated disk[8] was placed in the centre of one plate and a cephalothin-impregnated disk[9] on the other. The plates were incubated at 37 ℃ in CampyGen for 48 hours.

A generous loopful of several colonies was taken from a 48 hour culture and inoculated into 0.4 mℓ of a 1 % aqueous sodium hippurate[10] solution in a test tube. Inoculated test tubes were incubated at 37 ℃ for two hours in normal atmosphere. After incubation 0.2 mℓ of ninhydrin solution[11] was added to the test tubes. Then the test tubes were incubated at 37 ℃ for ten minutes. The formation of a deep purple or blue colour was considered to be indicative of hippurate hydrolysis (Prescott 1990).

The *Campylobacter* spp. were then identified using the criteria in Table 1.

Table 1. Differential characteristics of *C. jejuni* and *C. coli*

| | Catalase | Oxidase | Growth at 42° C | Growth at 37° C | Growth at 25° C | Hippurate hydrolysis | Nalidixic acid susceptibility | Cephalothin susceptibility |
|---|---|---|---|---|---|---|---|---|
| ***C. jejuni*** | + | + | ++ | + | - | + | S | R |
| ***C. coli*** | + | + | ++ | + | - | - | S | R |

(Adapted from Prescott 1990; Quinn, Carter, Markey & Carter 1999)

[5] Hydrogen peroxide solution 6%, LOB-179, B&M Scientific, P.O. Box 196 - Athlone 7760 - South Africa
[6] 11330, Bactident®Oxidase, Merck Chemicals, Frankfurter Str. 250, 64293 Darmstadt, Germany
[7] CM0337, Oxoid Limited, Wade Road, Basingstoke, Hampshire, RG24 8PW, United Kingdom
[8] A NA, Oxoid Limited, Wade Road, Basingstoke, Hampshire, RG24 8PW, United Kingdom
[9] A KF30, Oxoid Limited, Wade Road, Basingstoke, Hampshire, RG24 8PW, United Kingdom
[10] 88449, Hippurate hydrolysis broth, Sigma-Aldritch, USA

After confirmation, isolates were frozen on Microbank beads[12]. Following the manufacturer's instructions, cryovials containing porous beads and cryopreservative were inoculated with a 24 hour culture, closed and inverted 4-5 times. Then the cryopreservative was drawn off and the vials were frozen at –70 ℃ in an ultra low temperature freezer[13].

**Minimum Inhibitory Concentration tests**

Broth dilution testing of *Campylobacter* isolates was done in a series of wells (96-well microtitre plates) containing broth medium (CLSI 2008). Standardised concentrations of antimicrobial agents were added to the wells and diluted in serial two fold dilutions across the plate. The dilution range included quality control ranges as well as any available breakpoints (CLSI 2008). A standard concentration of the test *Campylobacter* sp. was added and the plates were incubated for 48 hours under microaerophilic conditions.

*Campylobacter* panels were designed for chlortetracycline[14], doxycycline[15], enrofloxacin HCL[16], erythromycin[17], fosfomycin calcium[18], lincomycin[19], norfloxacin[20], tiamulin[21] and tylosin[22].

[11] N7285, 2 % Ninhydrin solution, Sigma-Aldritch, USA
[12] PL.170/M Microbank®. Prolab Diagnostics, 9701 Dessau Road, Suite #802, Austin, TX, 78754-3941, USA.
[13] Premium ULT Laboratory Freezers, New Brunswick Scientific , 44 Talmadge Road, Edison, New, Jersey 08817 USA
[14] Fujian Fukang Pharmaceutical Co. Ltd, 138, Xiangban Road, Taijiang District, Fushou, China
[15] Yancheng Suhai Pharmaceutical Company, 92, E. Tiankang Road, Dafeng, Hangsu, China
[16] Kirsch Pharma, Gewel Street, Isando, South Africa
[17] Ercros Industrial, South Africa
[18] Hangzhou Chyszem Biotech Co., LTD., Chaohui Rd, Hangzhou, Zhejiang 310014, China
[19] Nanyang Pukang Pharmaceutical Co., LTD., 11 Jinyi Road, Zheng Zhou, China
[20] Dankong Industry & Trade Group, Co., LTD., No.183,Central Avenue,E.D.Z.,Taizhou , 18000,Zhejiang,China
[21] Shandong Lukang Shelile, 173 West Taibailou Road, Jining, Shandoung, China.
[22] Biesterfeld, Ferdinand Street, Hamburg, Germany.

Table 2. Breakpoints and quality control ranges in µg/mℓ

| | Resistant | Sensitive | *Escherichia coli* ATCC 25922 37°C/24 hours | *Staphylococcus aureus* ATCC 29213 37°C/24 hours | *Enterococcus faecalis* ATCC 29212 37°C/24 hours | *C. jejuni* ATCC 33560 37°C/48 hours | *C.jejuni* ATCC 33560 42°C/24 hours |
|---|---|---|---|---|---|---|---|
| **Doxycycline** | ≥8 | ≤4 | 0.5-2 | 0.12-1 | 8-32 | 0.12-0.5 | 0.12-0.5 |
| **Enrofloxacin** | ≥4 | ≤1 | 0.008-0.03 | 0.03-0.12 | 0.12-1 | - | - |
| **Erythromycin** | ≥8 | ≤1 | - | 0.25-1 | 1-4 | 0.5-2 | 0.25-2 |
| **Tetracycline** | ≥8 | ≤4 | 0.5-2 | 0.12-1 | 8-32 | 0.25-2 | 0.25-1 |
| **Tiamulin** | ≥1* | ≤0.1 | - | 0.5-2 | - | - | - |
| **Tylosin** | ≥64[#] | ≤32 | - | 0.5-4 | 0.5-4 | - | - |
| **Tilmicosin** | ≥32 | ≤16 | - | 1-4 | 8-32 | - | - |
| **Tulathromycin** | ≥64 | ≤16 | - | 2-8 | 4-32 | - | - |
| **Fosfomycin** | ≥128[§] | ≤128 | - | - | - | - | - |
| **Lincomycin/ Clindamycin** | ≥4 | ≤0.5 | - | 0.06–0.025 | 4-16 | 0.12-1 | 0.12-0.5 |
| **Gentamicin** | ≥8 | ≤4 | 0.25-1 | 0.1-2 | 4-6 | 0.5-2 | 0.25-2 |
| **Spectinomycin** | ≥128 | ≤64 | 8-64 | 64-256 | 64-256 | - | - |
| **Penicillin** | ≥16 | ≤8 | - | 0.25-1 | 1-4 | - | - |
| **Ampicillin** | ≥16 | ≤8 | 2-8 | 0.5-2 | 0.5-2 | - | - |
| **Ceftiofur** | ≥8 | ≤2 | 0.25-1 | 0.25-1 | - | - | - |
| **Florfenicol** | ≥32 | ≤8 | 2-8 | 2-8 | 2-8 | 1-4 | 0.5-2 |

A dash (-) indicates that no acceptable range has been established

Adapted from Antibiogram Committee of the French Society for Microbiology (1999, cited by Avrain 2003; CLSI 2008 )

* Islam, Klein & Burch 2009

[#] Aaestrup, Nielsen, Madsen & Engberg 1997

[§]Andrews, Baquero, Beltran, Canton, Crokaert & Gobernado 1983

The dilution range of antibiotics for testing was designed to include both the available quality control ranges (CLSI 2008) and breakpoints according to the Antibiogram Committee of the French Society for Microbiology (1999, cited by Avrain *et al.* 2003),

Islam, Klein & Burch 2009, Aaestrup, Nielsen, Madsen & Engberg 1997 and Andrews *et al* 1983.

In order to accomodate the available quality control ranges and breakpoints, the minimum inhibitory concentration panel was designed to run across two U-bottom 96-well microtitre plates[23] (Table 3) starting at Row A on the first plate (A – H) and ending at row F on the second plate (A2 – F2).

*Stock solutions*

Stock solutions of antibiotics for use in the assays were made up at concentrations of at least 1280 µg/mℓ or 40x the highest concentration tested (CLSI 2008).

The potencies of antibiotics were calculated using data from certificates of analysis and the proportion of active antibiotic in the compound, using the following formula:

> Potency = Molecular weight of active antibiotic/Molecular weight of total molecule (i.e. with the salt) x % purity (usually provided with the product) (CLSI 2008)

The amount of antibiotic powder to be used to make up 100 mℓ of stock solution was calculated using the following formula:

> Amount of powder = Weight (mg) = Volume (mℓ) x Concentration (µg/mℓ)/ Potency (µg/mg) (CLSI 2008)

The potency, amount of powder weighed and solvents are shown in Table 4. The stock solutions were aliquotted and stored frozen at –70 ℃ until use. When stock solutions were required, only enough was defrosted for a single day's use. Any stock solution left at the end of the procedure was discarded (CLSI 2008).

[23] 611U96, Bibby Sterilin LTD., Angel Lane, Aberbargoed, Bargoed, Caerphilly, CF81 9FW, United Kingdom

Table 3. MIC panel (µg/mℓ)

| | Chl | Dox | Enr | Em | Fos | L | Nor | Tia | Ty |
|---|---|---|---|---|---|---|---|---|---|
| A | 43 | 43 | 43 | 43 | 43 | 43 | 43 | 43 | 43 |
| B | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 |
| C | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| D | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| E | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| F | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| G | 0.67 | 0.67 | 0.67 | 0.67 | 0.67 | 0.67 | 0.67 | 0.67 | 0.67 |
| H | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 |
| A2 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 |
| B2 | 0.083 | 0.083 | 0.083 | 0.083 | 0.083 | 0.083 | 0.083 | 0.083 | 0.083 |
| C2 | 0.042 | 0.042 | 0.042 | 0.042 | 0.042 | 0.042 | 0.042 | 0.042 | 0.042 |
| D2 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 | 0.021 |
| E2 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 |
| F2 | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 | GC |

| | | | |
|---|---|---|---|
| Chl | Chlortetracycline | L | Lincomycin |
| Dox | Doxycycline | Nor | Norfloxacin |
| Enr | Enrofloxacin | Tia | Tiamulin |
| Em | Erythromycin | Ty | Tylosin |
| Fos | Fosfomycin | GC | Growth control |

*Preparation of MIC panels*

Fifty microlitre of cation adjusted Mueller-Hinton Broth (CAMHB)[24] was pipetted into each of the wells in rows A to H.

Stock solutions of the antibiotics to be tested were defrosted and diluted 1:10 in CAMHB to obtain a working dilution at four times the concentration of the first well. Fifty microlitre of the working dilution of each antibiotic was pipetted into the appropriate well in row A. The antibiotics were serially diluted in 50 µℓ amounts from row A to F2 to obtain a two-fold

dilution series of 43 to 0.005 µg/mℓ for all the antibiotics tested. Well F2.12 (growth control) received 100 µℓ of CAMHB only.

Table 4. Potencies, final amount of powder, solvents, diluents and volume of diluents

| Antibiotic | Potency (µg/mg) | Amount of powder (mg) | Solvent | Diluent | Volume of diluent (mℓ) |
|---|---|---|---|---|---|
| Chlortetracycline | 852 | 160 | Water | Water | 106.5 |
| Doxycycline | 847 | 180 | Water | Water | 119.1 |
| Enrofloxacin | 996 | 150 | ½ volume water, then add 1mol/L NaOH dropwise to dissolve | Water | 116.7 |
| Erythromycin | 655 | 210 | 95% Ethanol | Water | 107.5 |
| Fosfomycin | 761 | 170 | 95% Ethanol | Water | 101.1 |
| Lincomycin | 786 | 160 | Water | Water | 98.3 |
| Norfloxacin | 998 | 130 | ½ volume water, then add 1mol/L NaOH dropwise to dissolve | Water | 101.4 |
| Tiamulin | 986 | 170 | Water | Water | 105.7 |
| Tylosin | 978 | 170 | 95% Ethanol | Water | 129.9 |

In the case of *Campylobacter* species from Gauteng Province the Trek Sensititre Bovine/Porcine format plates[25] were used instead.

