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Full Length Research Paper

Potentially pathogenic *Campylobacter* species among farm animals in rural areas of Limpopo province, South Africa: A case study of chickens and cattles

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This study investigated the prevalence of thermophilic pathogenic *Campylobacter* in farm settlements in South Africa. Six hundred freshly voided faeces samples were collected from chicken and cattle (300 each) and analyzed on mCCDA supplemented with campylobacter supplement and incubated at 42℃. Out of this, 106 (35.3%) of the chicken faeces were positive for *Campylobacter*, 58 (19.3%) from cattle faeces were positive for *Campylobacter*. Ninety (84.9%) isolates from chicken were *C. jejuni*, while 16 (15.1%) were *C. coli*. Of the 58 isolates from cattle, 42 (72.4%) were *C. jejuni*, while 16 (26.7%) were *C. coli*. *C. jejuni* was more prevalent in chicken and cattle. The prevalence was higher in chicken than cattle, the prevalence was statistically significant at *P<0.005*. Diarrhea faeces from chicken and cattle contain more *C. jejuni* than *C. coli*. The prevalence skewed more to chicken than cattle. Different levels of resistance were noted amongst isolates from chicken and cattle. Study of prevalence of resistance to ciprofloxacin (a fluoroquinolone) showed that *C. coli* from cattle were more resistant to this antibiotic (*C. jejuni*, 33.3%; *C. coli,* 56.3%); from chicken (*C. jejuni*, 29%; *C. coli*, 37.5%). Varied resistance was shown to other antibiotics by the isolates. The resistance by isolates to ciprofloxacin, a fluoroquinolone is worrisome since it is used as therapeutic agent against campylobacteriosis. Of more concern is the multiple resistances shown by these isolates to the applied antimicrobials as resistance genes can be transferred to other microbes in the environment horizontally.

Key word: Prevalence, resistance, faeces, significant, campylobacteriosis, thermophilic.

INTRODUCTION

Campylobacters are widespread in the environment and may be commensals of the intestinal tracts of a wide range of birds and mammals, including domestic animals used for food production (Inglis et al., 2005). The thermotolerant species, *C. jejuni* and *C. coli* account for most of the human foodborne infections, which mostly appear sporadically.

Apart from poultry, other reservoirs of infections have been identified, examples are domestic pets, food and wild animals (Steinhauserova et al., 2002; Clark et al., 2003; Broman et al., 2004; Siemer et al., 2005; Cornelius et al., 2005; Devane et al., 2005; Workman et al., 2005; Uaboi-Egbenni et al., 2010, 2011). There are several reports indicating a high contamination rate of retail poultry meat in different countries (Corry and Atabay, 2001) including Slovenia (Zorman and Smole Možina, 2002) and BIH (Uzunović-Kamberović et al., 2004). Furthermore, an unusually high proportion of *C. coli* were found among poultry meat as well as among human clinical isolates from Southern Europe/ Balkan regions. The only form of Campylobacteriosis of major public health importance is *Campylobacter* enteritis due to *C. jejuni* and *C. coli* (Nachamkin et al., 2000).

The gastrointestinal tracts of other food animal species have also been shown to be frequently colonized with campylobacters, particularly, *C. jejuni* and *C. coli*

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(Minihan et al., 2004). Reported rates of *Campylobacter* carriage in food animals have varied widely between studies (Busato et al., 1999). Campylobacters have been isolated from the intestines of healthy calves and adult cattle (Ono et al., 1995; Inglis et al., 2005; Stanley and Jones, 2003), as well as from calves exhibiting signs of enteritis (Inglis et al., 2005b; Morris et al., 2011; Feodoroff et al., 2010; Hakkinen, 2010). Cattle and sheep have also been reported to excrete *Campylobacter* organisms but at less rate (Stanley and Jones, 2003). There are divergent reports from different countries on the shedding pattern of campylobacters amongst farm animals. Account of cross contamination/ zoonosis has been given by Stanley and Jones (2003) in their review.

