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

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ABSTRACT

Campylobacter jejuni is one of the leading causes of sporadic food-borne bacterial disease in humans. In intensive poultry and pig rearing systems the use of oral antibiotics is essential to maintain health. Consequently, there is a high risk for the thermophilic *Campylobacter jejuni* and *C. coli* resident in the intestinal tract of food animals to develop resistance to commonly used antibiotics. Contamination of meat or eggs with pathogenic strains of resistant *Campylobacter* could, therefore, result in a form of campylobacteriosis in humans that is difficult to treat. The aim of this investigation was to determine the antimicrobial susceptibility of thermophilic *Campylobacter* spp. isolated from pigs and poultry by the broth microdilution minimum inhibitory concentration (MIC) test. A total of 482 samples from the Western Cape and Gauteng provinces was collected and analysed. Thirty-eight *Campylobacter* isolates were obtained. Analysis of data revealed that *C. jejuni* strains mainly of poultry origin were more resistant to the fluoroquinolones, macrolides and tetracyclines and the *C. coli* strains were more resistant to the macrolides and lincosamides. Multi-resistance was also detected in 4 *Campylobacter* strains from the Western Cape. With the exception of tetracyclines, strains from high health Gauteng broiler farms were susceptible to antibiotics used to treat *Campylobacter* infections.

Keywords: antimicrobial susceptibility, broth microdilution, *Campylobacter*, minimum inhibitory concentration, thermophilic.

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INTRODUCTION

The thermophilic campylobacters *Campylobacter jejuni* and *C. coli* occur worldwide as commensals in the digestive tracts of healthy animals, especially birds[38]. *Campylobacter jejuni* is isolated most commonly from broilers and *C. coli* from pigs[11,42].

Although of low virulence in animals, some strains of both these species have the ability to cause potentially serious diarrhoeal illness in humans[26]. In fact, campylobacteriosis is considered to be one of the most common causes of sporadic food-borne bacterial illness worldwide[2,16,20]. Animal-derived foods, especially poultry products, are thought to be the major source of *Campylobacter* infections in humans[2,9,38].

Campylobacter jejuni predominates in human infections where it accounts for approximately 95 % of cases campylobacteriosis. This disease in humans is acute and tends to be self-limiting, but serious complications such as Guillain-Barré syndrome may develop in a small number of patients[5,38].

Macrolides such as erythromycin, azithromycin and clarithromycin are preferred in human cases that require medication[2,3]. Furthermore, *C. coli* and *C. jejuni* are also susceptible to the aminoglycosides chloramphenicol, clindamycin, nitrofurans and imipenem. Varying rates of resistance have been recorded in several countries to tetracyclines, erythromycin, fluoroquinolones, the beta-lactams and metronidazole[9,24,28,41]. Intrinsic resistance to vancomycin, rifampin, trimethoprim[3], bacitracin and novobiocin exists[39].

Three minimum inhibitory concentration methods have been validated to test the susceptibility of thermophilic campylobacters to antibiotics, namely, broth microdilution, agar dilution and the epsilometer (E-test) tests [28]. Both the broth microdilution and agar dilution are recommended by the Clinical and Laboratory Standards Institute (CLSI)[7].

The primary aim of this investigation was to isolate and determine the antimicrobial susceptibility patterns of thermophilic *Campylobacter* spp. isolated from the intestinal tract of poultry and pigs, both important food animals that are known to have a high carriage of these intestinal bacteria[5,12,24,36].

MATERIALS AND METHODS

Collection and culture

During November 2007 to June 2008, 226 intact chicken caecae and 256 10 cm lengths of porcine colons were collected during necropsy for a non-enteric disease and from healthy animals at abattoirs. These samples were placed individually in sterile plastic containers, sealed and transported on ice without preservatives or transport media to the Western Cape Provincial Laboratory or Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, Pretoria, where they were cultured within 3 hours of collection.

To improve the sensitivity of isolation, 2 methods were used. In the 1st method the intestinal mucosa was rubbed with a cotton-tipped swab. This swab was used to inoculate a plate of Skirrow's agar (SA) (CM0935 & SR 0069, Oxoid Ltd). In the second method a cellulose nitrate filter with pore size 0.65 μm (Sartorius Stedim Biotech) was placed on a plate of Columbia agar (CM0331, Oxoid Ltd) containing 5 % defribrinated sheep's blood (BCA) and a generous sample (approximately 0.5 mℓ) of intestinal content placed on it[33]. The plates were incubated in *Campylobacter* gas (CampyGen) (CN0225, Oxoid Ltd) at 42 °C for 48 to 72 hours. After 24 hours incubation the filter on the BCA was removed, the inoculum streaked out to obtain single colonies of bacteria and re-incubated under the same conditions as previously.

