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Original Article

Antibiotic resistance profiles of Campylobacter species in the South Africa private health care sector

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Abstract

Introduction: There is a dearth of surveillance data on clinical *Campylobacter* in South Africa, particularly in the private healthcare environment. We investigated the prevalence of resistance to first-line antibiotics used to treat campylobacterioses in clinical *Campylobacter* isolates from a private pathology laboratory.

Methodology: Identification of the *Campylobacter* specific genes were confirmed by PCR. Minimum inhibitory concentrations were determined using the broth micro-dilution method against macrolides (erythromycin, azithromycin), fluoroquinolones (ciprofloxacin, gatifloxacin) and tetracycline.

Results: Seventy-two *Campylobacter* isolates were identified by PCR, with 54 (75%) being classified as *C. jejuni* and 18 (25%) as *C. coli.* Of these, 11 (20.4%) *C. jejuni* and six (33.3%) *C. coli* strains were resistant to ciprofloxacin and three (7.41%) *C. jejuni* and three (16.7%) *C. coli* strains were resistant to gatifloxacin. The number of *C. jejuni* strains resistant to erythromycin and azithromycin was 17 (31.5%) and 36 (50%) respectively, while the resistance of *C. coli* strains to erythromycin and azithromycin were seven (38.9%) and 14 (77.8%) respectively. Resistance to tetracycline was detected in 10 (55.6%) *C. coli* and 14 (25.9%) *C. jejuni* strains.

Conclusion: In the light of these resistant profiles, the lack of a South African *Campylobacter* surveillance program is of concern. Relatively high prevalence of resistance in clinical isolates of *C. jejuni* and *C. coli* to the fluoroquinolones, macrolides and tetracycline used in first line treatment is of great concern. The efficacy treating human campylobacteriosis should thus be revisited.

Key words: *Campylobacter*; antibiotic resistance; fluoroquinolones; macrolides; tetracycline.

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Introduction

Campylobacter species are the most prevalent and widespread enteric bacteria pathogens in both industrialized and developing countries [1]. It accounts for most cases of human gastrointestinal infections worldwide, causing 400-500 million cases of diarrhea each year [2]. In the European Union (EU) in 2008, 190,566 cases of campylobacteriosis was confirmed [3], while in the United States (USA), an estimated 2.4 million incidents occur each year [4]. A total of 220,209 *Campylobacter* cases were reported and confirmed by the EU in humans in 2011, and 212,064 established cases in 2010 [5]. In developing countries, *Campylobacter*-related gastroenteritis rates are most common amongst children less than five years old [6].

Campylobacter infection is primarily a zoonotic disease as it is a commensal of food animals, particularly poultry, which serves as the main reservoir for human infection [7]. Other sources of transmission, include water, milk, and food animal meat products [8]. The disease characteristics vary from watery, non-bloody, non-inflammatory diarrhea to a severe inflammatory diarrhea followed by abdominal pain and fever [9]. Amongst all the species of *Campylobacter*, *Campylobacter jejuni* subsp *jejuni, Campylobacter coli,* and *Campylobacter lari* are the most prevalent pathogens in human [10, 11], with *C. jejuni* being responsible for 80-90% and *C. coli* for 5-10% infections in human [12]. *Campylobacter* spp, especially *C. jejuni* subsp. *jejuni,* has been linked to extensive intestinal infections, including gastrointestinal infections,

myocarditis, hepatitis, meningitis, myelitis, pancreatitis, haemolytic-uraemia syndrome and secondary complications, such as Guillain-Barré syndrome [13].

The recommended drugs for treating campylobacteriosis are macrolides (erythromycin, azithromycin and clarithromycin), amoxicillin, fluoroquinolones (ciprofloxacin) and tetracycline [14]. However, the emergence of antibiotic resistant strains poses a challenge in the management of *Campylobacter* infections. Isolates of *C. jejuni* and *C. coli* with resistance to various antimicrobial agents have been reported in both developed and developing countries [15] due to the extensive and unrestrained use of antibiotics especially in developing countries [16]. Fluoroquinolone use in food animals (administration in poultry flocks which is the main reservoir of *Campylobacter* spp.) may be associated with increases of resistant *Campylobacter* strains in human health care [12,17]. The latter has been well documented in several countries [12,18,19]. Mechanism of fluoroquinolone resistance was found to be chromosomally mediated through mutation of the *gyrA* gene and *parC* gene while macrolides resistance in *C. jejuni* is through the 23S rRNA. Resistance to tetracycline in *C. jejuni* and *C. coli* has been found to be positioned on a transmissible plasmid encoding ribosomal protection gene [16].