The recommended drugs for treatment of Campylobacteriosis are erythromycin, or amoxicillin or a fluoroquinolone (ciprofloxacin, norfloxacin) or tetracycline, provided the bacterium has not acquired a resistance. However, the emergence of antibiotic resistant strains has further opened a new dimension as to how to combat the disease. Isolates of *C. jejuni* and *C. coli* with resistance to various antimicrobial agents have been reported in both developed and developing countries (Hart and Kariuki, 1998). Since the 1990s, a significant increase in the prevalence of resistance to macrolides among *Campylobacter* spp. has been reported, and this is recognized as an emerging public health problem (Engberg et al., 2001). It has been suggested by some investigators that resistance to macrolides is mainly found in isolates of animal origin; especially *C. coli* form pigs and also *C. jejuni* from chickens (Van Looveren et al., 2001; Aarestrup et al., 1997; Chuma et al., 2001).

There is a dearth of information and research on the epidemiology and prevalence of campylobacters in animals in Africa, particularly, South Africa. The little available information on prevalence of campylobacters in animals is the report of Uaboi-Egbenni et al. (2008) on dogs and guinea-fowl. Most published information on human campylobacteriosis are in HIV/AIDS patients in the Vhembe District Limpopo, South Africa (Samie et al., 2006), and campylobacteriosis in children in Cape Town (Lastovica et al., 1997). Added to these are the reports of Uaboi-Egbenni et al. (2010) on *Campylobacter* in sheep, pigs and goats. Apart from these reports, we are not aware of any information on the prevalence and distribution of *Campylobacter* species and their drug resistance amongst chicken and cattle in sub-Saharan Africa. The objective of the present study was to determine the prevalence, haemolytic activities and antimicrobial profiles of pathogenic thermophilic *Campylobacter* spp. in chicken and Cattle in farm settlements in Venda region in South Africa.

MATERIALS AND METHODS

Age of animals

The Chickens used in this study were all four (4) months old, while the cows were six (6) months old (male and females). This information was given by the farm owners and was therefore considered to be authentic. So, the animals used in the study were actually at their state of maturity. As the study commenced, the animals were tagged properly to separate them of those that were not understudy. This was particularly important to the cattle that were led on grazing. The chickens were in their respective cages.

Collection of faeces

Samples were collected from three farms (designated A, B and C for confidentiality). Farms A and B were about 70 Km apart, while farm C was about 200 Km from the previous two farms. Briefly, one hundred (100) freshly voided faeces were collected from chickens and cows presenting with symptoms of diarrhea and from apparently healthy ones from each of the farms with the aid of oven-sterilized spatula (scrubbed with cotton wool soaked in absolute alcohol prior to collection of faeces) in sterile 50 ml plastic containers containing ice chips and transported to the laboratory within 2 h for initial processing. A total of 300 samples consisting of diarrheic and non-diarrheic faeces were collected. One hundred faeces samples were collected from each of the farms for chicken and cattle. Once in the laboratory, the faeces were immediately processed. About 2 g of each sample was transferred to 6 ml of sterile brain heart infusion broth (BHI) and left to emulsify at room temperature for 10 to 20 min to release the bacteria. The suspension was used directly on mCCDA agar for detection of *Campylobacter*.

The $\chi 2$ test was used to compare chicken and cattle isolates in relation to the prevalence of *Campylobacter* according to antibiotic resistance. Student's test was used to evaluate the significance of the differences in the prevalent rate of *Campylobacter* in chickens and cattle.

Isolation of *Campylobacter* by conventional culture methods

Twenty microlitres of faecal suspension was spread on the surface of a charcoal cefoperazone deoxycholate agar plates (CM 739 [Oxoid] with cefoperazone supplement SR 155E). The plates were incubated in 2.5 litres anaerobic jar under microaerophilic conditions employing the Campygen gas generating kit (Oxoid CM025) at 42°C for 48 h. Colonies suspected to be *Campylobacter* were further purified on blood agar plates (Blood Agar Base No.2 (Oxoid) supplemented with 5% sterile laked horse blood). All isolates were characterized by their catalase, oxidase reactions, hydrogen sulphide generation and susceptibility to nalidixic acid by standard procedures (Baker et al., 2008; Chaban et al., 2010). The resulting isolates were subsequently stored at -80°C in brain heart infusion broth with 15% glycerol until further investigation.