Any small, dew-like colonies isolated were identified as *Campylobacter*-like if they comprised of Gram-negative curved bacteria that were catalase- and oxidase-positive. Such colonies were then identified to species level by means of biochemical analyses[33]. After identification and follow-

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Table 1: **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 1 mol/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 1 mol/L NaOH dropwise to dissolve	Water	101.4
Tiamulin	986	170	Water	Water	105.7
Tylosin	978	170	95 % Ethanol	Water	129.9

ing the manufacturer's instructions, isolates were frozen on Microbank® beads (Prolab Diagnostics) at –70 °C in an ultra-low-temperature freezer (New Brunswick Scientific).

Minimum inhibitory concentration tests

Susceptibility of isolates to a selection of commonly used veterinary antimicrobial drugs in poultry and pigs was determined by broth microdilution as recommended by the CLSI[7]. For the testing of Western Cape isolates, stock solutions of the following analytical grade antimicrobial drugs were made: chlortetracycline (Fujian Fukang Pharmaceutical Co. Ltd, China), doxycycline (Yancheng Suhai Pharmaceutical Co. China), enrofloxacin (Kirsch Pharma, South Africa), erythromycin (Ercros Industrial, South Africa), fosfomycin (Hangzhou Chyszem Biotech Co. Ltd, China), lincomycin (Nanyang Pukang Pharmaceutical Co. Ltd, China), norfloxacin (Dankong Industry & Trade Group, Co. Ltd, China), tiamulin (Shandong Lukang Shelile, China) and tylosin (Biesterfeld, Germany).

Isolates from poultry farms in Gauteng were tested using a commercial MIC test (Trek Sensititre Bovine/Porcine plate format BOP06F, Trek Diagnostic Systems, Separation Scientific) which contained ceftiofur, gentamicin, neomycin, spectinomycin, florfenicol, chlortetracycline, oxytetracycline, penicillin, ampicillin, enrofloxacin, danofloxacin, tiamulin, tylosin, tulathromycin, tilmicosin and lincomycin.

Methodologies differ between the laboratories in the Western Cape and Gauteng, because the most conveniently available methods were used.

Preparation of MIC panels

Stock solutions of antimicrobials (refer to Table 1 for potency, amount of powder weighed and solvents) were prepared, aliquotted and frozen[7]. The stock solutions were defrosted and diluted 1:10 in cation-adjusted Mueller Hinton broth (CAMB) (CM 0405, Oxoid Ltd) to obtain working dilutions. The working dilutions were added to the first column of wells on a 96-well 'U'-bottomed microtitre plate and diluted in serial 2-fold dilutions using cation-adjusted Mueller-Hinton broth (CAMHB) as the diluent (CM 0405, Oxoid Ltd). The dilution range included quality control ranges as well as any available breakpoints. One well was used as a growth control and received 100 µℓ of CAMHB only. Plates were prepared the day before testing and stored in a refrigerator.

Antimicrobial susceptibility testing

Microbank beads containing frozen isolates were streaked on BCA without antibiotics. The plates were incubated in CampyGen at 42 °C for 48 hours. Two subsequent subcultures were made and incubated in CampyGen at 42 °C for 48 hours[10]. One full loop of culture was picked from 48-hour old cultures and suspended in 2 mℓ of 0.9 % saline to obtain a turbidity approximately equal to a 0.5 McFarland standard. This suspension was initially diluted 1:100 in CAMHB to obtain the final inoculum of approximately 10^5 colony forming units (cfu/mℓ)[5,10]. 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 to obtain the final inoculum of approximately 2×10^5 cfu/mℓ.

One hundred microlitre volumes of inoculum were pipetted into each well of the testing panels and the plates were covered with a lid. The inoculated panels were incubated microaerophilically at 37 °C for 48 hours, after which the panels were read[7,10].

Quality control procedures simultaneously performed with each batch of tests were as follows. Batch control using reference strains (*Escherichia coli* ATCC 25922 (American Type Culture Collection, USA), *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *Campylobacter jejuni* ATCC 33560). An inoculum density/purity control was performed for each isolate and a growth control well was carried out on each plate (Table 2). The goal of quality control was to monitor the precision and accuracy of the test as well as the performance of reagents, viability of organisms and the performance of persons carrying out the tests and interpreting results[8].

