Antimicrobial resistance in human isolates of *Campylobacter jejuni*, 2015

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DISCLAIMER

CONTENTS

LIST OF TABLES

SUMMARY

Historically rates of antimicrobial resistance, including resistance to fluoroquinolones such as ciprofloxacin, have been low among *Campylobacter* isolated from both human and animal sources in New Zealand (NZ). However in late 2014, the Institute of Environmental Science and Research was alerted to a possible cluster of fluoroquinolone-resistant *Campylobacter jejuni* in South Auckland. A subsequent preliminary study undertaken at Labtests in Auckland in February 2015 found a rate of 30% fluoroquinolone resistance among *C. jejuni* and 77% of the fluoroquinolone-resistant isolates were also tetracycline resistant. Therefore this survey was undertaken to (i) determine the antimicrobial resistance patterns, specifically to ciprofloxacin and tetracycline, in *C. jejuni* isolates from throughout NZ, and (ii) establish whether fluoroquinolone resistance in human isolates of *C. jejuni* is due to one or several clones.

Five sentinel site laboratories, processing community specimens from Northland District Health Board (DHB), the three DHBs in the greater Auckland area, Lakes and Bay of Plenty DHBs, the two DHBs in the greater Wellington area, Canterbury DHB and Southern DHB, referred approximately 50 *C. jejuni* isolates from each DHB/area between May and October 2015. Susceptibility to ciprofloxacin, erythromycin and tetracycline was determined using either Etest or disc diffusion susceptibility test methods. Multilocus sequence types were determined either by traditional PCR and sequencing of the seven target loci or derived *in silico* from assembled Illumina short-read data.

15.5% (46) of the 297 *C. jejuni* isolates included in the survey were ciprofloxacin resistant. Of these ciprofloxacin-resistant isolates, 87.0% (40) were co-resistant to tetracycline. There was a higher prevalence of ciprofloxacin/tetracycline co-resistance in the Auckland (31.1%) and Wellington (21.7%) areas compared with Northland (10.4%), Canterbury (9.4%), Southern (3.7%) and Bay of Plenty/Lakes (2.7%) DHBs. All isolates were susceptible to erythromycin.

Twenty-nine (72.5%) of the 40 ciprofloxacin/tetracycline co-resistant isolates were MLST type ST6964. Notably this type was identified only among *C. jejuni* with ciprofloxacin/tetracycline co-resistance. The remaining 11 ciprofloxacin/tetracycline co-resistant isolates were a diverse range of types. All or the majority of the ciprofloxacin/tetracycline co-resistant isolates from Northland DHB (100%), the Auckland area (64.3%) and the Wellington area (92.3%) were ST6964. In contrast, each of the five co-resistant isolates from Canterbury DHB was a different MLST type, only one of which was ST6964.

This survey demonstrates there has been a recent and rapid increase in fluoroquinolone resistance in *C. jejuni* from humans in NZ, largely driven by the emergence of a single ST6964 clone. The emergence of this resistant clone in human populations has implications for the treatment and surveillance of campylobacteriosis in NZ. Fluoroquinolone antimicrobials can no longer be 'taken for granted' as empiric treatment for campylobacteriosis in our setting. Ongoing periodic surveillance of antimicrobial resistance in *Campylobacter* will be important to better track the emergence and possible further spread of resistant clones in NZ. Such surveillance may be a challenge in the future given the proposed introduction of culture-independent diagnostic testing for enteric pathogens. Measures to control and mitigate the further spread of the fluoroquinolone/tetracycline co-resistant ST6964 clone should include appropriate 'source control' and increased public awareness of appropriate food hygiene in the prevention of campylobacteriosis.

1. INTRODUCTION

Campylobacteriosis is the most common cause of bacterial gastroenteritis in New Zealand. In 2014 alone, there were 6766 notified cases of campylobacteriosis in New Zealand, representing an incidence of 150.3 cases per 100 000 population [1]. The majority of campylobacteriosis is caused by *Campylobacter jejuni* and, to a lesser extent, by *Campylobacter coli*. Approximately two-thirds of cases of campylobacteriosis in New Zealand are thought to be due to ingestion of contaminated food, typically undercooked poultry, although other modes of transmission include direct or indirect consumption of faecally contaminated water and contact with colonised domestic or wild animals [1, 2].