Confirmation of positive *Campylobacter* isolates

Identification of *Campylobacter* isolates was done using Dryspot *Campylobacter* test kit (Oxoid Basingstoke, Hampshire, England). The test is specific for pathogenic *Campylobacter* strains belonging to *C. jejuni, C. coli, C. upsaliensis* and *C. lari.* The Oxoid agglutination test was done according to the manufacturer's instructions. Agglutination under normal lighting condition indicated that the test organism was *Campylobacter* and belongs to any of the four species mentioned earlier (Baker et al., 2008; Chaban et al., 2010).

Discrimination of *Campylobacter* species

The dryspot positive campylobacters were further subjected to Mast

diagnostic *Campylobacter* kits consisting of urease, indoxyl acetate and hippurate test (ampoules) and/or indoxyl acetate, urease and hippurate strips. Briefly, 24 h cultures of the *Campylobacter* were inoculated into the urease, indoxyl acetate and hippurate test solutions according to the manufacturer's instructions. These were then incubated for 4 h for colour development. For urease, development of pink colour was indicative of urease enzyme production (*C. lari*), development of pink colour in hippurate solution indicated production of hippuricase enzyme (*C. jejuni*). In the case of indoxyl acetate solution, change of colour from colourless to blue/purple was indicative of the presence of *Campylobacter jejuni/ Campylobacter coli*. A reaction positive for Indoxyl acetate reaction but negative for hippurate test solution, confirmed C*. coli*. A reaction positive for both reaction was indicative of *C. jejuni* (Baker et al., 2008; Chaban et al., 2010).

Alternatively, the indoxyl acetate strips and hippurate strips were impregnated with wet cultures and allowed to stay for 3-5 min. Development of blue/purple colour in the case of indoxyl acetate strips and development of pink colour in the case of hippurate strips within this period was indicative of positive reaction (*C. jejuni* and *C. coli*) and *C. jejuni* respectively.

Preparation of bacterial genomic deoxyribonucleic acid (DNA)

Genomic DNA was obtained by the whole-cell lysate method as described by Marshall et al. (1999). Briefly, cells from a 24 – 48 h culture grown on Columbia blood agar were re-suspended in sterile distilled water to an equivalent of 2.5 McFarland value. The suspensions were boiled to 100°C for 20 min in Eppendorff tube. The resulting templates were either used immediately for PCR or were kept at 4°C for up to 1 month.

Polymerase chain reaction (PCR) confirmation of *Campylobacter*

Genetic identification of the *Campylobacter* isolates was by PCR reaction using the general primers for the identification of campylobacteria. These primers are also specific for other members of the campylobacteriaceae (*Helicobacter* and *Arcobacter*). However, *Arcobacter* and *Helicobacter* spp. show negative reaction to the *Campylobacter* dryspot kit. Hence, any amplification of the primer sequences at the 1,004 bp fragment within the coding region of 16S rRNA confirms that such isolates are *Campylobacter* spp. and not *Helicobacter* or *Arcobacter* spp. The PCR reaction employed was as previously described by Marshall et al. (1999). Briefly, amplification was done in 50 µl reaction volume containing 5 µl of whole-cell lysate, 1 µl each primer, 10x buffer (invitrogen), 1.5 mM $MgCl_2$, 200 µM each deoxynucleotide (invitrogen) and 5U Taq DNA polymerase (invitrogen). The PCR amplification was performed with a thermocycler (ESCO Swift Mini Thermal Cycler Version 1.0, ESCO Technologies, Philadelphia U.SA). The samples were subjected to an initial denaturation for 2 min at 95°C, followed by 30 amplification cycles, each consisting of 94°C for 30 s, 52°C for 30 s, and 72°C for 90 s. A final primer extension at 72°C for 10 min was included. Oligonucleotides primers employed in this study are CAH16S 1a (5' – AAT ACA TCA AAG TCG AAC GA – 3') and CAH16S 1b (TTA ACC CAA CAT CTG ACG AC – 3'), respectively. The Oligonucleotides used in this study were synthesized by Inqaba Biotechnologies (Pretoria, South Africa).