*Inoculum*

Microbank beads containing frozen isolates were streaked on BCA without antibiotics. The plates were incubated in CampyGen at 42 ºC for 48 hours. Two subsequent subcultures were made and incubated in CampyGen at 42 ºC for 48 hours (SVA 2007).

One full loop of culture was picked from 48 hour cultures and suspended in two millilitre of 0.9 % saline to a turbidity approximately equal to 0.5 McFarland standard. This suspension was initially diluted 1:100 (100 µℓ in 10 mℓ) in CAMHB to obtain the final

[24] CM 0405, Oxoid Limited, Wade Road, Basingstoke, Hampshire, RG24 8PW, United Kingdom
[25] Trek Diagnostic Systems, Sensititre bovine/porcine plate format BOP06F, Separation Scientific, Johannesburg

inoculum of approximately $10^5$ colony forming units (cfu)/mℓ (Avrain *et al.* 2003, National Veterinary Institute (SVA) 2007). However, as the *Campylobacter* field strains yielded hardly any visible growth in CAMHB and very few colonies on the purity control plate, a decision was made to dilute the initial suspension 1:50 (200 µℓ in 10 mℓ) to obtain the final inoculum of approximately 2 x $10^5$ cfu/ mℓ.

One hundred microlitre of inoculum was pipetted into each well of the testing panels and the plates were covered with a lid. The inoculated panels were incubated microaerophilically at 37 ℃ for 48 hours after which the panels were read (SVA 2007; CLSI 2008).

*Batch control*

To ensure that the plates were not contaminated a plate in each batch of plates was incubated using only CAMHB as the inoculum.

Each batch of microtitre plates was tested with the reference strains mentioned in Table 2 to determine if minimum inhibitory concentrations fall within the expected range. Twenty-four hour cultures of *Escherichia coli* ATCC 25922[26], *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and 48 hour cultures of *Campylobacter jejuni* ATCC 33560 was used to prepare inoculums. Inoculums were made by suspending cultures of the reference strains in two millilitre of 0.9 % saline to a turbidity approximately equal to 0.5 McFarland's Standard.

In the case of *E. coli*, *E. faecalis* and *S. aureus,* ten microlitre of the suspension was pipetted into ten millilitres of CAMHB to obtain the final inoculum. Two hundred microlitre of *C. jejuni* suspension was pipetted into ten millilitre CAMHB to obtain the final inoculum for that isolate. Each well in the appropriate plates were inoculated with 100 µℓ of inoculum.

*Escherichia coli*, *E. faecalis* and *S. aureus* plates were incubated in normal air at 37 ℃ for 16-20 hours. The *C. jejuni* plates were incubated in a microaerophilic atmosphere at 37 ℃ for 48 hours.

[26] American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, VA 20108, USA

Only when it was shown that the MICs of the quality control strains fell within the expected test range (Table 2) and that the plates were sterile, was the batch of plates accepted as conforming to a standard and the MIC testing done.

*Inoculum density and purity control*

Four microlitre of the final inoculum was diluted in four millilitre of 0.9 % saline. One hundred microlitre of this dilution was used to inoculate a BCA plate. The colony count on this plate had to be about 100 colony-forming units (cfu) for *Campylobacter* species and 50 colony-forming units (cfu) for other species (SVA 2007; CLSI 2008).

If the inoculum was contaminated or the bacteria numbers too high or low the test results were disregarded and repeated.

*Growth control*

One well in each *Campylobacter* panel did not contain antimicrobials and was used to monitor growth (SVA 2007). Growth in this well not only determined the end point, either 24 or 48 hours after inoculation, but failure to grow within 72 hours of incubation led to rejection of the test.

The lowest concentration of antimicrobial that prevented visible growth was taken as the minimum inhibitory concentration and recorded (CLSI 2008).

**Data Analyses**

All the data was entered into an Microsoft™Excel 2003 spreadsheet. Descriptive statistics were predominantly used to perform inter-host; inter-provincial and inter-species comparisons. They included determining the percentage resistance using published breakpoint values (Table 2) for the tested antibiotics, the $MIC_{50}$ (median value) and $MIC_{90,}$ as well the the distribution percentages of the MICs. Using an internet calculator[27], The Mann-Whitney U test was used to determine whether there were any statistical differences (Mann & Whitney 1947). The non-parametric Mann-Whitney U test (synonym Wilcoxon rank-sum test)[28], was selected as it is best suited to compare two sets of independent data that does not have a normal distribution.

[27] http://faculty.vassar.edu/lowry/utest.html (accessed 26/01/2010)
[28] http://en.wikipedia.org/wiki/Mann%E2%80%93Whitney_U (accessed 26/01/2010)

# CHAPTER 3

## RESULTS/ OBSERVATIONS

### Isolation and identification

Three hundred and sixty-two samples were obtained from pigs (n = 256) and chickens (n = 106) originating from a total of 24 farms in the Western Cape. Thirteen farms were piggeries and 11 were poultry farms. A total of 120 caeca were also collected from 6 poultry farms in Gauteng Province.

The results are shown in Table 5.

Table 5. A summary of *C. jejuni* and *C. coli* cultured from the intestinal tract of healthy broilers and pigs

| | **Porcine** | | **Poultry** | | **Total** |
|---|---|---|---|---|---|
| | ***C. jejuni*** | ***C. coli*** | ***C. jejuni*** | ***C. coli*** | |
| Western Cape | 1 | 5 | 5 | 5 | 16 |
| Gauteng | 0 | 0 | 18 | 4 | 22 |
| Total | 1 | 5 | 23 | 9 | 38 |

Thirteen of the 30 farms (43 %) sampled yielded thermophilic *Campylobacter* isolates. Five of these farms were piggeries and eight were poultry farms. Thirty eight *Campylobacter* isolates were obtained from the 482 samples (7.88 %), six from pigs and 32 from chickens.

Based on the hippurate test, 23 of the isolates were identified as *C. jejuni* and the other 15 isolates were identified as *C. coli*. Of the six isolates from pigs, one was *C. jejuni* and five were *C. coli*. Of the 32 isolates from chickens, 23 were *C. jejuni* and 9 were *C. coli*. Sixty-five percent of isolates were obtained from carcasses presented for necropsy.

Unusually four *C. jejuni* isolates and one *C. coli* isolate from the Western Cape was nalidixic acid resistant on the disk diffusion sensitivity test (Table 6). Three of these isolates had MICs of 11 µg/mℓ for enrofloxacin and three had MICs of ≥ 11 µg/mℓ for norfloxacin. Two isolates had MICs of ≥ 11 µg/mℓ for both antibiotics.

Table 6. Identification of the thermophilic *Campylobacter* species isolated

| Isolate | Animal | Hippurate hydrolysis | Cephalothin sensitivity | Nalidixic acid sensitivity | Identification |
|---|---|---|---|---|---|
| 1 | Porcine | - | Resistant | Susceptible | *Campylobacter coli* |
| 2 | Chicken | + | Resistant | **Resistant*** | *Campylobacter jejuni* |
| 3 | Chicken | - | Resistant | **Resistant** | *Campylobacter coli* |
| 4 | Chicken | - | Resistant | Susceptible | *Campylobacter coli* |
| 5 | Chicken | - | Resistant | Susceptible | *Campylobacter coli* |
| 6 | Chicken | - | Resistant | Susceptible | *Campylobacter coli* |
| 7 | Chicken | - | Resistant | Susceptible | *Campylobacter coli* |
| 8 | Porcine | - | Resistant | Susceptible | *Campylobacter coli* |
| 9 | Porcine | - | Resistant | Susceptible | *Campylobacter coli* |
| 10 | Porcine | - | Resistant | Susceptible | *Campylobacter coli* |
| 11 | Chicken | + | Resistant | **Resistant** | *Campylobacter jejuni* |
| 12 | Chicken | - | Resistant | Susceptible | *Campylobacter coli* |
| 13 | Chicken | + | Resistant | **Resistant** | *Campylobacter jejuni* |
| 14 | Chicken | + | Resistant | **Resistant** | *Campylobacter jejuni* |
| 15 | Porcine | + | Resistant | Susceptible | *Campylobacter jejuni* |
| 16 | Porcine | - | Resistant | Susceptible | *Campylobacter coli* |
| B1 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |
| B2 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |
| B5 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |
| B9 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |
| B12 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |
| B15 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |
| B29 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |
| B33 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |
| B38 | Chicken | - | Resistant | Susceptible | *Campylobacter coli* |
| B43 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |
| B46 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |
| B49 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |
| B50 | Chicken | - | Resistant | Susceptible | *Campylobacter coli* |
| B51 | Chicken | - | Resistant | Susceptible | *Campylobacter coli* |
| B52 | Chicken | - | Resistant | Susceptible | *Campylobacter coli* |
| B69 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |
| B71 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |
| B73 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |
| B74 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |
| B89 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |
| B92 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |
| B94 | Chicken | + | Resistant | Susceptible | *Campylobacter jejuni* |

*The figures in bold indicate an unsual resistance to nalidixic acid

**Antimicrobial susceptibility testing**

The *C. jejuni* reference strain (ATCC 33560) grew very well in CAMHB at both 42 ℃ and 37 ℃. To the contrary, most field isolates did not grow well in CAMHB and especially not at 42 ℃. It was found that two passages on BCA, a more concentrated inoculum (1:50) and incubation at 37 ℃ for 48 hours improved the readability of MIC results while MIC results for the *Campylobacter* control culture remained within the published acceptable quality control ranges (CLSI 2008). With the exception of nr 17, a *C. coli* of porcine origin all of the isolates grew in CAMHB.

**Antimicrobial susceptibility results**

MIC results for the *Campylobacter* isolates are shown in Table 7 for the Western Cape and for Gauteng in Table 8.

The $MIC_{50}$ and $MIC_{90}$ are minimum inhibitory concentrations at which growth of 50 % and 90 % of organisms respectively, were inhibited by the antibiotics in the growth medium (Luangtongkum *et al.* 2006). These results together with the percentage distribution and percentage resistance (Avrain *et al*., 2003, CLSI, 2008) are shown in Tables 9, 10 and 11 for the Western Cape and in Tables 12 and 13 for Gauteng. The 4 strains of *C. coli* and 4 strains of *C. jejuni* (Table 8) isolated from healthy broiler caeca in Gaueng had the same MIC values.

When $MIC_{50}$ and $MIC_{90}$ values and the percentage distribution graphs were compared, it was revealed that *C. coli,* the predominant isolate from pigs, tended to be, with the exception of resistance to the lincosamides (lincomycin/clindamycin) and macrolides, more susceptible to antimicrobials than *C. jejuni*. Although four *C. coli* were isolated from broilers on a farm in Gauteng, it is believed that they belong to the same clone, as they have the same MIC values for all the antibiotics tested. These isolates, unlike those from the Western Cape, were highly susceptible to the lincosamides and macrolides. Interestingly only the thermophilic *Campylobacter* species originating from the Western Cape revealed any resistance to the fluoroquinolones, 31.25% in the case of enrofloxacin and 37.5% in the case of norfloxacin. The $MIC_{50}$ and $MIC_{90}$ values were also higher from animals in the Western Cape.