There is a dearth of surveillance data on clinical *Campylobacter* in South Africa, particularly in the private healthcare environment. We therefore investigated the prevalence of resistance to ciprofloxacin and erythromycin, as well as against newer antibiotics in these classes, viz., gatifloxacin, azithromycin and tetracycline in clinical *Campylobacter* isolates from a private pathology laboratory in South Africa.

Methodology

Sample collection

Ethical approval was granted by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (reference number BE084/14). The laboratory samples were collected from October 2013 to September 2014, and each person's demographic and clinical information on age, sex of patient, date of specimen collection and whether an inpatient/outpatient was obtained. During this period, 72 clinical isolates from patients with diarrhea/dysentery were collected and collated by a private pathology laboratory, based in Durban, South Africa. The bacterial samples were stored at -60°C in Brucella broth (Becton Dickinson and Company, Sparks, USA) supplemented with 10% (v/v) glycerol (ACE, Southdale, Johannesburg) until analysis. To confirm culture purity, bacteria were cultured on *Campylobacter* blood-free Selective Agar Base (Oxoid Ltd, Basingstoke, England), supplemented with CCDA Selective Supplement (Oxoid LTD, Basingstoke, Hampshire, England, SR155E.) and incubated at 37°C in a microaerobic atmosphere (CampyGen; Oxoid Ltd, Basingstoke, UK) for 48 hours. This was followed by sub-culturing on Triptose Blood Agar Base (Biolab, Longmeadow Business Estate South, Modderfontein, South Africa) supplemented in 7% defibrinated sheep blood and incubated at 42ºC for 24 hours in microaerobic atmosphere (CampyGen, Oxoid Ltd, Basingstoke, UK).

Species identification

The *Campylobacter* species were screened using conventional methods that included the catalase, oxidase and hippurate hydrolysis tests, according to the Cape Town protocol [20]. PCR was used to confirm species identifications using primer *hip*O for *C. jejuni* and putative aspartokinase for *C. coli* as indicated in Table 1. DNA was extracted using a modified heat lysis method; briefly, a bacterial cell suspension was boiled for 10 minutes followed by freeze-thaw for five minutes and then centrifuged at 5000 rpm for 10 min. The supernatant was collected and stored at -40ºC until analysis. The concentration and purity of the DNA was determined spectrophotometrically using the Nanodrop ND-1000 Spectrometer (Thermo Scientific, Waltham, USA), and all samples had an *A* 260/280 ratio ranging from 1.7 to 2.1. PCR was performed using a ThermoCycler (Applied Biosystems, Foster City, USA), the primers 2.5µl and sample DNA (10ng) were added to dreamTaq DNA polymerase master mix (Thermo Scientific, Waltham, USA) to a final reaction volume of 50µl. The reaction template for all primers was the same, with an initial denaturation at 95ºC for 5 min and final elongation at 72ºC for 4 min, with 35 cycles of PCR. The PCR consisted of denaturation at 95ºC for 1 min, followed by annealing for 30s (temperature for each primer shown in Table 1), and 30s elongation at 72ºC [21]. The primer sequences primer *hip*O and putative aspartokinase with their annealing temperatures are shown in Table 1.

Antibiotic susceptibility testing

The minimum inhibitory concentrations (MICs) were determined by broth micro-dilution, as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines [22]. The samples were tested against macrolides (erythromycin and

azithromycin) (Sigma-Aldrich, Steinheim, Germany), fluoroquinolones (ciprofloxacin (Fluka Analytical, Sigma-Aldrich Buchs, Steinheim, Germany) and gatifloxacin (DLD Scientific, Durban North, South Africa) in order to determine whether there was a significant difference between newer generations of the same antibiotic class and tetracycline (Fluka Analytical, Gillingham, UK). The two-fold dilution range of antibiotics was as follows: for ciprofloxacin and gatifloxacin 8 - 0.003µg/ml, for erythromycin and azithromycin 128 - 0.06µg/ml, and for tetracycline, 128 - 0.06µg/ml. The bacterial inoculate was adjusted to a 0.5 McFarland, as recommended by CLSI guidelines [22] using a McFarland densitometer DEN 1B, Bioscan (Bioscan, Riga, Latvia), and inoculated in each well containing the diluted antibiotic concentrations. One set of wells was left blank as media controls and another as growth controls. *E. coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 strains served as controls for all antibiotics except azithromycin where *Staphylococcus aureus* ATCC 29213 was used instead of *E. faecalis* ATCC 29212 (Rapidmicrobiology, Manassas, USA)

Minimum Inhibitory Concentration determinations were done in replicate. Breakpoints for ciprofloxacin (MIC ≤1), gatifloxacin (MIC ≤2), tetracycline (MIC ≤4), and erythromycin (MIC ≤8) were used, as described in the CLSI M100-S24 for *Enterobaceriaceae* [22]. Due to lack of breakpoint in the CLSI guideline, the breakpoint for azithromycin (MIC 0.5 for *C. coli* and 0.25 for *C. jejuni*) was used as described in "Clinical breakpoints, epidemiological cut-off (ECOFF) values and EUCAST disk diffusion methodology for *Campylobacter jejuni* and *Campylobacter coli"* [23]. Multidrug resistance was defined as resistance to all three of the antibiotics classes that the antibiotics represent *i.e*. fluoroquinolones, macrolides and tetracycline.