Blood haemolysis

To ascertain the pathogenic status of the isolates, the *Campylobacter* spp were subjected to haemolytic test according to the procedure of Samie et al. (2006). Briefly, a 24 h broth culture of *Campylobacter* spp. was inoculated onto Columbia agar base plates supplemented with sheep blood. Plates were incubated at 35°C for 24 h. Thereafter, plates were observed for complete, partial and no haemolysis.

Antimicrobial agents

The antibiotics tested in this study were: Nalidixic acid (30 µg), ciprofloxacin (5 µg), gentamycin (10 µg), tetracycline (30 µg), ampicillin (10 µg), erythromycin (15 µg), imipenem (µg) (Oxoid, Unipath Ltd, Basingstoke, England).

Antimicrobial susceptibility testing

The method of Gaudreau and Gilbert (1997) was used. Briefly, the confirmed *Campylobacter* isolates were inoculated onto Mueller-Hinton agar plates carrying a maximum of six (6) discs. All plates were incubated at 42°C under a microaerophilic atmosphere obtained with a Campygen gas generator envelope (Oxoid) for 24 h. The resulting zone diameters were measured with a graduated metre rule. Analysis of diameter was done according to the procedural methods of CLSI (2010) for enterobacteriaceae.

RESULTS

Prevalence of *Campylobacter* in chicken

Out of a total of six hundred freshly voided faecal samples collected from three (3) farm settlements in the Venda Region of South Africa, consisting of 300 each from both chicken and cattle. One hundred and six (106) (35.3%) of the 300 samples from chicken were positive for campylobacters. Out of three hundred (300) samples from cattle, 58 (19.3%) were positive for campylobacters. From the 106 positive *Campylobacter* strains from chicken, 90 (84.9%) were *C. jejuni*, while 16 (15.1%) were *C. coli*. Similarly, from the 58 positive campylobacters strains from cattle faeces, 42 (72.4%) were *C. jejuni* and 16 (26.7%) were *C. coli*. There was a higher prevalence of *C. jejuni* than *C. coli* in chicken as well as in cattle. However, the prevalence rate was higher in chicken than in cattle. The prevalent rate was statistically significant at $P<0.005$. The prevalence of *C. jejuni* in chicken over cattle was highly significant at $P<0.005$ (Table 1).

Diarrheic and non-diarrheic faeces

In chicken, out of the 300 faecal samples analyzed, 98 (32.7%) were diarrheic. Of these, 72.5% (71 of 98) were positive for *C. jejuni*, while 4.1% (4 of 98) and 23.5% (23 of 98) were devoid of *Campylobacter* organisms. Of the remaining 202 non-diarrheic faeces, 9.4% (19 of 202) were positive for *C. jejuni*, 5.9% (12 of 202) were positive for *C. coli* and the remaining 171 (84.7%) were void of *Campylobacter* organisms. In cattle, of the 300 faecal samples, 12% (36 of 300) faeces were diarrheic of which

Table 1. Prevalence of *Campylobacters* in chicken faeces, incidence of *Campylobacter* dryspot positive strains and β-, α- and non- sheep red cell haemolytic *Campylobacter.*

No. of positive faeces samples		No. of *C. jejuni* isolated		No. of *C. coli* isolated		No. of positive diarrhea samples		No. of positive non-diarrhea samples		No. of isolates that were β-haemolytic	
Chicken	Cattle	Chicken	Cattle	Chicken	Cattle	Chicken	Cattle	Chicken	Cattle	Chicken	Cattle
106	58	82	36	16	20	59	35	31	23	82	38

Table 1. Continued.