Table 7. MIC values of *Campylobacter* isolates (µg/mℓ) from the Western Cape Province for a test range of 0.005 to 43 to µg/mℓ

| Isolate | Animal | Species | Chlortetracycline | Doxycycline | Erythromycin | Fosfomycin | Lincomycin | Enrofloxacin | Norfloxacin | Tiamulin | Tylosin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Porcine | *C. coli* | 0.042 | 0.010 | >43 | 0.67 | 43 | 0.042 | 0.33 | 0.17 | 43 |
| 2 | Chicken | *C. jejuni* | 43 | 43 | 0.33 | 11 | 0.17 | 11 | 11 | 0.17 | 5 |
| 3* | Chicken | *C. coli* | 11 | 21 | >43 | 21 | >43 | 11 | 0.33 | 3 | 11 |
| 4 | Chicken | *C. coli* | 11 | 11 | >43 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | >43 |
| 5 | Chicken | *C. coli* | 11 | 0.083 | >43 | 11 | >43 | 11 | 43 | 3 | >43 |
| 6 | Chicken | *C. coli* | 5 | 11 | >43 | 11 | 43 | 5 | 11 | 5 | 21 |
| 7 | Chicken | *C. coli* | 0.33 | 0.042 | 0.33 | 0.17 | 0.33 | 0.33 | 0.33 | 0.083 | 0.17 |
| 8 | Porcine | *C. coli* | 5 | 0.33 | >43 | 11 | 21 | 0.33 | 0.33 | 0.33 | 21 |
| 9 | Porcine | *C. coli* | 21 | 11 | 1.5 | 5 | 11 | 0.083 | 0.33 | 0.33 | 11 |
| 10 | Porcine | *C. coli* | 3 | 11 | >43 | 21 | >43 | 0.021 | 0.33 | 1.5 | >43 |
| 11 | Chicken | *C. jejuni* | 21 | 21 | >43 | >43 | >43 | 11 | >43 | 3 | 43 |
| 12 | Chicken | *C. coli* | 21 | 0.17 | 0.083 | 21 | 3 | 3 | 21 | 0.12 | 5 |
| 13 | Chicken | *C. jejuni* | >43 | >43 | 1.5 | 43 | 11 | 0.33 | 21 | 1.5 | 0.17 |
| 14 | Chicken | *C. jejuni* | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 |
| 15 | Porcine | *C. jejuni* | >43 | 5 | 0.33 | 0.33 | 0.33 | 0.17 | 0.33 | 0.33 | 0.33 |
| 16 | Porcine | *C. coli* | 3 | 3 | >43 | 0.33 | 43 | 0.083 | 0.67 | 0.33 | 0.33 |

*The shaded areas indicated *Campylobacter* species that are resistant to three or more classes of antibiotics.

The Western Cape *Campylobacter coli* isolates yielded a $MIC_{50}$ and $MIC_{90}$ of >43 µg/mℓ to erythromycin and $MIC_{90}$ of >43 µg/mℓ to lincomycin/clindamycin (lincosamides) and tylosin. In Figure 2a this is illustrated by the high peak at the >43 µg/mℓ category for the *C. coli* group. The *C. coli* were considered to be more susceptible than the *C. jejuni* to the tetracyclines: chlortetracycline ($p$= 0.0307) and doxycycline ($p$= 0.0446). There was a tendency for *C. coli* to be more resistant than *C. jejuni* to the macrolides: erythromycin ($p$= 0.0708) and tylosin ($p$= 0.063) (Table 16).

The lowest $MIC_{50}$ for *C. coli* (Table 10) was 0.33 µg/mℓ to enrofloxacin, norfloxacin and tiamulin, and the lowest $MIC_{90}$ was to tiamulin at 3 µg/mℓ. $MIC_{50}$ and $MIC_{90}$ values were not calculated for *C. coli* isolated in Gauteng as the numbers were too low and it was suspected that they belonged to the same clone. In contrast, the $MIC_{50}$ and $MIC_{90}$ of *C.*

*jejuni* strains from the Western Cape generally had lower $MIC_{50}$ values (Table 11) than *C. coli*, but at the same time also had higher $MIC_{90}$ values, indicating that there there was not a normal population distribution.

Table 8. MIC values of *Campylobacter* isolates (µg/mℓ) isolated from poultry caeca from Gauteng.

| Identification | Species | Ceftiofur (.25-8*) | Gentamicin (1-16) | Neomycin (4-32) | Spectinomycin (8-64) | Florfenicol (.25-8) | Chlortetracycline (.5-8) | Oxytetracycline (.5-8) | Penicillin (.12-8) | Ampicillin (.25-16) | Enrofloxacin (.12-2) | Danofloxacin (.12-1) | Tiamulin (.5-32) | Tylosin (0.5-32) | Tulthromycin (1-64) | Tilmicosin (4-64) | Lincomycin (.25-16) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B38 | *C.coli* | >8 | 1 | 4 | 8 | 2 | >8 | >8 | >8 | 8 | .12 | 1 | .5 | 1 | 2 | 4 | .25 |
| B50 | *C.coli* | >8 | 1 | 4 | 8 | 2 | >8 | >8 | >8 | 8 | .12 | 1 | .5 | 1 | 2 | 4 | .25 |
| B51 | *C. coli* | >8 | 1 | 4 | 8 | 2 | >8 | >8 | >8 | 8 | .12 | 1 | .5 | 1 | 2 | 4 | .25 |
| B52 | *C.coli* | >8 | 1 | 4 | 8 | 2 | >8 | >8 | >8 | 8 | .12 | 1 | .5 | 1 | 2 | 4 | .25 |
| B1 | *C.jejuni* | >8 | 1 | 4 | 8 | 4 | >8 | >8 | >8 | 8 | .25 | .5 | .5 | 2 | 1 | 4 | .25 |
| B12 | *C. jejuni* | >8 | 1 | 4 | 8 | 4 | >8 | >8 | >8 | 8 | .25 | .5 | .5 | 2 | 1 | 4 | .25 |
| B15 | *C.jejuni* | >8 | 1 | 4 | 8 | 4 | >8 | >8 | >8 | 8 | .25 | .5 | .5 | 2 | 1 | 4 | .25 |
| B2 | *C.jejuni* | >8 | 1 | 4 | 8 | 4 | >8 | >8 | >8 | 8 | .25 | .5 | .5 | 2 | 1 | 4 | .25 |
| B29 | *C.jejuni* | >8 | 1 | 4 | 8 | 4 | >8 | >8 | >8 | 8 | .25 | .5 | .5 | 2 | 1 | 4 | .25 |
| B33 | *C.jejuni* | >8 | 1 | 4 | 8 | 2 | >8 | 8 | >8 | 4 | .25 | .5 | .5 | 1 | 1 | 4 | .25 |
| B43 | *C.jejuni* | >8 | 1 | 4 | 8 | 2 | >8 | >8 | >8 | 8 | .12 | 1 | .5 | 1 | 2 | 4 | .25 |
| B46 | *C. jejuni* | >8 | 1 | 4 | 8 | 2 | >8 | >8 | >8 | 8 | .25 | 1 | .5 | 2 | 1 | 4 | .25 |
| B49 | *C.jejuni* | >8 | 1 | 4 | 8 | 2 | >8 | >8 | >8 | 8 | .12 | 1 | .5 | 1 | 2 | 4 | .25 |
| B5 | *C.jejuni* | >8 | 1 | 4 | 8 | 2 | >8 | >8 | 8 | 1 | .12 | .5 | .5 | 4 | 1 | 4 | .25 |
| B69 | *C.jejuni* | >8 | 1 | 4 | 8 | 2 | >8 | >8 | >8 | 8 | .25 | .5 | .5 | 2 | 1 | 4 | .25 |
| B71 | *C.jejuni* | .25 | 1 | 4 | 8 | .25 | 1 | .5 | .12 | .25 | .25 | .25 | .5 | .5 | 1 | 4 | .25 |
| B73 | *C.jejuni* | 8 | 1 | 4 | 8 | .25 | >8 | 8 | >8 | 8 | .12 | .5 | .5 | 1 | 1 | 4 | .25 |
| B74 | *C.jejuni* | 8 | 1 | 4 | 8 | .25 | >8 | 8 | >8 | 8 | .12 | .5 | .5 | 1 | 1 | 4 | .25 |
| B89 | *C. jejuni* | >8 | 1 | 4 | 8 | 2 | >8 | >8 | >8 | 8 | .25 | .5 | .5 | 1 | 1 | 4 | .25 |
| B9 | *C.jejuni* | >8 | 1 | 4 | 8 | 2 | >8 | >8 | >8 | 8 | .12 | 1 | .5 | 1 | 2 | 4 | .25 |
| B92 | *C. jejuni* | >8 | 1 | 4 | 8 | 2 | >8 | >8 | >8 | 16 | .5 | 1 | .5 | 1 | 1 | 4 | .25 |
| B94 | *C.jejuni* | >8 | 1 | 4 | 8 | 4 | >8 | >8 | >8 | 8 | .25 | 1 | .5 | 2 | 1 | 4 | .25 |

* The figures in brackets denote the concentration range tested in µg/mℓ

This is clearly shown, with the exception of tiamulin, in Figure 2a and 2b that there were two populations of both *C. jejuni* and *C. coli*: resistant and non-resistant. This was not observed in the microorganisms from poultry samples from Gauteng (Table 12), where resistance to antimicrobials was limited to the tetracyclines (95.5 %) and β-lactams. *Campylobacter* species isolated from the Western Cape, had a lower percentage

resistance to the tetracyclines (56.25 %) (Tables 10, 11 and 16) which was considered to be statistically significant ($p$ = <0.0001) and a higher level of resistance to enrofloxacin ($p$= 0.0392), macrolides ($p$= 0.0262) and lincosamides ($p$ = 0.0001).

Table 9. Percentage distribution of *Campylobacter* species (n=16), $MIC_{50}$, $MIC_{90}$ and percentage resistant strains from the Western Cape

| Antibiotic | % resistant | $MIC_{50}$ (µg/mℓ) | $MIC_{90}$ (µg/mℓ) | Percentage of isolates at each concentration (µg/mℓ) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.01 | 0.02 | 0.04 | 0.083 | 0.17 | 0.33 | 0.67 | 1.5 | 3 | 5 | 11 | 21 | 43 | >43 |
| Chlortetracycline | 56.25 | 11 | 43 | | | 6.25 | | | 12.5 | | | 12.5 | 12.5 | 18.8 | 18.8 | 6.25 | 12.5 |
| Doxycycline | 50 | 5 | 21 | 6.25 | | 6.25 | 6.25 | 6.25 | 12.5 | | | 6.25 | 6.25 | 25 | 12.5 | 6.25 | 6.25 |
| Erythromycin | 56.25 | >43 | >43 | | | | 6.25 | | 25 | | 12.5 | | | | | | 56.3 |
| Fosfomycin | 6.25 | 11 | 21 | | | | 0 | 12.5 | 18.8 | 6.25 | | | 6.25 | 25 | 18.8 | 6.25 | 6.25 |
| Lincomycin | 62.5 | 11 | 43 | | | | 0 | 12.5 | 18.8 | | | 6.25 | | 12.5 | 6.25 | 18.8 | 25 |
| Enrofloxacin | 31.25 | 0.33 | 11 | 0 | 6.25 | 6.25 | 12.5 | 12.5 | 25 | | | 6.25 | 6.25 | 25 | | | |
| Norfloxacin | 37.5 | 0.33 | 21 | | | | 0 | 6.25 | 50 | 6.25 | | 0 | 0 | 12.5 | 12.5 | 6.25 | 6.25 |
| Tiamulin | 37.5 | 0.33 | 3 | | | | 12.5 | 18.8 | 31.3 | | 12.5 | 18.8 | 6.25 | | | | |
| Tylosin | 18.75 | 11 | >43 | | | | | 12.5 | 18.8 | | | | 12.5 | 12.5 | 12.5 | 12.5 | 18.8 |

