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Rates of fluoroquinolone resistance in domestically acquired *Campylobacter jejuni* are increasing in people living within a model study location in Canada

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**Running Title:** Fluoroquinolone resistance in *Campylobacter jejuni*
Abstract: Antimicrobial resistance was evaluated in *Campylobacter jejuni* isolated from 1,291 diarrheic people over a 15 year period (2004-2018) in Southwestern Alberta, a model location in Canada with a high rate of campylobacteriosis. The prevalence of resistance to chloramphenicol, clindamycin, erythromycin, and gentamicin was low during the examination period (≤4.8%). Resistance to tetracycline remained consistently high (41.6-65.1%), and resistance was primarily conferred by plasmid-borne *tetO* (96.2%). Resistance rates to ciprofloxacin and nalidixic acid increased substantially over the examination period, with a maximal fluoroquinolone resistance (FQR) prevalence of 28.9% in 2016. The majority of *C. jejuni* isolates resistant to ciprofloxacin (93.9%) contained a C257T mutation within the *gyrA* chromosomal gene. Follow up with infected people indicated that the observed increase in FQR was primarily due to domestically acquired infections. Moreover, the majority of FQR *C. jejuni* subtypes (82.6%) were endemic in Canada, primarily linked to cattle and chicken reservoirs; 18.4% of FQR isolates were assigned to three subtypes, predominantly associated with cattle. Study findings indicate the need to prioritize FQR monitoring in *C. jejuni* infections in Canada, and to elucidate the dynamics of the emergence and transmission of resistant *C. jejuni* strains within and from cattle and chicken reservoirs. [197 words]

Keywords: Antimicrobial resistance, Human health risk, Zoonotic reservoirs, Cattle, One health.
Introduction

Campylobacteriosis is a disease of the intestine that can result in enteritis in both the small and large intestine, and colitis may be manifested as bloody diarrheal syndrome (Skirrow and Blaser 2000). Campylobacteriosis is one of the most prevalent bacterial enteric diseases in Alberta, and elsewhere in Canada and the world (Inglis et al. 2019; Kaakoush et al. 2015; Thomas et al. 2013). The genus *Campylobacter* currently contains ≥38 species (https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi), but the primary incitant of campylobacteriosis in people is *C. jejuni*, and to a lesser extent *C. coli*, *C. lari*, and *C. upsaliensis* (Platts-Mills et al. 2020). Rates based on classical microbiological diagnosis vary, but can exceed 100 cases per 100K (Baker et al. 2007). There is no standard detection method used for campylobacteria in stools (M’ikanatha N et al. 2012). Moreover, it is recognized that current diagnostic methods are not comprehensive (Inglis et al. 2019; Lastovica and le Roux 2000), and that many people afflicted with campylobacteriosis do not seek medical attention (Mead et al. 1999). Adjustments for under-reporting have estimated the true per capita rate of campylobacteriosis in Canada to be in the range of 450 cases per 100K (Thomas et al. 2013). The epidemiology of campylobacteriosis is enigmatic at present. The majority of cases of human illness incited by *C. jejuni* are sporadic (Silva et al. 2011); however, recent evidence has indicated that spatial and temporal case clusters (i.e. “mini-outbreaks”) may be important in the epidemiology of campylobacteriosis (Inglis et al. 2019; Joensen et al. 2018). Conventional thought is that poultry is a primary reservoir of *C. jejuni*, and that handling of and/or consumption of undercooked poultry are the principal mechanisms by which the pathogen is transmitted to people (Skarp et al. 2016). However, *C. jejuni* is readily found in an array of animal and environmental reservoirs (Whiley et al. 2013). It is recognized that consumption of unpasteurized milk and untreated surface water can result in campylobacteriosis (Clark et al. 2003; Pintar et al. 2017). Although the mechanisms of transmission are poorly understood, emerging evidence is implicating beef cattle as an important reservoir of *C. jejuni* infecting people, although not likely via consumption of meat (Inglis et al. 2020a; Inglis et al. 2020b).

Although campylobacteriosis is considered to be a self-limiting enteric disease, there are instances where illness is severe and medical intervention is necessary. Furthermore, infection by *C. jejuni* can lead to secondary sequelae (e.g. Guillain-Barré syndrome) (Keithlin et al. 2014) and campylobacteriosis is a risk factor for inflammatory bowel disease (Garcia Rodriguez et al. 2006) and irritable bowel syndrome (Marshall et al. 2006).

At present, the primary treatment option for acute campylobacteriosis that is available to physicians is the administration of antibiotics, primarily macrolides and fluoroquinolones (FQs) (Platts-Mills et al. 2020). Individuals with bloody stools, high fevers, symptoms lasting >1 week, worsening symptoms, along with neonates, pregnant women, and immunocompromised individuals rely on effective therapeutic intervention.