Amplification of resistance genes

The extracted DNA was used to detect the frequently encountered genes conferring resistance to ciprofloxacin, erythromycin and tetracycline. The presence of the *tetO* gene, which is responsible for tetracycline resistance and Thr-86-Ile mutations that are found in the quinolones resistance determining region (QRDR) of the *gyr*A gene in *Campylobacter,* were investigated. The presence of erythromycin resistance was also determined by detecting point mutations at position 2075 and 2074 in the V 23S rRNA gene, which are associated with high levels of resistance [12]. The presence of the multidrug efflux pumps was investigated, specifically the *Campylobacter* multidrug efflux gene B (gene Cj366c), which was determined using a primer for *cmeB*. PCR, and master mix conditions were carried out as previously reported, with a 25 µl final reaction volume being used [24]. The primer sequences and PCR conditions are shown in Table 1.

Statistical analysis

Statistical analysis was undertaken using SPSS software (IBM version 23) to correlate demographic and susceptibility data. MIC_{50} and MIC_{90} values were determined using the MIC susceptibility values, and the two-tailed T tests were used to determine significance ($p \leq 0.01$) in resistance between antibiotics originating from the same class.

Table 1. Primer sequences used for species identification and detection of resistance genes together with annealing temperatures.

Target	Primer name	Primer sequence	Product size (bp)	Annealing temp (°C)	Reference
C. jejuni	HipO	F5'-GAAGAGGGTTTGGGTGGT3' R5'AGCTAGCTTCGCATAATAACTTG3'	750	58	[21]
C. coli	Putative aspartokinase	F5'GGTATGATTTCTACAAAGCGAG3' R5'ATAAAAGACTATCGTCGCGTG3'	500	58	[21]
Thr-86-Ile mutations (*C.jejuni*)	*CjgyrA-F* *CjgyrA-R*	F5'TTTTTAGCAAAGATTCTGAT3' R5'CAAAGCATCATAAACTGCAA3'	265	48	[42]
Thr-86-Ile mutations (*C.coli*)	*CcgyrA-F* *CcgyrA-R*	F5'TATGAGCGTTATTATCGGTC3' R5'TAAGGCATCGTAAACAGCCA3'	192	48	[43]
23S rRNA at position 2074	*23SRNA-F* *ERY2074R*	F5'TTAGCTAATGTTGCCCGTACCG3' R5'AGTAAAGGTCCACGGGGTCTCG3'	485	59	[44]
23S rRNA at position 2075	*23SRNA-F* *ERY2075R*	F5'TTAGCTAATGTTGCCCGTACCG3' R5'TAGTAAAGGTCCACGGGGTCGC3'	486	59	[44]
Tet(O)	Tet1 Tet2	F5'GGCGTTTTGTTTATGTGCG3' R5'ATGGACAACCCGACAGAAGC3'	559	57	[45]
Effux pump	CmeB-F CmeB-R	F5'GACGTAATGAAGGAGAGCCA3' R5'CTGATCCACTCCAGCTATG3'	1,166	50	[24]

Sequencing

The *gyr*A and *tet*O genes were confirmed by sequencing 12% and 16% of the samples respectively (Inqaba Biotechnical Industries (Pty) Ltd., Pretoria, South Africa) and blasting on GENBank. Due to financial constraints, not all the samples were sequenced.

Results

Clinical profile

Table 2 shows the age, sex and in-patient/out-patient status of the sample. Of the 72 stool samples collected from patients, who were aged one month to 70 years, the infection rate was higher among males at 46 (63.9%) than females at 26 (36.1%). In terms of age, the most infected where 28 (38.9%) infants and toddlers aged 0-2 year(s), followed by 14 (19.4%) young to mature adults aged 21-40 years as indicated in Table 2. The majority of the samples were collected in spring; 34 (46.6%) and summer 16 (21.9%), with 15 (20.5%) isolated in winter and eight (11%) in autumn. Persons whose infections were severe enough to warrant hospitalization accounted for 36 (47.2%) of the patients.