Statistics

Descriptive statistics were predominantly used to perform inter-host, inter-provincial and inter-species comparisons. These included determining the percentage resistance using published breakpoint values[1,5,19] for the tested antibiotics, the MIC_{50} (equivalent to the median value) and MIC_{90}, as well as the distribution percentages of the MICs. Using an internet calculator (http://faculty.vassar.edu/lowry/utest.html), the Mann-Whitney *U*-test was used to determine whether there were any statistical differences[25]. The non-parametric Mann-Whitney *U*-test (synonym Wilcoxon rank-sum test)[27], was selected as it is best suited to compare 2 sets of independent data that do not have a normal distribution.

RESULTS

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 Province. Thirteen farms were piggeries and 11 were poultry farms. A total of 120 caeca

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

Antibiotic	Resistant	Susceptible	*Escherichia coli* ATCC 25922 37 °C/24 hours	*Staphylococcus aureus* ATCC 29213 37 °C/24 hours	*Enterococcus faecalis* ATCC 29212 37 °C/24 hours	*Campylobacter jejuni* ATCC 33560 37 °C/48 hours
Doxycycline	≥8	≤4	0.5–2	0.12–1	8–32	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
Tetracycline	≥8	≤4	0.5–2	0.12–1	8–32	0.25–2
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
Gentamicin	≥8	≤4	0.25–1	0.1–2	4–6	0.5–2
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

Adapted from Antibiogram Committee of the French Society for Microbiology (1999, cited by Avrain *et al.*[5]; CLSI[7]).
[†]A dash indicates that no acceptable range has been established.
*, #, ** = Refs 19, 1 and 4, respectively.

was also collected from 6 poultry farms in Gauteng Province.

Specimens from 13 of the 30 farms (43 %) sampled yielded thermophilic *Campylobacter* isolates. Five of these farms were piggeries and 8 were poultry farms. Thirty-eight *Campylobacter* isolates were obtained from the 482 samples (7.88 %), 6 from pigs and 32 from chickens.

Based on the hippurate hydrolysis test[33], 24 of the isolates were identified as *C. jejuni* and the other 14 isolates as *C. coli.* Of the 6 isolates from pigs, 1 was *C. jejuni* and 5 were *C. coli.* Of the 32 isolates from chickens, 23 were *C. jejuni* and 9 were *C. coli.* Sixty-five per cent of isolates were obtained from carcasses presented for necropsy. The distribution of the cultured *Campylobacter* spp. is shown in Table 3.

Antimicrobial susceptibility testing results

The MIC_{50} and MIC_{90} are minimum inhibitory concentrations of an antimicrobial at which growth of 50 % and 90 % of isolates respectively, are inhibited[24]. The MIC_{50} and MIC_{90} values were higher from *Campylobacter* spp. isolated in the Western Cape Province than those from Gauteng Province (for those that could be compared.). *Campylobacter* spp. from the Western Cape, as revealed in Figs 1 and 2, and with the exception of tiamulin resistance, tended to be divided into resistant and non-resistant populations. This was not observed in isolates from poultry samples from Gauteng, where resistance to antimicrobials was limited to the tetracyclines (95.5 %), β-lactams (95.4 %), ceftiofur (95.5 %) and ampicillin (85.5 %). *Campylobacter* spp. isolated from the Western Cape, had a lower percentage of resistance to the tetracyclines (57.7 %), which is 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 ($P = 0.0001$). There was also a tendency for these bacteria to be more resistant to the pleuromutilins (tiamulin) ($P = 0.0985$).

When MIC_{50} and MIC_{90} values, and the percentage distribution graphs were compared (Tables 4, 5 and 6), it was revealed that *C. coli,* the predominant isolate from pigs, tended to be, with the exception of resistance to the lincosamides (lincomycin) and macrolides, more susceptible to antimicrobials than *C. jejuni.* The Western Cape *C. coli* isolates yielded a MIC_{50} and MIC_{90} of >43 µg/mℓ to erythromycin and a MIC_{90} of >43 µg/mℓ to the lincosamides and tylosin. In Fig. 1 this is illustrated by the high peak at the >43 µg/mℓ category for the *C. coli* group. Consequently there was a tendency for *C. coli* to be more resistant than *C. jejuni* to the macrolides, erythromycin ($P = 0.0708$) and tylosin ($P = 0.063$). However, the 4 *C. coli* isolated from broilers on a farm in Gauteng, unlike those from the Western Cape, were highly susceptible to the lincosamides and macrolides. The *C. coli* were considered to be more susceptible than the *C. jejuni* to the tetracyclines chlortetracycline ($P = 0.0307$) and doxycycline ($P = 0.0446$).