Typically, infection with *C. jejuni* or *C. coli* causes an acute, self-limiting gastroenteritis, and treatment is largely supportive. However, antimicrobial treatment is indicated in certain circumstances, such as invasive infection, severe and persistent gastroenteritis, and patients with immunocompromise [2]. The recommended treatment of choice for *Campylobacter* gastroenteritis is a macrolide, such as erythromycin, although fluoroquinolones may also be used [2]. However, high rates of fluoroquinolone resistance in *Campylobacter* in other geographic settings have precluded their use in many parts of the world [3, 4].

In general, rates of antimicrobial resistance, including to fluoroquinolones, in *Campylobacter* have historically been considered low in New Zealand. To date, antimicrobial resistance data in human isolates of *Campylobacter* has been collected annually from diagnostic laboratories, and reported by the Antibiotic Reference Laboratory, Institute of Environmental Science and Research (ESR) (see Antimicrobial resistance data from hospital and community laboratories. Available at https://surv.esr.cri.nz/antimicrobial/general_antimicrobial_susceptibility.php). For the past 13 years, fluoroquinolone resistance in human isolates of *Campylobacter* has been reported at a prevalence of <6% (Table 1).

Table 1. Antimicrobial resistance in human isolates of *Campylobacter* collated from diagnostic hospital laboratories in New Zealand, 2002–2013

Year	Percent resistant (number tested)	
	Erythromycin	Fluoroquinolone
2002	2.0 (303)	2.8 (318)
2003	1.1 (367)	1.9 (367)
2004	0 (392)	2.3 (384)
2005	1.1 (351)	2.0 (351)
2006	0.1 (693)	1.0 (687)
2007	0.5 (376)	2.4 (378)
2008	0 (229)	1.3 (228)
2009	0 (318)	1.6 (318)
2010	0.4 (276)	3.6 (276)
2011	0.8 (255)	2.0 (255)
2012	1.4 (218)	5.1 (216)
2013	0.8 (238)	2.1 (236)

In addition, a systematic survey of antimicrobial resistance in animal isolates (from 'bobby' calves and poultry) of *Campylobacter*, performed in 2009-2010, found no erythromycin resistance in *C. jejuni*, and a fluoroquinolone resistance rate of only 2.3% [5].

In November 2014, ESR was alerted to a possible cluster of fluoroquinolone-resistant *C. jejuni* in South Auckland. A preliminary study in the Auckland area was subsequently undertaken in conjunction with Labtests in February 2015. One hundred consecutive isolates of *C. jejuni* underwent antimicrobial susceptibility testing using disc diffusion testing (see Methods). Of the 100 isolates, 30 were resistant to fluoroquinolones, representing a significant increase compared to historical patterns of resistance. Interestingly, of these 30 fluoroquinolone-resistant isolates, 23 (77%) were also resistant to tetracycline. Accordingly, the aims of this present survey were to (i) determine the antimicrobial resistance patterns, specifically to ciprofloxacin and tetracycline, in *C. jejuni* isolates from throughout New Zealand, and (ii) establish whether fluoroquinolone resistance in human isolates of *C. jejuni* is due to one or several *C. jejuni* clones.

2. METHODS

2.1 BACTERIAL ISOLATES, IDENTIFICATION AND SAMPLING

Five New Zealand community diagnostic laboratories were asked to refer *C. jejuni* isolates for the survey between May and October 2015. The laboratories that referred isolates were Labtests, Auckland; Pathlab Bay of Plenty; Aotea Pathology, Wellington; Canterbury Southern Community Laboratories; and Southern Community Laboratories, Dunedin. Each laboratory was asked to refer approximately 50 consecutive isolates, except for Labtests which referred approximately 50 isolates from the three district health boards (DHBs) in the Auckland area (ie, Waitemata, Auckland and Counties Manukau) and another approximately 50 isolates from the Northland DHB. Isolates identified as a species other than *C. jejuni* were excluded. When referring isolates for the survey, laboratories supplied sufficient patient identifiers to allow duplicates from the same patient to be excluded.

2.2 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Susceptibility to ciprofloxacin, erythromycin and tetracycline was determined by the methods described by the Clinical and Laboratory Standards Institute (CLSI), using either Etest or disc diffusion [6]. Tests were performed on Mueller-Hinton agar with 5% sheep blood and incubated at 36-37ºC for 48 hours in microaerobic conditions. Minimum inhibitory concentrations were interpreted according to CLSI breakpoints [6]. Disc diffusion results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints [7].