Species distribution of Campylobacter by PCR

From the 72 *Campylobacter* isolates tested by PCR, 54 (75%) were identified as *C. jejuni* using the *hip*O gene, and 18 (25%) were identified as *C. coli* with the putative aspartokinase gene amplification.

Minimum inhibitory concentration (MIC)

MIC's of the isolates showed resistance to ciprofloxacin of 11 (20.4%) *C. jejuni* and six (33.3%) *C. coli* strains. In comparison, resistance to gatifloxacin was shown in three (7.41%) *C. jejuni* and three (16.7%) *C. coli* strains, albeit not significantly ($p \geq 0.557$). Notably, the majority (MIC_{90}) of the *Campylobacter strains (C. jejuni* and *C. coli)* presented MIC≤ 2 µg/ml for ciprofloxacin and gatifloxacin, indicating low-level resistance to fluoroquinolones. The number of *C. jejuni* strains resistant to erythromycin and azithromycin was 17 (31.5%) and 36 (50%) respectively. In addition, the numbers of *C. coli* strains resistant to erythromycin and azithromycin were seven (38.9%) and 14 (77.8%) respectively. The MIC_{90} of the *Campylobacter* against erythromycin and azithromycin were approximately 2-folds apart (≥ 32 µg/ml) and not significantly different on comparison ($p \geq 0.497$). Resistance to tetracycline was detected in nearly a third of the samples, 24 (33.3

Table 2. Demographic characteristics of patients.

Age (years)	No. of individuals (%)	No. of male	No. of female	No. of out-patient	No. of in-patient	No. of unknown
≤ 2	28 (38.9%)	16	12	9	19	0
3 - 12	10 (13.9%)	7	3	4	3	3
13 - 20	6 (8.3%)	4	2	3	2	1
21 - 40	14 (19.4)	12	2	5	3	5
41 - 59	10 (13.9%)	6	4	3	7	0
≥ 60	4 (5.6%)	1	3	2	2	0
Total	**72 (100%)**	**46 (63.9 %)**	**26 (36.1%)**	**27 (37.5%)**	**36 (50%)**	**9 (12.5%)**

Table 3. MIC percentiles and resistance frequencies of clinical *Campylobacter* spp.

Antimicrobial	MIC_{50}	MIC_{90}	MIC range	*C. jejuni* No. (%)	*C. coli* No. (%)
Ciprofloxacin	0.5	2	≤ 0.25 - 8	11(20.4)	6(33.3)
Gatifloxacin	1	2	≤ 0.25 - 8	3(7.41)	3(16.7)
Erythromycin	2	32	≤ 0.25 - 32	17(31.5)	7(38.9)
Azithromycin	2	54.4	0.25 - 128	36(50)	14(77.8)
Tetracycline	2	32	≤ 0.25 - 32	14(25.9)	10(55.6)

Table 4. Frequency of common mutations conferring resistance to fluoroquinolones, tetracycline and macrolides in *C. jejuni* and *C. coli*.

	Fluoroquinolones		Tetracycline		Macrolides		Efflux Pump	
Gene/mutation	Resistance No (%)	Thr-86-Ile	Resistance No (%)	*Tet*(O)	Resistance No (%)	2075/2074	Resistance No (%)	*cme*B
C. jejuni (n 54)	14 (25.9)	16 (29.6)	14 (25.9)	14 (25.9)	40 (74)	1 (5.9)	2 (3.7)	8 (14.8)
C. coli (n 18)	9 (50)	9 (50)	10 (55.6)	10 (55.6)	14 (77.8)	7 (38.9)	1 (5.6)	5 (27.8)

%), with a higher prevalence in *C. coli* 10 (55.6%) than *C. jejuni* 14 (25.9%). MIC_{50} and MIC_{90} percentile values were 2 and 32 µg/ml respectively. A tableted MIC percentiles and resistance frequencies of the clinical *Campylobacter* spp. are shown in Table 3.

Antibiotic resistance genes

Table 4 shows the results of the common mutations conferring resistance to fluoroquinolones, tetracycline and macrolides in *C. jejuni* and *C. coli* as was found in this study. Frequency of common mutations conferring resistance to fluoroquinolones, tetracycline and macrolides in *C. jejuni* and *C. coli*. The quinolone resistance determining region (QRDR) of the *gyr*A of all the fluoroquinolone resistance strains showed a mutation at the Thr-86-Ile region. In addition, two isolates that were susceptible to ciprofloxacin with an MIC value of 0.5 µg/ml also showed mutations at this region. The transitional mutations A2075G and A2074C in the 23S rRNA gene were only amplified in seven (7) *C. coli* strains and one isolate of *C. jejuni*. All isolates resistant against tetracycline were shown to carry the *tet*O gene. *Campylobacter* multi-resistance gene B (*cme*B) was amplified in 13 (18.1%) strains. The sequenced samples all showed similarity with known *gyr*A and *tet*O genes of *Campylobacter jejuni* and *Campylobacter coli* sequences in GenBank.