Interestingly only the thermophilic *Campylobacter* spp. originating from the Western Cape revealed any resistance to the fluoroquinolones, 33.65 % in the case of enrofloxacin and 43.65 % in the case of norfloxacin.

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

Unusually, 4 *C. jejuni* isolates and 1 *C. coli* isolate from the Western Cape (all from poultry) were nalidixic acid resistant

Table 3: **A summary of *Campylobacter 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	4	6	16
Gauteng	0	0	18	4	22
Total	1	5	22	10	38

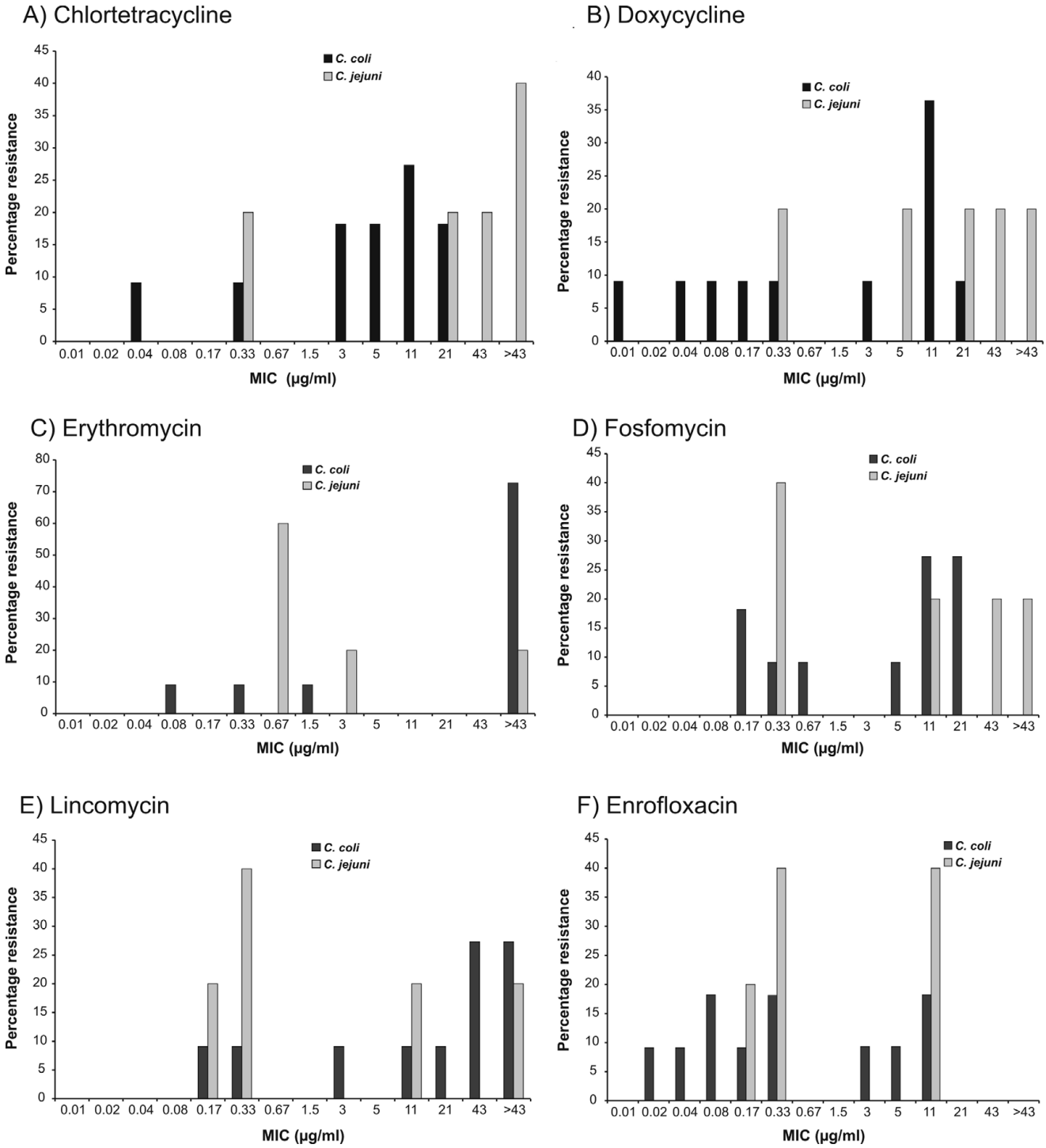

Fig. 1: **Percentage distribution of MIC values to the indicated antibiotics of *Campylobacter jejuni* (*n* = 5) and *C. coli* (*n* = 11) isolated from the Western Cape.**

on the disk diffusion sensitivity test. Three of these isolates had MICs of 11 µg/mℓ for enrofloxacin and 3 had MICs of ≥11 µg/mℓ for norfloxacin. Two isolates had MICs of ≥11 µg/mℓ for both antibiotics.