The shaded areas indicate the susceptibility range of each antibiotic tested (refer to Table 2)

Table 10. Percentage distribution, $MIC_{50}$, $MIC_{90}$ and percentage resistant strains from the Western Cape of *C. coli* (n=11)

| Antibiotic | % resistant | $MIC_{50}$ (µg/mℓ) | $MIC_{90}$ (µg/mℓ) | Percentage of isolates at each concentration (µg/mℓ) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.01 | 0.02 | 0.04 | 0.08 | 0.17 | 0.33 | 0.67 | 1.5 | 3 | 5 | 11 | 21 | 43 | >43 |
| Chlortetracycline | 45.5 | 5 | 21 | | | 9.1 | | | 9.1 | | | 18.2 | 18.2 | 27.3 | 18.2 | | |
| Doxycycline | 45.5 | 4 | 21 | 9.1 | | 9.1 | 9.1 | 9.1 | 9.1 | | | 9.1 | | 36.4 | 9.1 | | |
| Erythromycin | 72.7 | >43 | >43 | | | | 9.1 | | 9.1 | | 9.1 | | | | | | 72.7 |
| Fosfomycin | 0 | 11 | 21 | | | | | 18.2 | 9.1 | 9.1 | | | 9.1 | 27.3 | 27.3 | | |
| Lincomycin | 72.8 | 21 | >43 | | | | | 9.1 | 9.1 | | | 9.1 | | 9.1 | 9.1 | 27.3 | 27.3 |
| Enrofloxacin | 27.5 | 0.33 | 11 | | 9.1 | 9.1 | 18.2 | 9.1 | 18.1 | | | 9.3 | 9.3 | 18.2 | | | |
| Norfloxacin | 27.3 | 0.33 | 21 | | | | | 9.1 | 54.6 | 9.2 | | | | 9.1 | 9.1 | 9.1 | |
| Tiamulin | 36.6 | 0.33 | 3 | | | | 18.1<br>8 | 18.2 | 27.3 | | 9.2 | 18.3 | 9.1 | | | | |
| Tylosin | 27.2 | 21 | >43 | | | | | 9.1 | 9.1 | | | | 9.1 | 18.2 | 18.2 | 9.1 | 27.2 |

The shaded areas indicate the susceptibility range of each antibiotic tested (refer to Table 2)

Table 11. Percentage distribution, $MIC_{50}$, $MIC_{90}$ and percentage resistant strains from the Western Cape of *C. jejuni* (n=5)

| Antibiotic | % resistant | $MIC_{50}$ (µg/mℓ) | $MIC_{90}$ (µg/mℓ) | Percentage of isolates at each concentration (µg/mℓ) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.01 | 0.02 | 0.04 | 0.08 | 0.17 | 0.33 | 0.67 | 1.5 | 3 | 5 | 11 | 21 | 43 | >43 |
| Chlortetracycline | 80 | 43 | >43 | | | | | | 20 | | | | | | 20 | 20 | 40 |
| Doxycycline | 60 | 21 | >43 | | | | | | 20 | | | | 20 | | 20 | 20 | 20 |
| Erythromycin | 20 | 0.33 | >43 | | | | | | | 60 | | 20 | | | | | 20 |
| Fosfomycin | 20 | 11 | >43 | | | | | | 40 | | | | | 20 | | 20 | 20 |
| Lincomycin | 40 | 0.33 | >43 | | | | | 20 | 40 | | | | | 20 | | | 20 |
| Enrofloxacin | 40 | 0.33 | 11 | | | | | 20 | 40 | | | | | 40 | | | |
| Norfloxacin | 60 | 11 | >43 | | | | | | 40 | | | | | 20 | 20 | | 20 |
| Tiamulin | 40 | 0.33 | 3 | | | | | 20 | 40 | | 20 | 20 | | | | | |
| Tylosin | 0 | 0.33 | 43 | | | | | 20 | 40 | | | | 20 | | | 20 | |

Table 12. Percentage distribution of *Campylobacter* spp. (n=22), $MIC_{50}$, $MIC_{90}$ and percentage resistant strains from broiler caeca in Gauteng

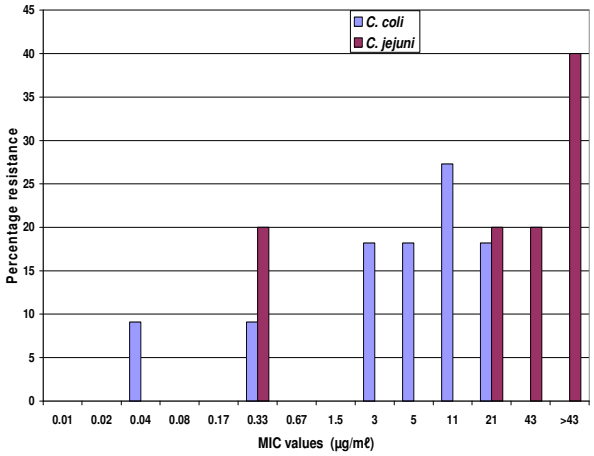
| | | | | Percentage of isolates at each concentration (µg/mℓ) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Antibiotic** | **%resistance** | **$MIC_{50}$** | **$MIC_{90}$** | **0.12** | **0.25** | **0.5** | **1** | **2** | **4** | **8** | **>8** |
| Ceftiofur | 95.5 | >8 | >8 | | 4.5 | | | | | 9.1 | 86.4 |
| Florfenicol | 0 | 2 | 4 | | 13.6 | | | 59.1 | 27.3 | | |
| Chlortetracycline | 95.5 | >8 | >8 | | | | 4.5 | | | | 95.5 |
| Oxytetracycline | 95.4 | >8 | >8 | | | 4.5 | | | | 13.6 | 81.8 |
| Penicillin | 95.4 | >8 | >8 | 4.5 | | | | | | 4.5 | 90.9 |
| Ampicillin/ Amoxicillin | 85.7 | 8 | 8 | 45.5 | 50 | | 4.8 | 4.8 | 4.8 | 85.7 | |
| Enrofloxacin | 0 | 0.12 | 0.25 | | | 4.5 | | | | | |
| Norfloxacin | 0 | 0.5 | 1 | | | 54.5 | 45.5 | | | | |
| Gentamicin | 0 | 1 | 1 | | 4.5 | | 100 | | | | |
| Neomycin | 0 | 4 | 4 | | | | | | 100 | | |
| Spectinomycin | 0 | 8 | 8 | | | | | | | 100 | |
| Tylosin | 0 | 1 | 2 | | | 4.5 | 54.5 | 36.4 | 4.5 | | |
| Tulthromycin | 0 | 1 | 2 | | | | 68.2 | 31.8 | | | |
| Tilmicosin | 0 | 4 | 4 | | | | | | 100 | | |
| Tiamulin | 0 | 0.5 | 0.5 | | | 100 | | | | | |
| Lincomycin | 0 | 0.25 | 0.25 | | 100 | | | | | | |

The shaded areas indicate the susceptibility range of each antibiotic tested (refer to Table 2).

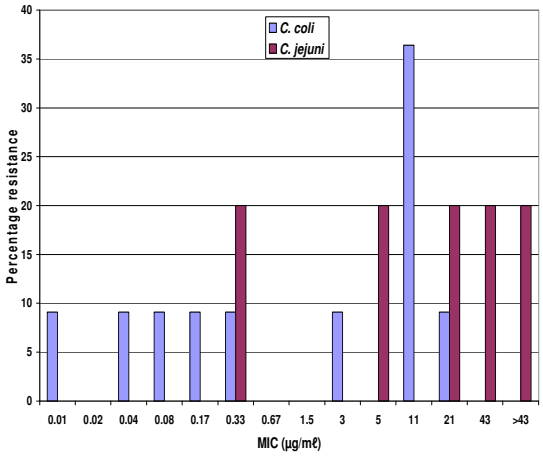
Table 13. Percentage distribution of *C. jejuni* (n=18), $MIC_{50}$, $MIC_{90}$ and percentage resistant strains from broiler caeca in Gauteng.

| | | | | Percentage of isolates at each concentration (µg/mℓ) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Antibiotic** | **%resistance** | **$MIC_{50}$** | **$MIC_{90}$** | **0.12** | **0.25** | **0.5** | **1** | **2** | **4** | **8** | **>8** |
| Ceftiofur | 94.4 | >8 | >8 | | 5.6 | | | | | 11.1 | 83.3 |
| Florfenicol | 0 | 2 | 4 | | 16.7 | | | 50 | 33.3 | | |
| Chlortetracycline | 94.4 | >8 | >8 | | | | 5.6 | | | | 94.4 |
| Oxytetracycline | 94.5 | >8 | >8 | | | 5.6 | | | | 16.7 | 77.8 |
| Penicillin | 94.5 | >8 | >8 | 5.6 | | | | | | 5.6 | 88.9 |
| Ampicillin/ Amoxicillin | 82.4 | 8 | 8 | | | | 5.9 | 5.9 | 5.9 | 82.4 | |
| Enrofloxacin | 0 | 0.12 | 0.25 | 33.3 | 61.1 | 5.6 | | | | | |
| Norfloxacin | 0 | 0.5 | 1 | | | | 100 | | | | |
| Gentamicin | 0 | 1 | 1 | | 5.6 | 61.1 | 33.3 | | | | |
| Neomycin | 0 | 4 | 4 | | | | | | 100 | | |
| Spectinomycin | 0 | 8 | 8 | | | | | | | 100 | |
| Tylosin | 0 | 1 | 2 | | | 5.6 | 44.4 | 44.4 | 5.6 | | |
| Tulthromycin | 0 | 1 | 2 | | | | 83.3 | 16.7 | | | |
| Tilmicosin | 0 | 4 | 4 | | | | | | 100 | | |
| Tiamulin | 0 | 0.5 | 0.5 | | | 100 | | | | | |
| Lincomycin | 0 | 0.25 | 0.25 | | 100 | | | | | | |

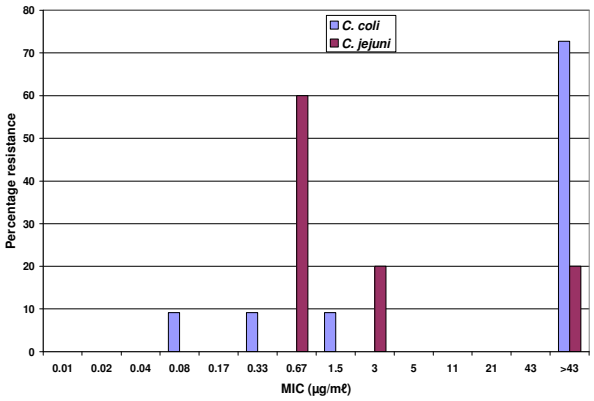
The shaded areas indicate the susceptibility range of each antibiotic tested (refer to Table 2).