No. of α-haemolytic		Diarrheic faeces with *C. coli*		Diarrheic faeces with *C. jejuni*		Non-diarrheic faeces with *C. coli*		Non-diarrheic faeces with *C. jejuni*	
Chicken	Cattle	Chicken	Cattle	Chicken	Cattle	Chicken	Cattle	Chicken	Cattle
33	11	4	2	71	34	12	12	19	10

34 (94.4%) were positive for *C. jejuni*, 2 (5.6%) were positive for *C. coli* and 1 (2.8%) was void of *Campylobacter* spp. (Table 1).

Haemolysis

Of the 106 *Campylobacter* strains isolated from chicken, 77.4% (82 of 106) were β-haemolytic, 18.9% (20 of 106) were α-haemolytic while 3.7% (4 of 106) were non-haemolytic. Out of the 58 *Campylobacter* isolates from cattle, 75.8% (44 of 58) were β-haemolytic, 20.7% (12 of 58) were α-haemolytic, while 3.5% (2 of 58) were non-haemolytic. The *Campylobacter* isolates from both animals understudy were more β-haemolytic than α-haemolytic (Table 1).

Antimicrobial susceptible profile

One hundred and six (106) and fifty-eight (58) *Campylobacter* strains from chicken and cattle faeces respectively were exposed to 7 antibiotics. The results from exposure are as shown in the analyses in Tables 2, 3, 4 and 5). Of the seven antibiotics tested, resistance was higher among *C. coli* than *C. jejuni* in most cases. However, the rate of resistance was not statistically significant.

Ciprofloxacin: The rate of resistance to this antibiotic was higher among cattle isolates than chicken isolates. Resistance rate was low among chicken (*C. jejuni*, 29%; *C. coli*, 37.5%) than cattle isolates (*C. jejuni,* 33.3; *C. coli*, 56.3%).

Tetracycline: Rate of resistance to the antibiotic was lower among *C. jejuni* isolates (31%) but higher among *C. coli* (62.5%) from cattle. In contrast, resistance rate was higher among *C. jejuni* (33.3%) than *C. coli* strains (43.8%). The rate of resistance to this antibiotic was highly variable among isolates from cattle and chicken from the farms.

Erythromycin: Rate of resistance to this antibiotic was highest among isolates from chicken, *C. jejuni* (56.7%) and *C. coli* (43.8%) than strains from cattle, *C. jejuni* (42.9%) and *C. coli* (6.8%).

Nalidixic acid: Rate of resistance to this antibiotic, a quinolone, was very high among chicken and cattle isolates. However, *C. jejuni* isolates from cattle had low resistance rate (26.2%) when compared with *C. jejuni* from chicken (47.8%). *C. coli* strains from cattle had higher rates of resistance (37.5%) compared to *C. coli* strains from chicken (31.3%).

Gentamycin: Rate of resistance was high with resistant rates more pronounced among *C. coli* strain from chicken (68.8%) than *C. coli* from cattle (62.5%). *C. jejuni* from cattle had a higher rate of resistance, 33.3%, than *C. jejuni* from chicken, 31.2% (Table 2, 3, and 4).

Multiple resistance

Although, multiple resistance was not a common feature, 12 (7.3%) of isolates were resistant to 2 antibiotics, 7 (4.3%) were resistant to 3 antibiotics, while 5 (3.1%) were resistant to 4 antibiotics (Tables 5 and 6). Most multiple resistant strains were isolated from cattle and were mostly *C. coli* strains.

DISCUSSION

The prevalence of *Campylobacter* in faecal specimens of chickens and cattle with and without diarrhea are presented in Table 1. The results of this study shows that the overall isolation rate of campylobacters were 3 5.5% (106 of 300) from

Table 2. Susceptibility profile of *C. jejuni* and *C. coli* isolated from chicken to seven antimicrobials using CLSI (2010) Guidelines.