As pigs are given different therapeutic regimens from those of poultry, it was also decided to determine whether there were any differences between the campylobacters of porcine and those of poultry origin. Porcine *Campylobacter* strains were considerably more susceptible to tetracyclines (percentage resistance 34.4 % and 33.3 % to chlortetracycline and doxycycline, respectively) than the poultry strains (70 % and 60 % percentage resistance to chlortetracycline and doxycycline, respectively). However, these differences were not statistically significant when the MIC values were compared (chlortetracycline $P = 0.2389$ and doxycycline $P = 0.1922$). The 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 (83.3 %) and erythromycin (66.7 %).

DISCUSSION

Worldwide, most poultry flocks are considered to be the natural hosts of *C. jejuni* with prevalence rates from 10 to 82 % in conventionally reared positive flocks and an even higher prevalence in free-range chickens (54 % to 100 %)[18]. Similarly, the prevalence of *Campylobacter* spp. may be as high as 100 % in piggeries, the only difference being that *C. coli* tends

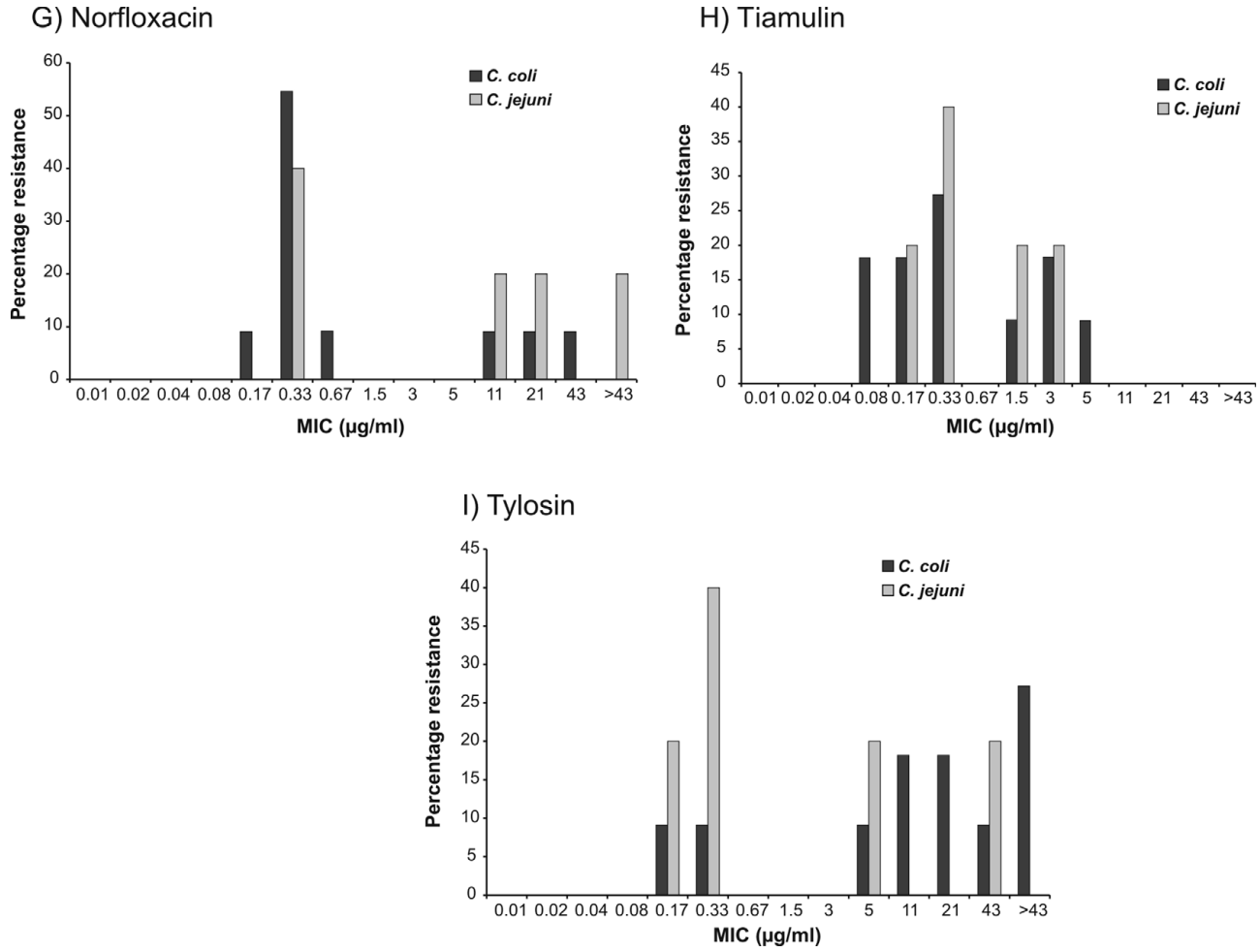

Fig. 2: **Percentage distribution of MIC values to the indicated antibiotics of *Campylobacter jejuni* (*n* = 5) and *C. coli* (*n* = 11) isolated from the Western Cape.**

to be the predominant species[15]. Even though the number of farms tested during this investigation was small, the 43 % infected farms was not unusual with *C. coli* (5 of 6 isolates) predominating in pigs and *C. jejuni* (23 of 32 isolates) predominating in poultry.

Poultry and pig farms in South Africa have over the years implemented more stringent infection control measures, such as the all-in-all-out system, in-line chlorination of drinking water, restricted access of humans to farms and high levels of hygiene. Furthermore, farm workers may only wear designated protective clothing and are only permitted to work in a specific area[20]. Under these circumstances the possibility of *Campylobacter* spp. being on a farm is greatly reduced and it is not unreasonable to expect a low prevalence, such as the 7.66 % obtained in this study. In the absence of infected animals, *Campylobacter* spp. can be introduced by, for example, outerwear of farm workers, transport vehicles, water, food, wild birds and, to a limited extent, rodents.

Furthermore, very few farms in South Africa practice 'thinning out', a procedure where some birds are removed from the flocks at 35 days of age, to allow the remaining birds to grow more rapidly. The crates that are used to remove these excess birds are often heavily contaminated, thus exposing the remaining birds to thermophilic *Campylobacter* spp. which spread to all of them by the time they are slaughtered[30].