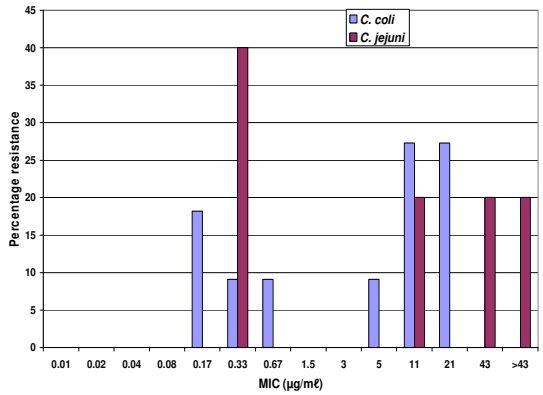

A) Chlortetracycline



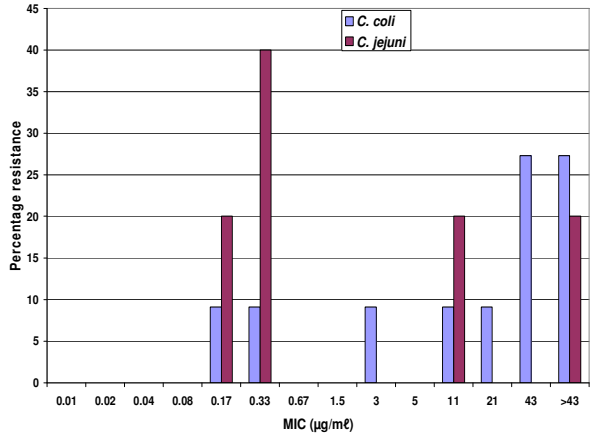

B) Doxycycline/Oxytetracycline



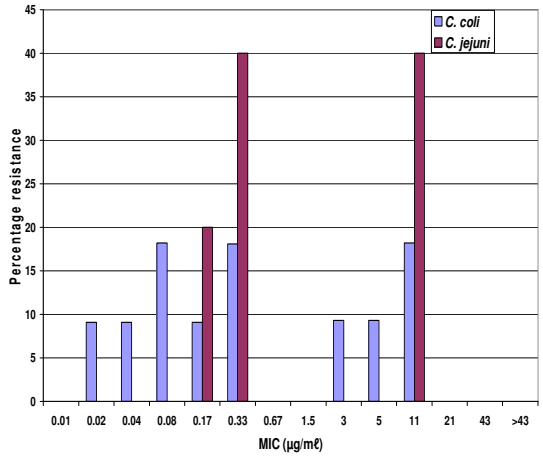

C) Erythromycin




D) Fosfomycin

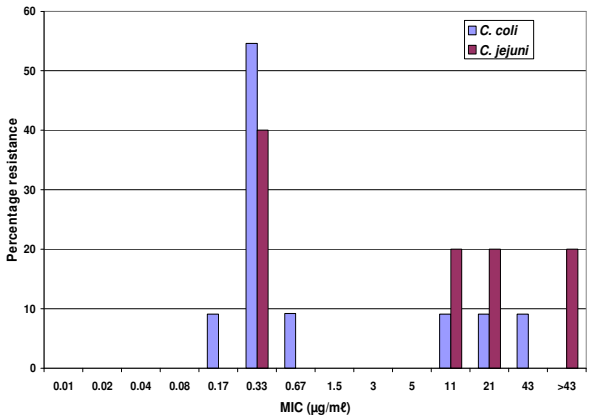



E) Lincomycin/Clindamycin

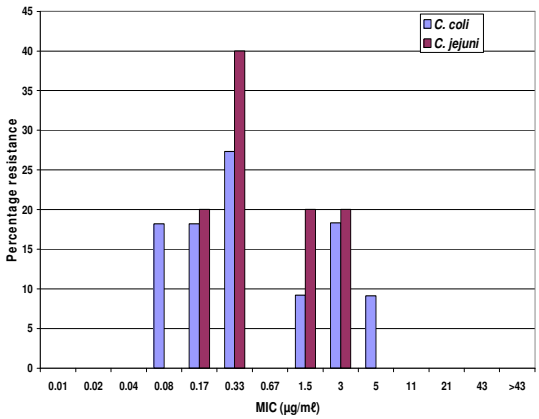



F) Enrofloxacin

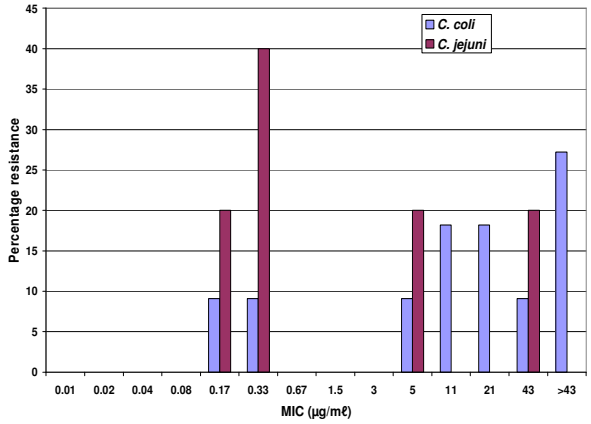
Figure 2a. Graphical representations (A to F) of the percentage distribution of the MIC values to the indicated antibiotics of *C. jejuni* (n=5) and *C. coli* (n=11) isolated from the Western Cape




G) Norfloxacin




H) Tiamulin




I) Tylosin

Figure 2b Graphical representations (G to I) of the percentage distribution of the MIC values to the indicated antibiotics of *C. jejuni* (n=5) and *C. coli* (n=11) isolated from the Western Cape

There was also a tendency to of these bacteria to be more resistant to the pleuromutilins

($p$= 0.0985). Furthermore *Campylobacter* strains from Gauteng tended to have a narrow MIC range (Table 12), indicating that only 1 population was present.

Four of the 16 (25 %) bacteria (3 *C. coli* and 1 *C. jejuni*) isolated from the Western Cape were resistant to three or more antibiotic classes, including the tetracyclines, macrolides, lincosamides, pleuromutulins and fluoroquinolones (Table 7). No multi-resistant *Campylobacter* species were isolated from the flocks in Gauteng.

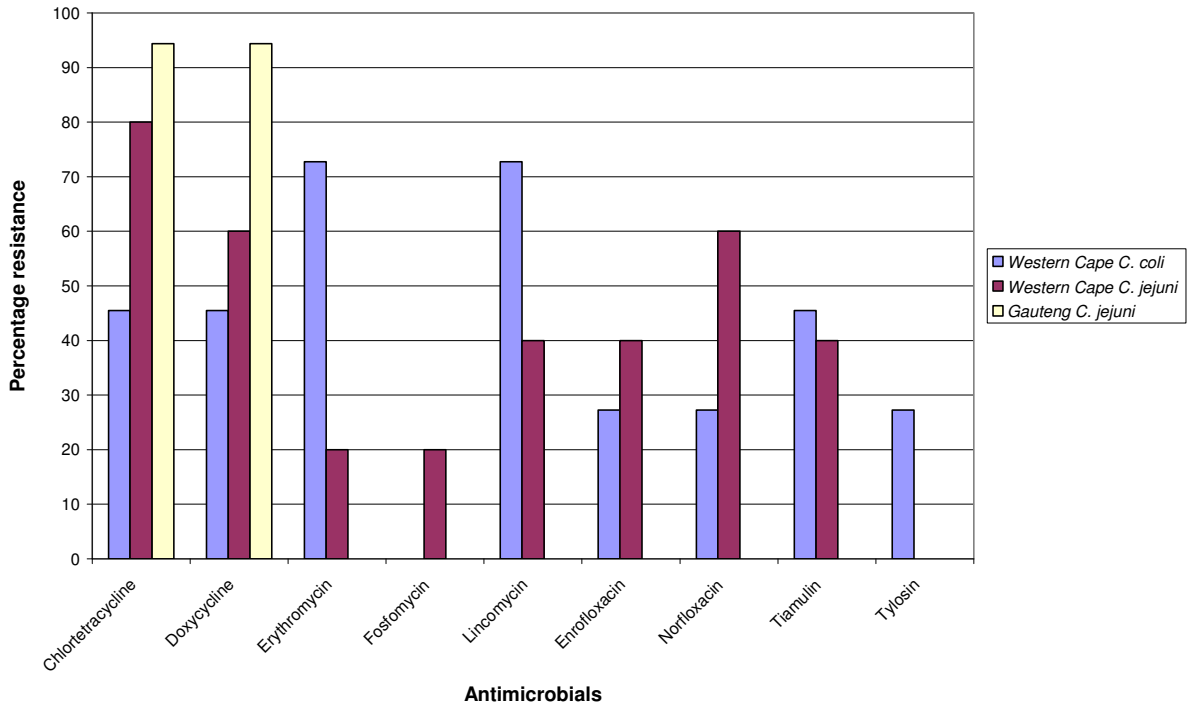



Figure 3. A comparison of the percentage resistance of *C. jejuni* and *C. coli* isolated from the Western Cape, and of *C. jejuni* isolated from Gauteng

As pigs are given different prophylactic and therapeutic antibiotic regimens to those of poultry, it was also decided to examine whether there were any differences in the antimicrobial susceptibility between campylobacters of porcine and poultry origin. For this exercise only isolates from the Western Cape were evaluated as shown in Tables 14 and 15. Marked differences were noted in that porcine *Campylobacter* spp. were much more susceptible to tetracyclines (percentage resistance 34.4 % and 33.3 % to chlortetracycline and doxycyline respectively) than the poultry strains (70 % and 60 % percentage resistance to chlortetracycline and doxycyline respectively). However, these differences were not statistically significant when the MIC values were compared (chlortetracycline $p$= 0.2389 and doxycycline $p$= 0.1922). Thermophilic campylobacters of poultry origin were more resistant to enrofloxacin ($p$= 0.0021) and tended to be resistant to norfloxacin ($p$=0.0793). Even though not statistically significant a higher percentage of porcine strains were resistant to the lincosamides (lincomycin) (83.3%) and erythromycin (66.7%).

Table 14. Percentage distribution of *Campylobacter* species (n=10), $MIC_{50}$, $MIC_{90}$ and percentage resistant strains from broilers in the Western Cape

| Antibiotic | % resistant | $MIC_{50}$ (µg/mℓ) | $MIC_{90}$ (µg/mℓ) | Percentage of isolates at each concentration (µg/mℓ) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.01 | 0.02 | 0.04 | 0.08 | 0.17 | 0.33 | 0.67 | 1.5 | 3 | 5 | 11 | 21 | 43 | >43 |
| Chlortetracycline | 70 | 11 | 43 | | | | | | 20 | | | | 10 | 30 | 20 | 10 | 10 |
| Doxycycline | 60 | 11 | 43 | | | | 10 | 10 | 10 | | | | | 20 | 20 | 10 | 10 |
| Erythromycin | 50 | 1.5 | >43 | | | | 10 | | 30 | | 10 | | | | | | 50 |
| Fosfomycin | 10 | 11 | 43 | | | | | 20 | 10 | | | 10 | | 30 | 20 | 10 | 10 |
| Lincomycin | 50 | 3 | >43 | | | | | 20 | 20 | | | 10 | | 10 | | 10 | 30 |
| Enrofloxacin | 50 | 3 | 11 | | | | | 10 | 30 | | | | 10 | 40 | | | |
| Norfloxacin | 60 | 11 | 43 | | | | 20 | 10 | 30 | | | 30 | | 20 | 20 | 10 | 10 |
| Tiamulin | 20 | 0.33 | 3 | | | | | 20 | 10 | | 10 | | 10 | | | | |
| Tylosin | 20 | 5 | >43 | | | | | 20 | 10 | | | | 20 | 10 | 10 | 10 | 20 |

The shaded areas indicate the susceptibility range of each antibiotic tested (refer to Table 2).