2.3 MULTILOCUS SEQUENCE TYPING (MLST)

DNA was extracted using a High Pure PCR purification kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. DNA was diluted to 30-50 µg/mL prior to being used as a source of DNA template in MLST PCRs. MLST was carried out using protocols and conditions described on the *C. jejuni* MLST website

(http://pubmlst.org/campylobacter/). The loci assessed were *aspA, glnA, gltA, glyA, pgm, tkt, uncA*. Sequences were analysed using BioNumerics software (Applied Maths, St-Martens-Latem, Belgium) and sequence types were assigned using the *C. jejuni* MLST website interface. MLST allelic data was also derived *in silico,* from assembled Illumina short-read data.

2.4 DATA ANALYSIS

Statistical analyses were performed with SAS software v.9.3 (SAS Institute Inc, Cary, NC, United States) [8]. The chi-square test was used to determine the significance of any observed differences. A *p* value of ≤0.05 was considered significant.

3. RESULTS

3.1 ANTIMICROBIAL SUSCEPTIBILITY BY GEOGRAPHIC LOCATION

All 297 viable, non-duplicate isolates referred as *C. jejuni* were included in the survey. The geographic source of the isolates, and ciprofloxacin and tetracycline resistance, is shown in Table 2. 15.5% of the isolates were ciprofloxacin resistant and 14.5% were tetracycline resistant. All 297 isolates were susceptible to erythromycin.

87.0% (40/46) of the ciprofloxacin-resistant isolates were also tetracycline resistant. Only 2.0% (6) and 1.0% (3) of the isolates had monoresistance to ciprofloxacin and tetracycline, respectively. Notably all ciprofloxacin-resistant isolates from Northland DHB and the three Auckland DHBs (Waitemata, Auckland and Counties Manukau) were also tetracycline resistant.

Table 2. Ciprofloxacin and tetracycline resistance among *Campylobacter jejuni*, by geographic location, 2015

District Health Board(s)	Number of isolates	Percent (number) resistant		
		Ciprofloxacin	Tetracycline	Co-resistance to ciprofloxacin and tetracycline
Northland	48	10.4 (5)	10.4 (5)	10.4 (5)
Waitemata/Auckland/ Counties Manukau	45	31.1 (14)	31.1 (14)	31.1 (14)
Bay of Plenty/Lakes	37	5.4 (2)	2.7 (1)	2.7 (1)
Capital and Coast/ Hutt Valley	60	25.0 (15)	23.3 (14)	21.7 (13)
Canterbury	53	9.4 (5)	11.3 (6)	9.4 (5)
Southern	54	9.3 (5)	5.6 (3)	3.7 (2)
Total	297	15.5 (46)	14.5 (43)	13.5 (40)

Ciprofloxacin resistance and tetracycline resistance was significantly higher ($p \leq 0.05$) among *C. jejuni* referred from the three Auckland DHBs and the two Wellington DHBs (Capital and Coast and Hutt Valley) compared with isolates collectively referred from other DHBs.

3.2 CLONALITY OF FLUOROQUINOLONE-RESISTANT ISOLATES

A subset of 76 of the 297 isolates underwent MLST analysis (Table 3). This subset included all isolates resistant to ciprofloxacin and/or tetracycline and a representative sample of susceptible isolates.

Table 3. Multilocus sequence types by antimicrobial resistance pattern among *Campylobacter jejuni*, 2015

Resistance pattern	Number of isolates typed	MLST types (number of isolates of each type)
Ciprofloxacin and tetracycline co-resistant	40	ST6964 (29), ST50 (2), ST42(1), ST331 (1), ST354 (1), ST1145 (1), ST1707 (1), ST4053 (1), ST4056 (1), ST4526 (1), ST8077 (1)
Ciprofloxacin resistant only	6	ST2343 (2), ST50 (1), ST61 (1), ST1726 (1), ST7323 (1)
Tetracycline resistant only	3	ST48 (2), ST4254 (1)
Susceptible to ciprofloxacin and tetracycline	27	ST48 (4), ST2345 (4), ST50 (2), ST190 (2), ST354 (2), ST486 (2), ST45 (1), ST53 (1), ST257 (1), ST474 (1), ST520 (1), ST677 (1), ST1911 (1), ST2256 (1), ST2350 (1), ST3230 (1), ST3528 (1)

Thirty-one MLST types were identified among the 76 isolates. The majority of isolates (29/40; 72.5%) that were resistant to both ciprofloxacin and tetracycline were ST6964, and this type was identified only among *C. jejuni* with ciprofloxacin/tetracycline co-resistance. ST6964 belongs to MLST clonal complex 354 (CC354). Among the remaining 11 ciprofloxacin/tetracycline co-resistant isolates, there was one isolate whose MLST type also

belongs to CC354 (ST354). None of the MLST types of the isolates with other resistance patterns belong to CC354.