It is also not surprising that a patchy distribution of *Campylobacter* spp. was found, as exemplified by those samples from Gauteng where only 2 of the 6 flocks tested were positive. For example, in a study in which poultry in 4 broiler houses were examined, it was found that those in the 1st broiler house to become infected had a low prevalence of *Campylobacter* spp. but by the time the birds were slaughtered 4 weeks later these bacterial species could not be isolated. This was not the case in the other houses that were suspected to have been infected by workers from the first house later in the grow-out cycle when 100 % of the birds tested at 4 weeks of age had evidence of intestinal colonisation[17]. It has been reported that proper cleaning and disinfection will destroy *Campylobacter* spp. in houses[30].

The nature of the samples and the sampling method and preservation of the specimens were similar to those of other studies in which high isolation rates of *Campylobacter* spp. were obtained. In addition, the use of both a non-selective culture medium with a filter as well as a selective isolation medium such as Skirrow's will ensure an optimal recovery of most strains of the enteric *Campylobacter* spp.[23]

There are currently no internationally accepted criteria for testing resistance to *Campylobacter* species, nor are there accepted breakpoint values[28]. The CLSI (2008) considers the agar diffusion test unreliable and recommends the use of either the agar dilution or broth dilution tests. There are, however, no specific breakpoints for this genus. Therefore if a published breakpoint could not be found (Table 2), the clinical breakpoint for an antimicrobial in the same group or for other Gram-negative bacteria was used in this study.

Therapeutic antimicrobials of choice in human patients suffering from life-threatening campylobacteriosis are initially the macrolides and thereafter the fluoroquinolones and gentamicin[11]. Resistance to these 2 classes of antibiotics in zoonotic *Campylobacter* species can

Table 4: **Percentage distribution, MIC_{50}, MIC_{90} and percentage resistant strains of *Campylobacter coli* from the Western Cape** (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	45.4	5	21			9.1			9.1			18.2	18.2	27.2	18.2		
Doxycycline	45.4	3	11	9.1		9.1	9.1	9.1	9.1			9.1		36.3	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	27.2	27.2		
Lincomycin	72.6	43	>43					9.1	9.1			9.1		9.1	9.1	27.2	27.2
Enrofloxacin	27.3	0.33	11		9.1	9.1	18.2	9.1	18.2			9.1	9.1	18.2			
Norfloxacin	27.3	0.33	21					9.1	54.5	9.1				9.1	9.1	9.1	
Tiamulin	36.4	0.33	3				18.2	18.2	27.2		9.1	18.2	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 for the breakpoint values)