Table 15. Percentage distribution of *Campylobacter* species (n=6), $MIC_{50}$, $MIC_{90}$ and percentage resistant strains from pigs in the Western Cape

| Antibiotic | % resistant | $MIC_{50}$ (µg/mℓ) | $MIC_{90}$ (µg/mℓ) | Percentage of isolates at each concentration (µg/mℓ) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.01 | 0.02 | 0.04 | 0.08 | 0.17 | 0.33 | 0.67 | 1.5 | 3 | 5 | 11 | 21 | 43 | >43 |
| Chlortetracycline | 34.4 | 3 | >43 | | | 16.7 | | | | | | 33.3 | 16.7 | | 16.7 | | 16.7 |
| Doxycycline | 33.3 | 3 | 11 | 16.7 | | | | | 16.7 | | | 16.7 | 16.7 | 33.3 | | | |
| Erythromycin | 66.7 | >43 | >43 | | | | | | 16.7 | 16.7 | | | | | | | 66.7 |
| Fosfomycin | 0 | 0.67 | 21 | | | | | | 33.3 | | 16.7 | | 16.7 | 16.7 | 16.7 | | |
| Lincomycin | 83.3 | 21 | >43 | | | | | | 16.7 | | | | | 16.7 | 16.7 | 33.3 | 16.7 |
| Enrofloxacin | 0 | 0.17 | 0.33 | | 16.7 | 16.7 | 33.3 | 16.7 | 16.7 | | | | | | | | |
| Norfloxacin | 0 | 0.33 | 0.67 | | | | | | 83.3 | 16.7 | | | | | | | |
| Tiamulin | 16.7 | 0.33 | 0.67 | | | | | 16.7 | 66.7 | | 16.7 | | | | | | |
| Tylosin | 16.7 | 11 | >43 | | | | | | 33.3 | | | | | 16.7 | 16.7 | 16.7 | 16.7 |

Table 16. P-values obtained at a 95% Confidence Level using the Mann-Whitney test when comparing inter-host; inter-provincial and inter-species MIC values

| **Antibiotic** | **Poultry vs porcine campylobacters (inter-host)** | **Gauteng vs Western Cape (inter-provincial)** | ***C. jejuni* vs *C. coli* in the WC (inter-species)** |
|---|---|---|---|
| Chlortetracycline | 0.2389 | <.0001 | 0.0307 |
| Doxycycline | 0.1922 | <.0001 | 0.0446 |
| Erythromycin | 0.2389 | n/a | 0.0708 |
| Fosfomycin | 0.2236 | n/a | 0.2676 |
| Lincomycin | 0.2743 | 0.0001 | 0.117 |
| Enrofloxacin | 0.0021 | 0.0392 | 0.1685 |
| Norfloxacin | 0.0793 | 0.2451 | 0.1539 |
| Tiamulin | 0.3707 | 0.0985 | 0.3897 |
| Tylosin | 0.3707 | 0.0262 | 0.063 |

# CHAPTER FOUR

## DISCUSSION

The primary aim of the project was to isolate and determine the antimicrobial susceptibility patterns of thermophilic *Campylobacter* species from the intestinal tract of poultry and pigs, both important food animals that are known to have a high carriage of these intestinal bacteria (Avrain *et al.* 2003; Frediani-Wolf & Stephan 2003; Luangtongkum *et al.* 2006; Shin & Lee 2007).

Worldwide, most poultry flocks are considered to be the natural hosts of especially *C. jejuni* with prevalence of positive flocks ranging from 10 to 82 % in conventionally reared flocks and an even higher flock prevalence in free-range chickens which can vary from 54 % to 100 % (Heuer, Pedersen, Andersen & Madsen, 2001).

Similar to another published study, the prevalence of *Campylobacter* spp. was up to 100 % in the piggeries, with *C. coli* being the predominant species (Gebreyes, Thakur & Morrow 2005). Even though the numbers of poultry farms tested were small, the percentage of infected flocks was not unusual being 43 %.

It is also not surprising that a patchy distribution of *Campylobacter* spp. was found as was noted in the samples from Gauteng where only 2 of the 6 flocks tested were positive. For example, in a study in which poultry in4 broiler houses were examined, it was found that those in the first broiler house to become affected had a low prevalence of *Campylobacter* spp. but by the time the birds were slaughtered four weeks later this bacterium could not be isolated. This was not the case in houses that were infected by workers from the first house later in the grow-out cycle where 100% of the birds tested at four weeks of age had evidence of intestinal colonization (Gregory, Barnhart, Dreesen, Stern & Corn 1997). It is reported that proper cleaning and disinfection will destroy *Campylobacter* spp. in houses (Newell & Fearnley 2003).

Therefore, in the all-in-all-out systems employed on many poultry and pig farms, the *Campylobacter* spp. has to be introduced by other means *e.g.* wild birds, outerwear of farm workers, transport vehicles, water, food, wild birds and, to a limited extent, rodents. Furthermore, colonization by thes bacteria begins when birds move to the broiler houses at 10 days of age and their prevalence increases so that by the time the birds are slaughtered up to 100% of them are colonized by these bacteria.

Very few farms in South Africa practice thinning out, a procedure in which some birds are removed from the flocks at 35 days of age, to allow the remaining birds to grow better. The crates that remove these birds are often heavily contaminated which result in the remaining birds becoming colonized with the thermophilic *Campylobacter* spp. by the time they are slaughtered.

Poultry and pig farms in South Africa have, over the years, increasingly implemented more stringent biosecurity measures, in that only all-in-all-out systems are practiced, in which there is in-line chlorination of drinking water, restriticion of access to the and a high level of hygiene. Furthermore, farm workers which may only wear the designated protective clothing are restricted to a specific area (Kapperud, Skjerve, Vik, Hauge, Lysaker, Aalmen, Ostroff & Potter 1993). Under these circumstances where the risk of *Campylobacter* being introduced onto a farm is greatly reduced, it is not unreasonable to expect that low numbers (7.66 %) of these bacteria will be cultured.

The samples, sampling method and preservation of the specimens were similar to those of other studies that had high isolation rates of *Campylobacter* spp'. It is also accepted that the use of both a non-selective culturing method *i.e.* the filter method and a selective isolation method such as Skirrow's medium, will effect an optimal recovery of most strains of the enteric *Campylobacter* spp. (Lastovica, 2006). However, both these methods only perform well in the presence of high numbers of *Campylobacter* spp. within the sample. In the presence of low numbers of *Campylobacter* spp., it has been shown that the incubating the samples in a broth enrichment-selective medium for 24 hours prior to streaking onto a solid medium encourages the selective growth of these bacteria and improves the overall sensitivity by 12.5 % (Hutchinson & Bolton 1983).

There are currently no internationally accepted criteria for the testing of anitimicrobial resistance in *Campylobacter* species, nor are there accepted breakpoint values (Moore *et al*. 2006). The CLSI (2008) is quite clear that the agar diffusion test is unreliable, and recommends the use of either the agar dilution or broth dilution tests. There are, however, no specific breakpoints for this genus. Therefore, unless published elsewhere (Table 2), the clinical breakpoints are the same as those used for other Gram-negative bacteria.

The antimicrobials of choice in human patients suffering from life-threatening campylobacteriosis are initially the macrolides and thereafter the fluoroquinolones and gentamicin (Engberg, Aarestrup, Taylor, Gerner-Smidt & Nachamkin 2001). Resistance to these two classes of antibiotics in zoonotic *Campylobacter* spp. can increase the rate s of hospitalization and the cost of therapy, and decrease the rate of survival of patients

(Engberg *et al*. 2001). Several countries, including Canada and the United States of America, have reported an increasing trend in the resistance of *C. jejuni* to the fluoroquinolones, whereas the prevalence of resistance to the macrolides and tetracylines has remained static (Gaudreau & Huguette 2003). The surveillance programme for resistance to *Campylobacter* spp. of human origin and commissioned surveys of resistance in poultry and pigs in France found that, from 1986 to 1998, the prevalence of resistance of *Campylobacter* spp. from humans to the fluoroquinolones increased, only to progressively decline over the next five years. This decline was partially associated with decreased fluoroquinolone resistance of these organisms in poultry and pigs in this country (Gallay, Prouzet-Mauléon, Kempf, Lehours, Labadi, Camou, Denis, de Valk, Desenclos & Mégraud 2007).

The banning of enrofloxacin in poultry by the Food and Drug Administration (FDA) in the USA in 2000 and effected in 2005 was a direct consequence of documented evidence showing increased resistance in disease causing strains of *Campylobacter* in humans as well as a 10 % resistance in those from poultry products (Moore *et al*. 2006; Nelson, Chiller, Powers & Angulo 2007). Since fluoroquinolones, especially enrofloxacin and norfloxacin, are used to treat resistant *E. coli* infection in birds, it would be expected that the same is true for South Africa. This was true for the few isolates (50 % to enrofloxacin and 60% to norfloxacin) from poultry in the Western Cape. None was noted in the poultry isolates from Gauteng, nor from the pig isolates in the Western Cape. Bester and Essack (2008) found that resistance to the fluoroquinolones was low at 8 % but much higher to nalidixic acid. This seems to point to differences in ttherapeutic regimens between the different regions and possibly farm management systems.

The farms tested in Gauteng had a niche market in that they supplied certain supermarket chains with so-called untreated birds. It is known that fluoroquinolone resistance develops rapidly, for, unlike other Gram-negative bacteria, the acquisition of fluoroquinolone resistance in *Campylobacter* spp. does not require stepwise accumulation of *gyrA* mutations and overexpression of efflux pumps, but is mainly mediated by single-step point mutations in *gyrA* in the presence of a constitutively expressed multidrug efflux pump, CmeABC (Zhang, Lin & Pereira 2003).

In South Africa, tylosin is used extensively by both the poultry and pig industry to treat *Mycoplasma* infections as well as spirochaete infections in pigs. It is also known to be used as a performance enhancer in sub-therapeutic doses. Therefore it was not surprising to detect cross-resistance to the parent macrolide erythromycin (56.25 %) in isolates from the Western Cape, The resistance was higher in *C. coli* (72.7 %) than in *C. jejuni* (20 %).

Resistance to tylosin was lower at 27.2 % in *C. coli* isolates. However, most probably due to the small sample size, these differences only tended to statistical significance ($p$= 0.0708 for erythromycin and $p$= 0.063 for tylosin). In a study where birds were either fed therapic or lower concentrations of antibiotics, the researchers found that 71 % of *C. coli* and only 37 % of *C. jejuni* that were isolated from birds fed diets supplemented with tylosin were resistant to erythromycin (Ladely, Harrison, Fedorka-Cray, Berrang, Englen & Meinersmann 2007). In studies by Engberg *et al*. (2001) and Luangtongkum *et al.* (2006), mention was made of high prevalence of resistance among *C. coli* isolates to erythromycin, as well as co-resistance between erythromycin and clindamycin. In this study both these tendencies were also observed although in this case lincomycin was used instead of clindamycin where the resistance was higher but not statistically significant ($p$= 0.117) in *C. coli* (72.8 %) compared to 40 % in *C. jejuni*. Shin & Lee (2007) reported a binomial pattern when examining resistance to the macrolides in *C. coli*. This pattern was observed for both *C. jejuni* and *C. coli* (Figure 2a and 2b) and clearly divides the resistant from the non-resistant populations.

An exception is the pleuromulutin, tiamulin, for although the AMR was relatively high in teh Western Cape strains at 37.5 %, there was a normal distribution of MIC values, so no real separation of the resistant and susceptible bacterial populations could be observed. This could be related to the fact that only small numbers of bacteria were tested. Of interest is the low breakpoint value of 1 µg/mℓ that tiamulin has in poultry compared to 32 µg/mℓ for other animal spp. (CLSI 2008). This is related to the fact that peak concentrations of this antibiotic at an oral dose of 25mg/mℓ in poultry serum are 1.7 µg/mℓ (Islam, Klein &. Burch 2009).