Antibiotic	Chicken [*C. jejuni* (n = 90); *C. coli* (n = 16)]								
	Sensitive			Intermediate			Resistant		
	C. jejuni		*C. coli*	*C. jejuni*		*C. coli*	*C. jejuni*		*C. coli*
Ciprofloxacin	64	≥ 21	10	6	16-20	2	20	≤15	4
Tetracycline	60	≥ 15	9	8	12-14	2	22	≤11	5
Erythromycin	39	≥23	9	17	14-22	3	34	≤13	4
Gentamycin	47	≥15	5	13	13-14	4	30	≤12	7
Ampicillin	41	≥17	6	17	14-16	4	32	≤13	6
Imipenem	42	≥16	2	15	14-15	3	33	≤13	11
Nalidixic acid	47	≥≤19	11	14	14-18	1	29	≤13	4

Table 3. Susceptibility profiles of *C. jejuni* and *C. coli* isolated from cattle to seven antimicrobials using CLSI (2010) Guidelines.

Antibiotic	Cattle [*C. jejuni* (n = 42); *C. coli* (n = 16)]								
	Sensitive			Intermediate			Resistant		
	C. jejuni		*C. coli*	*C. jejuni*		*C. coli*	*C. jejuni*		*C. coli*
Ciprofloxacin	20	≥ 21	7	8	16-20	2	14	≤15	7
Tetracycline	21	≥ 15	6	7	12-14	3	15	≤11	7
Erythromycin	26	≥23	15	8	14-22	_	8	≤13	1
Gentamycin	16	≥15	6	5	13-14	2	21	≤12	8
Ampicillin	15	≥17	4	4	14-16	1	23	≤13	11
Imipenem	19	≥16	7	7	14-15	1	17	≤13	8
Nalidixic acid	22	≥≤19	10	5	14-18	2	15	≤13	4

chicken faeces and 19.3% (58 of 300) from cattle faeces. Similar results were obtained by Baker et al. (2008), in their study of the prevalence of *Campylobacter* species in chickens. Also, Minihan et al. (2004) and Stanley and Jones (2003) working on cattle found a high pre-ponderance of *Campylobacter* in their faeces. Ninety (90) (84.9%) *C. jejuni* and 16 (15.1%) *C. coli* were isolated from chickens. Similarly, 42 (72.4%) *C. jejuni* and 16 (17.6%) *C. coli* were isolated from cattle. We observed statistically significant difference between prevalence of *Campylobacter* in chicken and cattle at ($P<0.005$). Despite the presence of *Campylobacter* in chicken and cattle, it is difficult to consider that this microorganism was the causal agent of diarrhea in these animals, apparently due to the broad spectrum of biological factors which influence the diarrhea process, association of enteric pathogens and the fact that not all diarrheic faeces were positive for *Campylobacter*. *Campylobacter jejuni* was more commonly isolated from diarrheic than non-diarrheic faeces in both chicken and cattle than *C. coli*.

The haemolysis of sheep red blood cells by *C. jejuni* and *C. coli* isolates from cattle and chickens is in line with similar studies done by Samie et al. (2007) on *C. jejuni* isolated from humans in Venda Region of South Africa. This attribute further confirms that most of these isolates were of public health significance.

Exposure of isolates to seven (7) antimicrobials popularly used as animals' growth promoters and prophylaxis revealed a discrepancy in the resistance patterns of chicken and cattle isolates to the different antibiotics. Prevalence of resistance to ciprofloxacin was higher among cattle isolates than chicken isolates, where chicken was (*C. jejuni*, 29%; *C. coli*, 37.5%) and cattle isolates (*C. jejuni*, 33.3%; C. coli, 56.3%). Gupta et al. (2004) reported increasing proportion of *Campylobacter* isolates around the world that were fluoroquinolone-resistant. The high prevalence of resistance observed in cattle isolates may have stemmed from the use of other fluoroquinolone-derivatives in cattle, which now confer observed resistance on the campylobacters isolated from the faeces of these animals. Increased resistance to fluoroquinolones was first reported for *Campylobacter* from chickens. Endtz et al. (1991) and Jacob-Reitsma et al. (1994) reported almost 30% fluoroquinolone resistant among *Campylobacter* isolates from broilers in the Netherlands, a result which is in line with our findings. This resistance of chicken isolates may have resulted from the use of sarafloxacin and enrofloxacin in broilers (since field investigation revealed that these farms use these antibiotics as growth promoters). Our findings on resistance of cattle isolates to ciprofloxacin was far higher

Table 4. Pooled values for the susceptibility profile of *C. jejuni* and *C. coli* exposed to seven (7) antimicrobials commonly used in animals.