Discussion

Campylobacter spp. isolated from patients with diarrhea by a private pathology laboratory in South Africa was studied. In this study, the infection rate was higher in male than in female compared to a study by Samie *et al* with the female having the highest rate of 21.7% [19]. *Campylobacter* spp. has been identified as the etiologic agent in outbreak and sporadic cases of gastroenteritis and gastrointestinal infection in both developed and developing counties [18]. Although this sample size collected from each season was too small for any statically significant seasonal distribution study, it did provide an indication that *Campylobacter* is likely to be more prevalent in summer and spring, as also reported by Mason *et al*. [25]. The most predominant of the *Campylobacter* spp. was *C. jejuni,* which is responsible for most infections in humans [12], as corroborated in this study, where the majority of *Campylobacter* isolates 54 (75%) were *C. jejuni* and 18 (25%) were *C. coli.* Similar *C. jejuni* to *C. coli* ratios (%) have been confirmed in studies from USA (66:2) [26], in the United Kingdom (90:10) [27] and China (76:19) [28]. This ratio of *C. jejuni* and *C. coli* has further been affirmed in developing countries. A study by Said et al. that was carried out in Egypt from 1998 to 2005 reported a ratio of 85:15 from 118 samples collected [18]. The Czech Republic reported similar ratios in two consecutive years, 46:3:other species in 2006 and 43:2:other species in 2007 [29]. Conversely, studies showing a greater prevalence of *C. coli* have also been documented, with a study by Maćkiw *et al* between January 2008 and December 2009 indicating the presence of *C. coli* in 108 samples out of 143 (75.5%), whereas *C. jejuni* was only found in 35 (24.5%) of the samples [12].

This study indicated that there was a higher rate of infection in children less than 3 years of age. This is comparable to results obtained by Samie *et al.,* in Vhembe District, South-Africa, who found a higher rate of infection in children less than two years (30.4%) [19]. A recent review article by Fletcher *et al.*, showed that *Campylobacter* gastroenteritis is particularly common between children less than 5 years old, with an isolation rate of approximately 58% in developing countries [30]. Another study conducted in New Delhi, India, showed a peak incidence of 19% in children below one year of age [31]. Infants and preschool children with compromised immune systems are more prone to infection than adults, thus control programs targeting the eradication of infection due to *Campylobacter* should prioritize children.

Increasing antibiotic resistance in *Campylobacter* spp., isolated from both humans and animals, has become a substantial and significant public health concern in both developed and developing countries [18,32]. While most *Campylobacter* infections are self-limiting, antibiotics may be prescribed in severe cases or in immunocompromised patients, with macrolides (erythromycin), fluoroquinolones (ciprofloxacin) and tetracycline are being recommended [33]. In *Campylobacter, E. coli*, and other Gram-negative bacteria, the fluoroquinolone mode of action is by interfering with DNA gyrase, a type II topoisomerase that catalyzes the negative supercoiling of relaxed or positively supercoiled, double-strand, covalently closed circular DNA [34]. Mutations in the *gyr*A gene of Gram-negative bacteria cause resistance to fluoroquinolones by altering the amino acid sequence near the putative active site of the *gyr*A protein. The main mechanism of ciprofloxacin resistance of *Campylobacter* spp. is the alteration of codon 86 from threonine to isoleucine in the *gyr*A of both *C. jejuni* and *C. coli* [18]. This was seen in the total number of *gyr*A genes subjected to sequencing.

A steady increase in fluoroquinolones, macrolides and tetracycline resistance has been observed in

Campylobacter in South-Africa. Lastovica observed that ciprofloxacin resistance increased from 1.4% to 29%, and to erythromycin it increased from 3.4% to 7.2% in a study conducted in Cape Town from 1998 to 2005 [35]. Studies carried out in the Vhembe district, South-Africa, by Samie *et al.* also showed an increase in erythromycin resistance from 25% to 53% and in ciprofloxacin from 8% to 13% from 2002 to 2007 [19]. Our study showed a 23.6% resistance to ciprofloxacin and 8.3% to gatifloxacin. Although the newer antibiotic i.e. gatifloxacin (A new 8- methoxyfluoroquinolone), appeared to be more effective, a significance difference could not be shown in this study. The difference in the resistance rate of ciprofloxacin and gatifloxacin has also been reported by Hayward *et al*., with an MIC of 50% and 90% for gatifloxacin for 0.094 µg/ml and 4 µg/ml respectively, while that of ciprofloxacin was 0.19 µg/ml and >32 µg/ml [36]. This could be attributed to older fluoroquinolones having greater potency against gyrase than against topoisomerase IV in many Gram-negative bacteria, and greater potency against topoisomerase IV than against gyrase in many Gram-positive bacteria. The newer quinolones seem to have a closely balanced activity against these enzymes [37].