Tetracyclines are extensively used in both the poultry and pig industries in South Africa, as they are broadspectrum in activity, and cheap, and can easily be administered in the food and water. It was, therefore, not surprising that 95.5% of the poultry isolates from Gauteng and 60% (doxycycline) and 70% (chlortetracycline) of the Western Cape isolates of *C. jejuni* were resistant to this class of antimicrobial. A recent study of *Campylobacter* spp. isolated from broilers and layer hens in Kwa-Zulu Natal Province also revealed the high level of resistance to the tetracyclines of up to 100 % (Bester & Essack 2008). It must be noted, however, they did use a lower breakpoint value of 4 µg/mℓ (this study used 8 µg/mℓ). Similar trends have been noted in the United Kingdom (Piddock, Briggs, Johnson, Ricci, Elviss, Williams, Jørgensen, Chisholm, Lawson, Swift, Humphrey & Owen 2008) anf the USA with prevalences of up to 99.5% being recorded in the latter country (Son, Englen, Berrang, Fedorka-Cray & Harrison 2007). This is thought to be due to the easy

transfer between bacteria of the conjugative plasmid with the tet(O) gene. Poultry products in countries in which tetracyclines are rarely used, such as Iceland, have negligible levels of resistance (0.3%) to it (Thorsteinsdottir, Kristinsson, Fridriksdottir & Gunnarsson 2008). This high level of tetracycline resistance is rarely recorded in humans, most probably due to the fact that tetracyclines are not used as first line therapy but are mainly used to treat vector-borne diseases, such as malaria and tick bite fever as well as certain skin and urinary tract diseases. Therefore, it is unusual to find that tetracycline resistance occurred in 70, 72 and 69 % of the *Campylobacter* spp. from humans in Israel, Spain and Japan respectively (Prats, Mirelis, Llovet, Munoz, Miro & Navarro 2001; Moore *et al*. 2006). At the same time, in Japan, tetracycline resistance was high in food-producing animals.

It is well known that *C. jejuni* produces β-lactamases that enable the bacterium to be resistant to the β-lactam drugs *i.e.* amoxicillin and ceftiofur at levels of between 83 to 92 % (LaChance, Gaudreau, Lamothe & Larivitre 1991). This was noted for the *C. jejuni* isolated from Gauteng where 82.4 % and 94.4 % were resistant to amoxicillin and ceftiofur respectively. Bester & Essack (2008) recorded up to 100 % resistance to ceftriaxone in *C. jejuni* isolated from layers and broilers originating from Kwa-Zulu Natal pointing to the possible presence of extended spectrum beta lactamases (ESBL) (CLSI 2008). Interestingly, isolates from children at the Red Cross hospital in Cape Town have also shown an increase in resistance from 3.6 % in 2002 to 24.6 % in 2006 (Moore *et al.* 2006). Treatment of *Campylobacter* spp. infections using the β-lactam drugs is not generally recommended as it is believed that the cell wall of *C. jejuni* is relatively impermeable to these antibiotics (LaChance *et al*. 1991).

Worldwide, the resistance of the thermophilic *Campylobacter* spp. to the aminoglycosides is very low (<1 %). In this study there was no resistance in the *Campylobacter* species isolated from birds in Gauteng to gentamicin, neomycin and spectinomycin. This was interesting, for although gentamicin is hardly ever used in poultry, both neomycin and spectinomycin are routinely used to treat intestinal disease. Unusually, a study done in a Swiss abattoir by Frediani-Wolf & Stephan (2003), revealed that 27.7% of the *C. jejuni* were resistant to streptomycin with a very low resistance to erythromycin and fluoroquinolones. However, technical errors and differences in breakpoint interpretative values may have accounted for the unusually high streptomycin resistance as the disk diffusion test was used (CLSI 2008).

*Campylobacter* species isolated from the two farms in Gauteng tended to have very similar AMR patterns. Therefore, it is possible that there was clonal expansion of the

strains on a farm. However, since the resistance was generally low, the clonal nature of the isolates can only be proven by genetic fingerprinting. These bacteria exhibited a significantly higher resistance to tetracyclines ($p$= <0.0001) and a lower resistance to tylosin ($p$= 0.0262), lincomycin/clindamycin ($p$= 0.0001) and enrofloxacin ($p$= 0.0392) than those originating from the Western Cape and even Kwa-Zulu Natal (Bester & Essack 2008). As mentioned above, it is possible that the high-level of management regarding farm biosecurity and that of consumer pressure to cease the treatment, of broilers prevented the selection of AMR.

Multi-resistance in both *C. jejuni* and *C. coli* has been reported both in human and animal isolates throughout the world. Resistance in all 4 *Campylobacter* spp. to tetracyclines, macrolides and fluoroquinolones has been recorded elsewhere. It is postulated that efflux pumps encoded either by the *Campylobacter*-specific *cme*ABC gene or by as yet unidentified genes, are responsible (Randall, Ridley, Cooles, Sharma, Sayers, Pumbwe, Newell, Piddock, Woodward 2003). Efflux pumps usually result in a low level of AMR to several antibiotics as they actively remove antibiotics from the bacterial cytosol.

## CONCLUSIONS AND RECOMMENDATIONS

Several studies, including this one, have shown that AMR of *Campylobacter* spp.isolated both from humans and animals are highly variable both geographically and from year to year (Moore *et al*. 2006). In animals, this is dependent on the level of disease and AMR in a flock or pig herd from year to year. However, it appears in South Africa that there is a general upward trend in resistance to the floroquinolones and macrolide s as well as multi-resistance. Therefore, a constant vigilance for *Campylobacter* spp. of public health significance should be maintained through the use of surveillance and the rapid reporting of trends (Moore *et al*. 2006). Economic restrictions have meant that studies in Africa, including South Africa ,have been done on an ad hoc basis and are few and far between. This is evidenced by the paucity of publications originating from this country as well as the fact that this genus has not been included in the fledgling South African antimicrobial surveillance programme (SANVAD 2008).

It is recommended; therefore, that surveillance be instituted for *Campylobacter* spp. originating especially from poultry and pigs in South Africa, and that the focus should be fluoroquinolone, macrolide and tetracycline resistance, in which a high resistance was observed for this study. The surveillance programme should also include the poultry and

pigs belonging to small scale farmers as the prevalence of these bacteria and AMR in South Africa is unknown. It is also likely that these animals will have a high carriage rate of thermophilic *Campylobacter* species.

It has been shown that on farms on which antibiotics are not used, the levels of AMR, although not absent, tend to be very low (Gebreyes *et al*. 2005). Therefore, producers should be encouraged by legislation or market pressures to reduce the use of therapeutic antimicrobials that are known to be effective against *Campylobacter* spp. as well as tylosin as a performance enhancer.

# CHAPTER 5

## REFERENCES

AARESTRUP, F.M., NIELSEN, E.M., MADSEN, M. & ENGBERG, J. 1997. Antimicrobial susceptibility patterns of thermophilic *Campylobacter* spp. from humans, pigs, cattle, and broilers in Denmark. *Antimicrobial Agents And Chemotherapy* 41:10:2244-2250.

ACHA, P.N. & SZYFRES, B. 2003. Campylobacteriosis in: Zoonoses and communicable diseases common to man and animals. *Bacterioses and Mycoses*. 3rd Ed. Washington D.C: Pan American Health Organization: pp67-72.

ALLOS, B.M. 2001. *Campylobacter jejuni* infections: Update on emerging issues and trends. *Clinical Infectious Diseases* 32: 1201-1206.

ANDREWS, J.M., BAQUERO, F., BELTRAN, J.M., CANTON, E., CROKAERT, F. & GOBERNADO, M. 1983. International collaborative study on standardization of bacterial sensitivity to fosfomycin. *Journal of Antimicrobial Chemotherapy* 12: 357-361.

AVRAIN, L., HUMBERT, F., L'HOSPITALIER, R., SANDERS, P., VERNOZY-ROZAND, C., & KEMPF, I. 2003. Antimicrobial resistance in *Campylobacter* from broilers: association with production type and antimicrobial use. *Veterinary Microbiology* 96: 267-276.

BESTER, L.A. & ESSACK, S.Y. 2008. Prevalence of antibiotic resistance in *Campylobacter* isolates from commercial poultry suppliers in KwaZulu-Natal, South Africa. *Journal of Antimicrobial Chemotherapy* 62: 1298-1300.

CLARK, C.G., PRICE, L., AHMED, R., WOODWARD, D.L., MELITO, P.L., RODGERS, F.G., JAMIESON, F., CIEBIN, B., LI A. & ELLIS, A. 2003. Characterization of waterborne outbreak-associated *Campylobacter jejuni,* Walkerton, Ontario. *Emerging Infectious Diseases* 9: 1232-1241.

CLINICAL AND LABORATORY STANDARDS INSTITUTE. 2002. *NCCLS. Performance Standards For Antimicrobial Disk And Dilution Susceptibility Tests For Bacteria Isolated From Animals; Approved Standard.* 2nd Ed. NCCLS document M31-A2.

CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI). 2008. *Performance Standards For Antimicrobial Disk And Dilution Susceptibility Tests For Bacteria Isolated From Animals; Approved Standard.* 3rd Ed. CLSI document M31-A3.

CORRY, J.E.L., POST, D.E., COLIN, P., LAISNEY, M.J. 1995. Culture media for the isolation of campylobacters. *International Journal of Food Microbiology* 26:43-76.

CUI, S., GE, B., ZHENG, J. & MENG, J. 2005. Prevalence and antimicrobial resistance of *Campylobacter* spp. and *Salmonella* serovars in organic chickens from Maryland retail stores. *Applied and Environmental Microbiology* 71: 7: 4108 – 4111.

DEPARTMENT OF ANTIBIOTICS. 2007. VetMIC™Camp (version 2) method. National Veterinary Institute. Uppsala. http://www.sva.se

ENGBERG, J., AARESTRUP, F.M., TAYLOR, D.E., GERNER-SCHMIDT, P. & NACHAMKIN, I. 2001. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: Resistance mechanisms and trends in human isolates. *Emerging Infectious Diseases* 7: 1: 24 – 34 http://www.cdc.gov/ncidod/EID. Accessed 27 January 2010.

EVANS, M.R., RIBEIRO,C.D & SALMON,R.L. 2003. Hazards of healthy living: bottled water and salad vegetables as risk factors for *Campylobacter* infection. *Emerging Infectious Diseases* 9:10. http://www.cdc.gov/ncidod/EID. Accessed 27 January 2010.

FOSGATE, G.T. 2009. Practical sample size calculations for surveillance and diagnostic investigations. *Journal of Veterinary Diagnostic Investigation* 21: 3-14.

FREDIANI-WOLF, V., STEPHAN, R. 2003. Resistance patterns of *Campylobacter* strains isolated from poultry carcasses in a big Swiss poultry slaughterhouse. *International Journal of Food Microbiology* 89: 233-240.

GALLAY, A., PROUZET-MAULEON, V., KEMPF, I., LEHOURS, P., LABADI, L., CAMOU, C., DENIS, M., DE VALK, H., DESENCLOS, J-C. & MÉGRAUD, F. 2007. *Campylobacter* antimicrobial drug resistance among humans, broiler chickens, and pigs, France. *Emerging Infectious Diseases* 13: February. www.cdc.gov/eid. Accessed 27 January 2010.

GAUDREAU, C. & HUGUETTE, G. 2003. Antimicrobial Resistance of *Campylobacter jejuni* subsp. *jejuni* strains isolated from humans in 1998 to 2001 in Montréal, Canada, *Antimicrobial Agents and Chemotherapy* 47: 2027–2029.