The geographic distribution of MLST types among the 40 *C. jejuni* isolates that had ciprofloxacin/tetracycline co-resistance is shown in Table 4. All ciprofloxacin/tetracycline co-resistant isolates from Northland DHB and Bay of Plenty/Lakes DHBs (albeit there was only one such resistant isolate from this area) were ST6964. In the two Wellington DHBs and the three Auckland DHBs, 92.3% (12/13) and 64.3% (9/14), respectively, of ciprofloxacin/tetracycline co-resistant isolates were ST6964. In contrast, each of the five co-resistant isolates from Canterbury DHB was a different MLST type, only one of which was ST6964, although the ST354 isolate was from Canterbury.

Table 4. Geographic distribution of multilocus sequence types among *Campylobacter jejuni* resistant to both ciprofloxacin and tetracycline, 2015

District Health Board(s)	Number (column % for ST6964) of ciprofloxacin- and tetracycline-resistant isolates of each type:											All
	ST6964	ST50	ST42	ST331	ST354	ST1145	ST1707	ST4053	ST4056	ST4526	ST8077	
Northland	5 (100)	0	0	0	0	0	0	0	0	0	0	5
Waitemata/Auckland/ Counties Manukau	9 (64.3)	2	0	0	0	0	0	1	1	0	1	14
Bay of Plenty/Lakes	1 (100)	0	0	0	0	0	0	0	0	0	0	1
Capital and Coast/ Hutt Valley	12 (92.3)	0	1	0	0	0	0	0	0	0	0	13
Canterbury	1 (20.0)	0	0	1	1	1	0	0	0	1	0	5
Southern	1 (50.0)	0	0	0	0	0	1	0	0	0	0	2
Total	29	2	1	1	1	1	1	1	1	1	1	40

4. DISCUSSION

In response to emerging data on a potential increase in fluoroquinolone resistance in *C. jejuni*, we performed a targeted period-prevalence survey using sentinel community laboratories in New Zealand. Overall, we found a fluoroquinolone resistance rate of 15%, a considerable increase on previously reported rates of fluoroquinolone resistance in *C. jejuni*. Of these fluoroquinolone-resistant isolates, 87% were co-resistant to tetracycline. There was a higher prevalence of fluoroquinolone/tetracycline co-resistance in the Auckland (31%) and Wellington (22%) areas compared with Northland (10%), Canterbury (9%), Southern (4%) and Bay of Plenty/Lakes (3%) DHBs. All isolates were susceptible to erythromycin.

The majority of the fluoroquinolone/tetracycline co-resistant isolates tested belonged to the ST6964 clone, while the MLSTs of representative fluoroquinolone-susceptible isolates were diverse and belonged to a range of MLST types. ST6964 is a rare ST among *C. jejuni,* with only one previously deposited ST6964 from China in the pubMLST database (last accessed 9 Sep 2015). The highly clonal nature of these co-resistant isolates, and the relatively rapid emergence among human populations in New Zealand (compared with historic rates of resistance) suggests a recent introduction from a common source. Concurrent investigations are underway to establish the genetic relatedness of these human cases to an emergent fluoroquinolone/tetracycline co-resistant ST6964 *C. jejuni* clone in poultry.

The emergence of this resistant clone in human populations has implications for surveillance and treatment of campylobacteriosis in New Zealand. Although all isolates tested in this survey were susceptible to erythromycin, the relatively high prevalence of fluoroquinolone resistance means that fluoroquinolone antimicrobials can no longer currently be 'taken for granted' as empiric treatment for campylobacteriosis in our setting. In this context, the proposed introduction of culture-independent diagnostic testing for enteric pathogens may have important implications for the surveillance of antimicrobial resistance in *Campylobacter*

(and other enteric pathogens), as the introduction of such methods may impact on the ability and capacity of diagnostic laboratories to routinely culture enteric pathogens [9]. Close liaison will be required between clinical and public health laboratories to ensure phased and responsible introduction of these tests.

In summary, this survey demonstrates a clear increase in fluoroquinolone resistance in isolates of *C. jejuni* from humans compared to historic data, largely driven by the emergence of a single clone, ST6964. Given the rapid emergence of this resistant clone, it is imperative that ongoing periodic surveillance of antimicrobial resistance in *Campylobacter* is performed to better track the emergence and possible further spread in the New Zealand setting. At present, the impact on public health is uncertain, but measures to control and mitigate the further spread of this clone would include appropriate 'source control', and increased public awareness of appropriate food hygiene in the prevention of campylobacteriosis.

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