For erythromycin, 33.3% resistance was observed and 69.4% for azithromycin in the present study, with no significant difference between these two antibiotics that belong to the same class. This is higher than previously reported by Lastovia and Samie *et al*, and may be attributed to selection pressure from the increasing use of these antibiotics since 2007. Global rises in antibiotic resistance have been noted in the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) report of 2012. Resistance as high as 35% to ciprofloxacin and 20% to tetracycline was evident in domestically-associated *C. jejuni* isolates [38]. In a study carried out in New Delhi, India, from September 2010 to April 2012, an increase in resistance of ciprofloxacin and erythromycin from 71.4% to 86.1% and 6.1% to 22.2% respectively compared to a formal study carried out in 2005 was observed [31].

In recent years, tetracycline resistance has occurred among many pathogenic and nonpathogenic species of bacteria, and it is due to different tetracycline resistance (*tet)* genes. The efflux pump and ribosomal protection genes are the two most important mechanism of tetracycline resistance in various genera of bacteria, and acquiring new tetracycline resistance genes is mostly associated with mobile components, such as plasmids or transposons, which are often conjugative elements [39]. In *Campylobacter* spp., tetracycline resistance is primarily mediated by a ribosomal protection protein (*tet*O) that is transferred as plasmid-encoded gene or in the chromosome where it is not self-mobile [40]. A 27% increase in tetracycline resistance was observed by Samie *et al.* while our study found 33.3% resistance. However, the study population and demographics differ, and additional studies are needed to confirm the tetracycline resistance increase in clinical samples in South-Africa.

A developing trend is the occurrence of multiple drug-resistant strains of zoonotic pathogens causing infections in humans in various developed countries [12]. Multi resistant *Campylobacter* spp. in South-Africa was first reported in 2006 by Lastovica [35]. Our study showed minimal multi-resistance in three (4.2%) isolates that were resistant to more than one of the antibiotic agents. This is comparable with the result noted in DANMAP of 4% multi-resistance of domestic acquired infection of *Campylobacter* in humans [38]. Higher levels of multi-drug resistance have been reported, e.g. 44.9% in Iran [41] and 86% in China [28]. Multi-resistance presents a risk to humans by limiting the therapeutic choice of antibiotics.

In conclusion, this study provides information on the prevalence of thermophilic *Campylobacter* spp. in private health sector in South-Africa. The antibiotic resistance profiles observed in clinical isolates of *C. jejuni* and *C. coli* to the fluoroquinolone, macrolides and tetracycline, which are often used as first line treatment is of concern. It is thus necessary to undertake continuous monitoring of both prevalence and molecular characteristics of antibiotic resistance to inform effective treatment regimens for *Campylobacter* infections.

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References

1. Coker AO, Isokpehi RD, Thomas BN, Amisu KO, Obi CL (2002) Human campylobacteriosis in developing countries-synopsis-statistical data included. Emerg Infect Dis 8: 237-243.
2. Mabote KI, Mbewe M, Ateba CN (2011) Prevalence of *Campylobacter* contamination in fresh chicken meat and milk obtained from markets in the North-West Province, South Africa. J Hum Ecol 36: 23-28.
3. The European Food Safety Authority (2010) Trends and sources of zoonoses and zoonotic agents and food-borne outbreaks in the European Union in 2008. EFSA journal 8: 1496.