Table 5: **Percentage distribution, MIC_{50}, MIC_{90} and percentage resistant strains of *Campylobacter jejuni* from the Western Cape** (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	

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

increase hospitalisation rates and the cost of therapy, and decrease the survival rate of patients[11]. Several countries, including Canada and the USA, reported trends of increased resistance of *C. jejuni* to the fluoroquinolones, whereas the prevalence of resistance to the macrolides and tetracyclines has remained static[14]. A surveillance programme for antibiotic resistance in *Campylobacter* spp. of human origin and similar commissioned surveys for 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 initially increased, only to progressively decline over the following 5 years. This decline was partially associated with decreased fluoroquinolone resistance in poultry and pigs in that country[13].

The banning of the incorporation of enrofloxacin in poultry feed in the USA in 2000 by the Food and Drug Administration (FDA) of the USA which was effected in 2005 was a direct consequence of documented evidence showing an increased resistance in disease-causing strains of *Campylobacter* isolated from humans as well as a 10 % resistance in poultry products[28,29]. Since fluoroquinolones, especially enrofloxacin and norfloxacin are used to treat resistant *E. coli* infection in birds, it would be expected that the same holds true for South Africa. This was true for the few isolates (50 % resistance to enrofloxacin and 60 % resistance to norfloxacin) from poultry in the Western Cape. None was, however, noted in the poultry isolates from Gauteng, nor from the pig isolates in the Western Cape. The farms tested in Gauteng have a niche market in that they supply certain supermarket chains with untreated birds. A study in KwaZulu-Natal found that resistance to the fluoroquinolones was low (8 %) but much higher to nalidixic acid[6]. This seems to point to differences in the nature of therapies used in the different provinces. It is known that fluoroquinolone resistance develops rapidly in *Campylobacter* spp., 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[42].

In South Africa, tylosin is used extensively by both the poultry and pig industries to treat *Mycoplasma* infections as well as spirochaete infections in pigs. It is also known to be used in sub-therapeutic doses as a performance enhancer. Therefore it was not surprising that cross-resistance to the parent macrolide erythromycin (46.35 %) in isolates from the Western Cape was detected. The resistance was higher in *C. coli* (72.73 %) than in *C. jejuni* (20 %). Resistance to tylosin was lower at 27.27 % in *C. coli* isolates. However, most probably due to the small sample size, these differences only tended toward statistical significance (P = 0.0708 for erythromycin and P = 0.063 for tylosin). It was found that 71 % of *C. coli* and only 37 % of *C. jejuni* isolated from birds fed diets supplemented with tylosin were resistant to erythromycin[22]. A high prevalence of resistance among *C. coli* isolates from humans and poultry to erythromycin, as well as co-resistance between erythromycin and clindamycin has been reported[11,24]. In our study *C. coli* was highly resistant to both erythromycin (72.7 %) and lincomycin (72.6 %), a lincosamide similar to clindamycin. A binomial pattern of resistance to the macrolides, which divides the bacteria into resistant and susceptible populations, has been reported in *C. coli*[36]; and occurred in both *C. jejuni* and *C. coli* in this study (Fig. 1). The resistance of the Western Cape strains of *Campylobacter* spp. to tiamulin (38.2 %) was unexpected as no cross-resistance has been reported between the macrolides and pleuromutulins. This could, therefore, be a direct consequence of the use of tiamulin in poultry and pigs in that province. Interestingly the

Table 6: **Percentage distribution of *Campylobacter* species** (n = 22), **MIC_{50}, MIC_{90} and percentage resistant strains from broiler caeca in Gauteng.**

Antibiotic	% Resistance	MIC_{50} (μg/mℓ)	MIC_{90} (μg/mℓ)	Percentage of isolates at each concentration (μg/mℓ)							
				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	85.5	8	8		4.5		4.5		4.5	81.8	4.5
Enrofloxacin	0	0.25	0.25	45.5	50	4.5					
Danofloxacin	0	0.5	1		4.5	50	45.5				
Gentamicin	0	1	1				100				
Neomycin	0	4	4						100		
Spectinomycin	0	8	8							100	
Tylosin	0	1	2			4.5	54.5	36.4	4.5		
Tulathromycin	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 1).

published breakpoint for *Campylobacter* is 1 μg/mℓ[19], much lower than what is used for other bacteria where the breakpoint is 32 μg/mℓ[7].