GEBREYES, W.A., THAKUR, S. & MORROW, W.E.M. 2005. *Campylobacter coli*: prevalence and antimicrobial resistance in antimicrobial-free (ABF) swine production systems. *Journal of Antimicrobial Chemotherapy* 56: 765–768.

GORMLEY, F.J., MACRAE, M., FORBES, K.J., OGDEN, I.D., DALLAS, J.F. & STRACHAN, N.J.C. 2008. Has retail chicken played a role in the decline of human campylobacteriosis. *Applied and Environmental Microbiology* 74: 2: 383-390.

GREGORY, E., BARNHART, H.B., DREESEN, D.W., STERN, N.J., & CORN, J.L., 1997. Epidemiological study of *Campylobacter* spp. in broilers: Source, time of colonization, and prevalence. *Avian Diseases* 41: 890-898.

GUPTA, A., NELSON, J.M., BARRETT, T.J., TAUXE, R.V., ROSSITER, S.P., FRIEDMAN, C.R., JOYCE, K.W., SMITH, K.E., JONES, T.F., HAWKINS, M.A., SHIFERAW, B., BEEBE, J.L., VUGIA, D.J., RABATSKY-EHR, T., BENSON, J.A., ROOT, T.P. & ANGULO, F.J. 2004. Antimicrobial resistance among Campylobacter strains, United States, 1997 – 2001. *Emerging Infectious Diseases* 10: 6: 1102 – 1109 http://www.cdc.gov/ncidod/EID. Accessed 27 January 2010.

HERENDA, D.C. & FRANCO, D.A.1996. Diseases and miscellaneous conditions, in: *Poultry Diseases and Meat Hygiene. A Colour Atlas*. 1st Ed. Ames, Iowa: Iowa State University Press: pp217 – 218.

HEUER, O.E., PEDERSEN, K., ANDERSEN J.S., & MADSEN, M. 2001. Prevalence and antimicrobial susceptibility of thermophilic *Campylobacte*r in organic and conventional broiler flocks. *Letters in Applied Microbiology*, 33, 269-274.

HUTCHINSON, D.N., & BOLTON, F.J. 1983. Is enrichment culture necessary for the isolation of *Campylobacter jejuni* from faeces? *Journal of Clinical Pathology* 36:1350-1352.

INGLIS,G.D., KALISCHUK, L.D. & BUSZ, H.W. 2004. Chronic shedding of *Campylobacter* species in beef cattle. *Journal of Applied Microbiology* 97: 410-420.

ISLAM, K.M.S., KLEIN, U. & BURCH, D.G.S. 2009. The activity and compatiblitiy of the antibiotic tiamulin with other drugs in poultry medicine – A review. *Poultry Science* 88:2353-2359.

JAY, J.M. 2000. *Modern Food Microbiology*. 6th Ed. Gaithersburg, Maryland: Aspen Publishers.

KAPPERUD, G. SKJERVE, E. VIK, L. HAUGE, K. LYSAKER, A. AALMEN, I. OSTROFF, S.M. & POTTER, M. 1993. Epidemiological investigation of risk factors for campylobacter colonization in Norwegian broiler flocks. *Epidemiology and Infection* 111: 245-255.

LADELY, S.R., HARRISON, M.A., FEDORKA-CRAY, P.J., BERRANG, M.E., ENGLEN, M.D. & MEINERSMANN, R.J. 2007. Development of macrolide-resistant *Campylobacter* in broilers administered subtherapeutic or therapeutic concentrations of tylosin. *Journal of Food Protection* 70: 1945–1951.

LASTOVICA, A.J. 2006. Emerging *Campylobacter* spp.: the tip of the iceberg. *Clinical Microbiology Newsletter* 28: 49 – 55.

LACHANCE, N., GAUDREAU, C., LAMOTHE, F. & LARIVItRE, L.A. 1991. Role of the β-lactamase of *Campylobacter jejuni* in resistance to β-lactam agents. *Antimicrobial Agents and Chemotherapy* 35: 813-818.

LUANGTONGKUM, T., MORISHITA,T.Y., ISON, A.J., HUANG, S., McDERMOTT, P.F & ZHANG, Q. 2006. Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of *Campylobacter* spp. in poultry. *Applied and Environmental Microbiology* 72: 5: 3600-3607.

MACGOWAN, A.P. & WISE, R. 2005. Establishing MIC breakpoints and the interpretation of *in vitro* susceptibility tests. British society for antimicrobial chemotherapy (BSAC) working party. http://www.bsac.org.uk

MANN, H.B., & WHITNEY, D.R., 1947. "On a test of whether one of two random variables is stochastically larger than the other". *Annals of Mathematical Statistics* 18: 50–60.

MANNING, G., DOWSON, G.D., BAGNALL, M.C., AHMED, H., WEST, M. & NEWELL, D.G. 2003. Multilocus sequence typing for comparison of veterinary and human isolates of *Campylobacter jejuni*. *Applied and Environmental Microbiology* 69: 11: 6370-6379

MOORE, J.E., BARTON, M.D., BLAIR, I.S., CORCORAN, D., DOOLEY, J.S.G., FANNING, S., KEMPF, I., LASTOVICA, A.J., LOWERY, C.J., MATSUDA, M., McDOWELL, D.A., McMAHON, A., MILLAR, B.C, RAO, J.R., ROONEY, P.J., SEAL, B.S., SNELLING, W.J. & TOLBA, O. 2006. The epidemiology of antibiotic resistance in *Campylobacter*. *Microbes and Infection* 8: 1955 – 1966.

NCBI Taxonomy Browser, 2008, http://www.ncbi.nlm.nih.gov. Accessed 27 January 2010.

NELSON, J.M., CHILLER, T.M., POWERS, J.H. & ANGULO, F.J. 2007. Fluoroquinolone-resistant *Campylobacter* species and the withdrawal of fluoroquinolones from use in poultry: a public health success story. *Clinical Infectious Diseases* 44: 977–980.

NEWELL D. G. & FEARNLEY C., 2003. Sources of *Campylobacter* Colonization in Broiler Chickens. *Applied and Environmental Microbiology* 69: 4343–4351.

PIDDOCK, L.J., GRIGGS, D., JOHNSON, M.M., RICCI, V., ELVISS, N.C., WILLIAMS, L.K., JØRGENSEN, F., CHISHOLM, S.A., LAWSON, A.J., SWIFT, C., HUMPHREY, T.J. & OWEN, R.J. 2008. Persistence of *Campylobacter* species, strain types, antibiotic resistance and mechanisms of tetracycline resistance in poultry flocks treated with chlortetracycline. *Journal of Antimicrobial Chemotherapy* 62: 303–315.

PRATS, G., MIRELIS, B., LLOVET ,T., MUNOZ, C., MIRO, E. & NAVARRO, F., 2000. Antibiotic resistance trends in enteropathogenic bacteria isolated in 1985–1987 and 1995–1998 in Barcelona, *Antimicrobial Agents and Chemotherapy* 44: 1140-1145.

PRESCOTT, J.F. 1990. Campylobacter, in: *Diagnostic Procedures in Veterinary Bacteriology and Mycology*, 5th Ed., edited by G.R. Carter & J.R. Cole. San Diego, California: Academic Press: 6: 61-75.

QUINN, P.J., CARTER, M.E, MARKEY, B. & CARTER, G.R. 1999. *Campylobacter* species, in: *Clinical Veterinary Microbiology*. Edinburgh, London, New York, Philadephia, St Louis, Sydney & Toronto: Harcourt Publishers: pp208-272

QUINN, P.J., MARKEY, B.K., CARTER, M.E., DONNELLY, W.J. & LEONARD, F.C. 2002. *Campylobacter* species, in: *Veterinary Microbiology& Microbial Diseases*. Cornwall, Great Britain: Blackwell Science Limited: 29: 168 – 172.

RANDALL, L.P., RIDLEY, A.M., COOLES, S.W., SHARMA, M., SAYERS, A.R., PUMBWE, L., NEWELL, D. G., PIDDOCK L J.V. & WOODWARD, M.J. 2003. Prevalence of multiple antibiotic resistance in 443 *Campylobacter* spp. isolated from humans and animals *Journal of Antimicrobial Chemotherapy* 52: 507–510.

SÁENZ, Y., ZARAZAGA, M., LANTERO, M., GASTAÑARES, M.J., BAQUERO, F. & TORRES, C. 2000. Antibiotic resistance in *Campylobacter* strains isolated from animals, foods, and humans in Spain in 1997-1998. *Antimicrobial Agents and Chemotherapy* 44: 267-271.

SANVAD 2007 *South African National Veterinary Surveillance and Monitoring Programme for Resistance to Antimicrobial Drugs*. ISBN: 978-1-86854-673.

SHIN, L. & LEE, Y. 2007. Antimicrobial resistance of 114 porcine isolates of *Campylobacter coli*. *International Journal of Food Microbiology* 118: 223-227.

SON, I., ENGLEN, M.D., BERRANG, M.E., FEDORKA-CRAY, P.J. & HARRISON, M.A. 2007. Antimicrobial resistance of *Arcobacter* and *Campylobacter* from broiler carcasses. *International Journal of Antimicrobial Agents* 29: 451-455.

SONGER, J.G. & POST, K.W. 2005. The genera *Campylobacter*, *Helicobacter* and *Arcobacter*, in: *Veterinary Microbiology*. St Louis, Missouri: Elsevier Saunders: 223-231.

STERN, N.J., BAILEY, J.S., BLANKENSHIP, L.C., COX, N.A. & MCHAN, F. 1988. Colonization characteristics of *Campylobacter jejuni* in chick caeca. *Avian Diseases* 32: 330-334.

TAYLOR, D.E. & COURVALIN, P. 1988. Mechanisms of antibiotic resistance in *Campylobacter* species. *Antimicrobial Agents and Chemotherapy* 32: 1107-1112.

THOMSON, J.R. 2006. Diseases of the digestive system, in: *Diseases of Swine*. 9th Ed, edited by B.E. Straw, J.J. Zimmerman, S. D'Allaire & D.J. Taylor. Ames, Iowa: Blackwell Publishing: pp37-55.

THORSTEINSDOTTIR, T.R., KRISTINSSON, K.G., FRIDRIKSDOTTIR, V. & GUNNARSSON, E. 2008. Antimicrobial resistance of *Campylobacter* spp. isolated from broiler flocks in Iceland 2001-2005. *Microbial Drug Resistance* 14: 49–53.

VAN DER WALT, M.L. 2004. *Campylobacter jejuni* infection, in: *Infectious diseases of livestock*. 2nd Ed, edited by J.A.W. Coetzer & R.C.Tustin. Cape Town: Oxford University Press. 3: pp1479-1483.

WITTWER, M., KELLER, J., WASSENAAR, T.M., STEPHAN, R., HOWALD, D., REGULA, G. & BISSIG-CHOISAT, B. 2005. Genetic diversity and antibiotic resistance patterns in a *Campylobacter* population isolated from poultry farms in Switzerland. *Applied and Environmental Microbiology* 71: 6: 2840-2847.

ZHANG, Q. 2008. Campylobacteriosis, in: *Diseases of poultry*, 12th Ed, edited by Y.M. Saif. Ames, Iowa: Blackwell Publishing: pp675-689.

ZHANG, Q., LIN, J. & PEREIRA, S. 2003. Fluoroquinolone-resistant *Campylobacter* in animal reservoirs: dynamics of development, resistance mechanisms and ecological fitness. *Animal Health Research Reviews* 4: 63–71.