	Chicken						Cattle					
	***C. jejuni* %R**	***C. jejuni* %S**	***C. jejuni*%I**	***C. coli* % R**	***C. coli* %S**	***C. coli* %I**	***C. jejuni* %R**	***C. jejuni* %S**	***C. jejuni* %I**	***C. coli* %R**	***C. coli* %S**	***C. coli* % I**
CIP	22.2	71.1	6.7	25	62.5	12.5	33.3	47.6	19.1	43.6	43.8	12.5
TE	24.4	66.7	8.9	31.3	56.3	12.5	35.7	50	16.7	43.6	37.5	18.8
E	37.8	43.3	18.9	25	56.3	18.8	19.1	57.1	19.1	18.8	81.3	0
GE	33.3	52.2	14.4	43.8	31.3	25	50	38.1	11.9	50	37.5	12.5
AMP	35.6	45.6	18.9	37.5	37.5	25	54.8	35.7	9.5	68.8	25	6.3
IMIP	36.7	46.7	16.7	68.8	12.5	18.8	40.5	45.2	14.3	50	43.8	6.3
NA	32.2	52.2	15.6	25	68.9	6.3	35.1	52.4	11.9	25	62.5	12.5

S = Susceptibility, R = resistance, CIP= ciprofloxacin, TE= tetracycline, IPM = imipenem, AMP = ampicillin, CN= gentamycin, E=erythromycin, NA= nalidixic acid.

than those reported by Aarestrup et al. (1997) who observed a resistance value of 7% to ciprofloxacin. The major difference between this study and the majority of previous studies performed in other countries is the absence of resistance to ciprofloxacin (Ronner et al., 2004). In contrast, ciprofloxacin resistance has been reported in the USA, 19% (Gupta et al., 2004) and a range of European countries (14.9% of *C. jejuni* and 39.6% of *C. coli* isolates) (Bywater et al., 2004). An absence or near absence of ciprofloxacin resistance has also been reported from Brazil (De Moura Oliviera et al., 2006), Canada (Guevremont et al., 2006) and Norway (Nostrom et al., 2006). The high level of resistance observed in this study of *C. coli* than *C. jejuni* is in line with other studies (Bywater et al., 2004; Van Looveren et al., 2001). Our finding indicates that resistant to fluoroquinolones has emerged as a significant problem in Venda region of South Africa.

A high prevalence of resistance to tetracycline for *C. jejuni* and *C. coli* isolated from cattle and chickens was recorded. This is in line with similar reports from a study in Australian, 15-36% (Barton and Wilkins, 2001). Higher levels of tetracycline resistance have been reported from four European countries, 35.4% (Bywater et al., 2004) and the USA, 43% (Gupta et al., 2004). Studies from other countries have reported relatively higher prevalence of resistance to tetracycline (Reina et al., 1994; Sjogren et al., 1992). Payot et al. (2004) also reported that a high proportion of their isolates were resistant to tetracycline (79%). This value is far higher than those reported in this study. There was a high prevalence of resistance to erythromycin by *C. jejuni* (42.9%) isolated from cattle than *C. coli* (6.8%). This is in line with previous reports by Sato et al. (2004) and Inglis et al. (2005), where prevalence of resistance was 45% for *C. jejuni* isolates. Similarly, there was a higher resistance to erythromycin by *C. jejuni* isolated from chicken (56.2%) than *C. coli* (43.8%). High prevalence of resistance to macrolides among *C. coli* isolates has been reported in previous studies (Cabrita et al., 1992; Sanchez et al., 1994). Cabrita et al. (1992) reported that a few *C. coli* isolates were resistant to tetracycline; however, this is in contrast to our observations and report from other studies (Sagara et al., 1987; Velazquez et al., 1995).