Tetracyclines are extensively used in both the poultry and pig industries in South Africa, as they are broad-spectrum in activity, cheap and can easily be administered in the food and water. It was, therefore, not surprising to find that 95.5 % of the poultry isolates from Gauteng and 52.7 % (doxycycline) and 62.7 % (chlortetracycline) of the Western Cape isolates were resistant to this class of antimicrobial. A recent study of *Campylobacter* spp. isolated from broilers and layer hens in KwaZulu Natal also revealed a high level of resistance to the tetracyclines of up to 100 %[6]. It must be noted, however, that this study used a breakpoint value of 8 μg/mℓ and not 4 μg/mℓ. Similar trends have been noted in the United Kingdom[31] and USA with prevalences of up to 99.5 % in the latter country[37]. This is thought to be due to the easy transfer between bacteria of the conjugative plasmid with the tet(O) gene[31]. Countries such as Iceland, in which tetracycline is rarely used, have negligible levels of resistance (0.3 %)[40]. This high level of tetracycline resistance is rarely recorded in humans and is most probably due to the fact that tetracyclines are not generally employed 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 conditions. 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[28,32]. Concurrently, in Japan, tetracycline resistance was high in food-producing animals[28].

It is well known that *C. jejuni* produces β-lactamases that confer resistance to the β-lactam drugs *i.e.* amoxicillin and ceftiofur at levels of between 83 to 92 %[21]. This was noted for *C. jejuni* isolated from Gauteng where 82.4 % and 94.1 % of *C. jejuni* isolates were resistant to amoxicillin and ceftiofur respectively. A study in KwaZulu-Natal recorded up to 100 % resistance to ceftriaxone in layers and broilers[6]. Interestingly, isolates from children at the Red Cross Hospital in the Western Cape have also shown an increase in resistance from 3.6 % in 2002 to 24.6 % in 2006[28]. 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 impermeable to these antibiotics[21].

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* spp. 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. Interestingly, a study done in a Swiss abattoir revealed an unusual resistance pattern in that 27.7 % of the *C. jejuni* were resistant to streptomycin with a very low resistance to erythromycin and fluoroquinolones[12]. However, technical errors may have accounted for the unusually high streptomycin resistance as the disk diffusion test which is considered to give erratic results was used[7].

Campylobacter spp. isolated from the 2 farms in Gauteng tended to have very similar antimicrobial resistance (AMR) patterns which indicates that possibly there was clonal expansion of the strains on the farms. 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 KwaZulu-Natal[6]. As mentioned above, it is possible that the high-level management and the consumer pressure to cease the treatment of broilers prevented the selection of antimicrobial resistance.

Multi-resistance in both *C. jejuni* and *C. coli* has been reported both in human and animal isolates throughout the world. The resistance pattern that was noted in the 4 multiresistant *Campylobacter* spp., especially to tetracyclines, macrolides and fluoroquinolones, has been recorded elsewhere[11,13,34]. It is postulated that efflux pumps either encoded by the *Campylobacter*-specific *cme*ABC gene or by as yet unidentified genes are responsible. Efflux pumps usually result in increased resistance to several antibiotics at once as they actively remove antibiotics from the bacterial cytosol[34].

CONCLUSIONS AND RECOMMENDATIONS

Several studies, including this one, have shown that antimicrobial resistance of *Campylobacter* spp. isolated from humans and animals is highly variable both geographically and from year to year[28]. In

poultry flocks or pig herds, antimicrobial resistance is dependent on the level of disease and antimicrobials used. In this study of only a few poultry and pig farms, resistance to not only the fluoroquinolones and macrolides, but also multi-resistance was found. Therefore, constant vigilance for *Campylobacter* spp. of public health significance should be maintained through the use of surveillance and the rapid reporting of trends[28]. Economic restrictions have meant that studies in Africa, including South Africa, are done on an *ad hoc* basis and are few and far between. This is evidenced by the paucity of publications originating from this continent and the fact that this genus was not included in the fledgling South African antimicrobial surveillance programme[35].

It is therefore recommended that surveillance is instituted for *Campylobacter* spp. originating especially from poultry and pigs in South Africa and that the focus should be fluoroquinolones, macrolides and tetracyclines, to which a high level of resistance was found in this study. The surveillance programme should also include 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 animal species will have a high carriage rate of thermophilic *Campylobacter* species[18].

It has been shown that on farms on which antibiotics are not used, the levels of antimicrobial resistance, although not absent, tend to be very low[15]. Therefore, producers should be encouraged by legislation or by market pressures to reduce not only the use of therapeutic antimicrobials that are known to be effective against *Campylobacter* spp. but also that of tylosin as a performance enhancer.

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