4. Bae W, Kaya KN, Hancock DD, Call DR, Park YH, Besser TE (2005) Prevalence and antimicrobial resistance of thermophilic *Campylobacter* spp. from cattle farms in Washington State. Appl and Environmental Microbiology 71: 169-174.
5. The European Food Safety Authority (2013) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011. The EFSA Journal, 11: 3129.
6. Sangaré L, Nikiméa A, Zimmermann S, Sanou I, Congo-Ouédraogo M, Diabaté A, Diandé S, Guissou P (2012) *Campylobacter* Spp. epidemiology and antimicrobial susceptibility in a developing country, Burkina Faso (West Africa). Afr J Clin Exp Microbiol 13: 110-117.
7. Alfredson DA, DA, Korolik V (2007) Antibiotic resistance and resistance mechanisms in *Campylobacter jejuni* and *Campylobacter coli.* FEMS Microbiol Lett 277: 123-132.
8. Taylor E, Herman K, Ailes E, Fitzgerald C, Yoder J, Mahon B, Tauxe R (2013) Common source outbreaks of *Campylobacter* infection in the USA, 1997–2008. Epidemiol Infect 141: 987-996.
9. Chen J, Sun XT, Zeng Z, Yu YY (2011) *Campylobacter* enteritis in adult patients with acute diarrhea from 2005 to 2009 in Beijing, China. Chin Med J: 124: 1508.
10. Keller JI, Shriver WG (2014) Prevalence of three *Campylobacter* species*, C. jejuni, C. coli and C. lari* using multilocus sequence typing in wild birds of the Mid - Atlantic Region, USA. J Wildlife Dis 50: 31-41.
11. Vencia W, Nogarol C, Bianchi D, Gallina S, Zuccon F, Adriano D, Gramaglia M, Decastelli L (2014) Validation according to ISO 16140: 2003 of a commercial real-time PCR-based method for detecting *Campylobacter jejuni, C. coli,* and *C. lari* in foods. Int J Food Microbiol 177: 78-80.
12. Maćkiw E, Korsak D, Rzewuska K, Tomczuk K, Rożynek E (2012) Antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli* isolated from food in Poland. Food Control. 23: 297-301.
13. Lynch ÓA, Cagney C, Mcdowell D, Duffy G (2011) Occurrence of fastidious *Campylobacter* spp. in fresh meat and poultry using an adapted cultural protocol. Int J Food Microbiol 150: 171-177.
14. Ruiz-Palacios GM (2007) The health burden of *Campylobacter* infection and the impact of antimicrobial resistance: playing chicken. Clin Infect Dis 44: 701-703.
15. Uaboi-Egbenni (2012) Potentially pathogenic *Campylobacter* species among farm animals in rural areas of Limpopo province, South Africa: A case study of chickens and cattles. Afr J Microbiol Res 6: 2835-2843.
16. Padungton P, Kaneene JB (2003) *Campylobacter* spp. in human, chickens, pigs and their antimicrobial resistance. J Vet Med Sci 65: 161-170.
17. Kaakoush NO, Castaño-Rodriguez N, Mitchell HM, Man SM (2015) Global epidemiology of *Campylobacter* infection. Clin Microbiol Rev 28: 687-720.
18. Said MM, El-Mohamady H, El-Beih F, Rockabrand D, Ismail T, Monteville M, Ahmed S, Klena J, Salama M (2010) Detection of gyrA mutation among clinical isolates of *Campylobacter jejuni* isolated in Egypt by MAMA-PCR. J Infect Dev Cntries 4: 546-554. doi:10.3855/jidc.963
19. Samie A, Guerrant R, Barrett L, Bessong P, Igumbor E, Obi C (2007) Prevalence, haemolytic and haemagglutination activities and antibiotic susceptibility profiles of *Campylobacter* spp. isolated from human diarrhoeal stools in Vhembe District, South Africa. J Health Popul Nutr 25: 406.
20. Lastovica AJ (2006) Emerging *Campylobacter* spp.: the tip of the iceberg. Clin Microbiol Newsl 28: 49-56.
21. On SL, Jordan PJ (2003) Evaluation of 11 PCR assays for species-level identification of *Campylobacter jejuni* and *Campylobacter coli.* J Clin Microbiol 41: 330-336.
22. Clinical and Laboratory Standards Institute (2014 Performance standards for antimicrobial susceptibility testing twenty-fourth informational supplement. CLSI document M100-S24.
23. European Committee on Antimicrobial Susceptibility Testing (2012) Clinical breakpoints, epidemiological cut-off (ECOFF) values and EUCAST disk diffusion methodology for *Campylobacter jejuni* and *Campylobacter coli* Available: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Consultation/Campylobacter_wide_consultation_August_2012.pdf Accessed 20 November 2015.
24. Pumbwe L, Piddock LJ (2002) Identification and molecular characterisation of CmeB, a *Campylobacter jejuni* multidrug efflux pump. FEMS Microbiol Lett 206: 185-189.
25. Mason J, Iturriza-Gomara M, O'Brien S, Ngwira B, Dove W, Maiden M, Cunliffe N (2013) *Campylobacter* infection in children in Malawi is common and is frequently associated with enteric virus co-infections. PloS one 8: e59663.
26. Nielsen H, Hensen K, Gradel K, Kristensen B, Ejlersen T, Østergaard C, Schonheyder H (2010) Bacteraemia as a result of *Campylobacter* species: a population-based study of epidemiology and clinical risk factors. Clin Microbiol Infect 16: 57-61.
27. Jorgensen F, Ellis-Iversen J, Rushton S, Bull S, Harris S, Bryan S, Gonzalez A, Humphery T (2011) Influence of season and geography on *Campylobacter jejuni* and *C. coli* subtypes in housed broiler flocks reared in Great Britain. Appl Environ Microbiol 77: 3741-3748.
28. Chen X, Naren G, Wu C, Wang Y, Dai L, Xia L, Luo P, Zhang Q, Shan J (2010) Prevalence and antimicrobial resistance of *Campylobacter* isolates in broilers from China.Vet Microbiol 144: 133-139.
29. Bardon J, Kolar M, Cekanova L, Hejnar P, Koukalova P (2009) Prevalence of *Campylobacter jejuni* and its resistance to antibiotics in poultry in the Czech Republic. Zoonoses Public Health 56: 111-116.
30. Fletcher SM, McLaws ML, Ellis JT (2013) Prevalence of gastrointestinal pathogens in developed and developing countries: systematic review and meta-analysis. J Public Health Res 2: 42.
31. Ghosh R (2013) Increasing antimicrobial resistance of *Campylobacter jejuni* isolated from paediatric diarrhea cases in a tertiary care hospital of New Delhi, India. J Clin Diagn Res 7: 247.
32. Luangtongkum T, Jeon B, Han J, Plummer P, Logue C, Zhang Q (2009) Antibiotic resistance in *Campylobacter:* emergence, transmission and persistence. Future Microbiol 4: 189-200.
33. Ge B, Wang F, Sjolund-Karlsson M, McDermott P (2013) Antimicrobial resistance in *Campylobacter:* susceptibility testing methods and resistance trends.J Microbiol Methods 95: 57-67.
34. Aldred KJ, Kerns RJ, Osheroff N (2014) Mechanism of quinolone action and resistance. Biochemistry 53: 1565-1574.
35. Lastovica AJ (2006) Antibiotic resistance patterns of *Campylobacter jejuni, C. upsaliensis* isolates from paediatric patients in Cape Town, South Africa, 1998 - 2005., in 106th General Meeting of the American Society for Microbiology W. American Society for Microbiology, DC, USA, Editor. Available at:

http://ieg.ou.edu/ASM2006/data/papers/C_038.htm: Orlando FL. Accessed on
36. Hayward C, Erwin M, Barrett M, Jones R (1999) Comparative antimicrobial activity of gatifloxacin tested against *Campylobacter jejuni* including fluoroquinolone-resistant clinical isolates. Diagn Microbiol Infect Dis 34: 99-102.
37. Hooper DC (2000) Mechanisms of action and resistance of older and newer fluoroquinolones. Clin Infect Dis 31(Supplement 2): 24-28.
38. DANMAP (2012) Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. ISSN 1600- 2032. Available: http://www.danmap.org/Downloads. Accessed January 2013.
39. Roberts MC (2005) Update on acquired tetracycline resistance genes. FEMS Microbiol lett 245: 195-203.
40. Abdi-Hachesoo B, Khoshbakht R, Sharifi Yazdi H, Tabatabaei S, Hossienzadeh S, Asasi K (2014) Tetracycline resistance genes in *Campylobacter jejuni* and *C. coli* isolated from poultry carcasses. Jundishapur J Microbiol **7**: 9.
41. Rahimi E, Ameri M (2011) Antimicrobial resistance patterns of *Campylobacter* spp. isolated from raw chicken, turkey, quail, partridge, and ostrich meat in Iran. Food Control 22: 1165-1170.
42. Zirnstein G, Li Y, Swaminathan B, Angulo F (1999) Ciprofloxacin resistance in *Campylobacter jejuni* isolates: detection of gyrA resistance mutations by mismatch amplification mutation assay PCR and DNA sequence analysis. J Clin Microbiol 37: 3276-3280.
43. Zirnstein G, Helsel L, Li Y, Swaminathan B, Besser J (2000) Characterization of gyrA mutations associated with fluoroquinolone resistance in *Campylobacter coli* by DNA sequence analysis and MAMA PCR. FEMS Microbiol lett 190: 1-7.
44. Alonso R, Mateo E, Churruca E, Martenez I, Girbau C, Fernandez-Astorga A (2005) MAMA-PCR assay for the detection of point mutations associated with high-level erythromycin resistance in *Campylobacter jejuni* and *Campylobacter coli* strains. J Microbiol Methods 63: 99-103.
45. Gibreel A, Tracz D, Nonaka L, Ngo T, Connell S, Taylor D (2004) Incidence of antibiotic resistance in *Campylobacter jejuni* isolated in Alberta, Canada, from 1999 to 2002, with special reference to tet (O)-mediated tetracycline resistance. Antimicrob Agents Chemother 48: 3442-3450.

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