C. jejuni and *C. coli* from cattle and chicken were highly resistant to ampicillin. Balton and Wikins (2001) reported high prevalence of resistance of *C. jejuni* and *C. coli* of between 50.4 – 63.6%. Resistance of *C. coli* isolates from chicken and cattle to nalidixic acid was ≤35.1%. However, the *C. coli* from chicken were more resistant to this antibiotic (25%) than those from cattle (12.5%). Payot et al. (2004) in their study observed that resistance to nalidixic acid went from 0 – 75% depending on the farm studied. Our findings in this study are at variance with their observation. The non-conformity of our results on the high prevalence of resistance of *C. coli* over *C. jejuni* as observed in other studies might have stemmed from the differences in policies involving the control use of antimicrobials, differences in farms, in addition to regional/geographical differences. Resistance to gentamycin by isolates were from chicken, *C. jejuni* (33.3%), *C. coli* (43.8%); from cattle, *C. jejuni* (38.1%) and *C. coli* (50%). Norma et al. (2007) in Canada reported a low level prevalence of resistance (0.2%) of *C. coli* to gentamycin.

Multi-drug resistance (for example, resistance to at least three different families of antimicrobial drugs) also showed a great variation from 25-37.5% among *C. jejuni* and *C. coli* isolates from chicken and 6.3-50% among *C. jejuni* and *C. coli* isolates from cattle, respectively (Tables 4 and 5).

Table 5. Multidrug resistance rate patterns of *C. jejuni* and *C. coli* isolated from chicken exposed to 7 antibiotics.

Resistance patterns	*C. jejuni*		*C. coli*	
	No. of strains	% of strains	No. of strains	% of strains
C,T,E,N,G,A	20	22.2	4	25.0
C, T	20	22.2	4	25.0
C, T, N	20	22.2	4	25.0
G, A	30	33.3	6	37.5
T.G.N	22	24.4	4	25.0
TEN	22	24.4	4	25.0

C = ciprofloxacin, T= tetracycline, E= erythromycin, N= nalidixic acid, G= gentamycin, A= ampicillin.

Table 6. Multidrug resistance rate patterns of *C. jejuni* and *C. coli* isolated from cattle exposed to 7 antibiotics.

Resistance patterns	*C. jejuni*		*C. coli*	
	No. of strains	% of strains	No. of strains	% of strains
C,T,E,N,G,A	8	19.0	1	6.3
C, T	14	33.3	7	43.8
C, T, N	14	33.3	4	25.0
G, A, I	15	35.7	8	50.0
T.G.N	15	35.7	4	25.0
TEN	8	19.0	1	6.3

Our finding is in near agreement with the observation of Payot et al. (2004) who observed multi-resistance from 5 to 75% High prevalence of resistance of *C. jejuni* and *C. coli* in extensively reared cattle could attribute to horizontal transmission of resistant strains with multi-drug resistant factors via the open environment where the cattle herds have unrestricted access to soil, forage for food and water. In case of chickens, the high prevalence may have resulted from the feed water given in the poultry. The erythromycin/nalidixic acid/tetracycline resistance pattern was the most common MDR pattern in our study and has also been reported by Payot et al. (2004) to be the most common MDR pattern.

In conclusion, this study highlights the prevalence of *C. jejuni* and *C. coli* from diarrheic and non-diarrheic faeces in the Vhembe district of the Limpopo province of South Africa. It also brings to the fore the occurrence of antimicrobial-resistant *C. jejuni* and *C. coli* from farm settlements in the country. The prevalence of MDR *C. jejuni* and *C. coli* isolates from chicken and cattle farms is alarming since these antimicrobials are used in the treatment of severe invasive cases of campylobacteriosis. Our study provides a sound insight into the prevalence of thermophilic *Campylobacter* species and antimicrobial resistance in *Campylobacters* associated with chickens and cattle in farm settlements in South Africa and has provided solid evidence that the majority of poultry and cattle isolates of *Campylobacter* showed remarkable resistance to antibiotics that are either used in the poultry or cattle industry.

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