The possibility for increasing rates of treatment failure due to resistance development is a significant issue in the treatment of campylobacteriosis, and the World Health Organization (WHO) recently identified resistance
to FQ antibiotics in Campylobacter spp. to be a high risk issue worldwide (World-Health-Organization 2017). It is now recognized that there is considerable genetic diversity within C. jejuni, and that a subset of subtypes pose an increased risk to human health (Mullner et al. 2009; Sheppard et al. 2009; Wilson et al. 2008). A multitude of surveillance studies have reported on resistance rates in C. jejuni at national (Centers-for-Disease-Control-and-Prevention 2020; Public-Health-Agency-of-Canada 2020) and local levels, but relatively few have linked resistance to specific subtypes. Moreover, few studies to date have examined resistance in model study locations in an attempt to resolve antimicrobial resistance development in an epidemiological context (e.g. identification of primary reservoirs and transmission mechanisms). Thus, the overarching goal of the current study was to use a model study location to examine temporal resistance rates to antibiotics in C. jejuni recovered from diarrheic people, and to identify potential sources from which the bacterium originated.

Specific objectives were: (i) measure resistance to antibiotics (i.e. representing different families of antibiotics) in C. jejuni obtained from diarrheic humans living in Southwestern Alberta (SWA), Canada as a model location over a 15-year period; (ii) characterize the genetics of resistance; (iii) subtype all C. jejuni isolates; (iv) ascertain the relative importance of domestically versus internationally acquired infections in resistance development; (v) use the subtype data to identify important reservoirs from which resistant C. jejuni isolates are likely to have originated.

Materials and methods

Ethical statement

Approval to transfer Campylobacter isolates from the Chinook Regional Hospital (CRH) to Agriculture and Agri-Food Canada (AAFC) Lethbridge Research and Development Centre (LeRDC) was obtained from the University of Lethbridge Office of Research Ethics (Certificate of Human Subject Research #715) and the University of Alberta Research Ethics Office (Health Research Ethics Board #Pro00094238). Information that was transferred with the isolates was restricted to the isolate identifier and the year of isolation. No information that could reveal the identity of the individual originally submitting the stool sample for diagnosis was disclosed to research personnel. The AAFC LeRDC is licensed by the Public Health Agency of Canada to conduct research with risk group 2 pathogens, for which Campylobacter spp. are designated. Approval to transfer the isolates from the CRH to LeRDC was obtained from the AAFC and Alberta Health Services (AHS) biological safety officers. Campylobacteriosis is a reportable disease in Canada, and all individuals determined to be infected by a Campylobacter species are subject to a retrospective interview by an AHS Public Health Officer. Approval to access selected information from the AHS Disease Incident Exposure Questionnaire (i.e. conclusion on whether the infection was internationally or domestically acquired) was obtained from the University of Alberta Research Ethics Office (#Pro00094238). Given that no patient specific information was
released to research personnel, a requirement for informed consent was waived by the two ethics review committees.

**Model study location**

SWA is a region of mixed agriculture, and it is delineated by the Montana border to the south, and by the border with the Canadian province of British Columbia to the west (i.e. continental divide) (Inglis et al. 2019). The location was chosen as a model study site for the following reasons: (i) there are high rates of enteric disease, including campylobacteriosis, among people living in the region; (ii) it contains a significant rural population of people, with a ≈60:40 urban:rural divide; (iii) the human population is moderate in size (180,994 people in 2016), thereby facilitating directed analyses; (iv) there is a single public diagnostic facility servicing the region facilitating comprehensive acquisition of *Campylobacter* isolates; (v) there is a high density of livestock production with ≈2.7M poultry, ≈0.39M pigs, and ≈1.1M cattle with 51% being beef cattle in confined feeding operations (CFOs) (Alberta-Government 2014); (vi) there is a single prominent watershed; (vii) there is a spatial gradation of human agricultural activity from west (mountains) to east (cultivated short grass prairie); and (viii) intensive research targeting *Campylobacter* has been conducted in the region.

**Isolation of *Campylobacter* species**

Human stool samples were processed immediately upon arrival at the CRH as described previously (Inglis et al. 2019). Briefly, a subsample from each stool was streaked onto Campy CVA Agar (Becton Dickinson, Oakville, ON); this medium consists of *Brucella* agar supplemented with cefoperazone (20 mg/L), vancomycin (10 mg/L), amphotericin B (2 mg/L) and 5% defibrinated sheep blood. Cultures were incubated at 42°C in an anaerobic atmosphere consisting of 10% CO₂, 10% H₂ and 80% N₂ (Inglis et al. 2019). Colonies exhibiting characteristic *Campylobacter* morphology were isolated, and isolates were identified using a VITEK® (bioMérieux Inc. Canada, St-Laurent. QC). Biomass of each isolate was stored at -80°C at the CRH until transferred to AAFC.

**Confirmation of *Campylobacter* identification**

After transfer to AAFC, isolates were streaked for purity, accessioned in the LeRDC Intestinal Bacterial Collection (IBaC), and stock cultures were lyophilized and/or stored over liquid nitrogen. Actively growing isolates were tested for their ability to hydrolyze hippurate using a disc method (Dalynn Biologicals, Calgary, AB) (1). Genomic DNA of isolates was extracted using an AutoGen 740 robot (Holliston, MA) according to the manufacturer’s protocol, and genomic DNA was subjected to diagnostic PCR for *Campylobacter* genus (16S rRNA gene) *C. jejuni* (*hipO* and *ipxA* genes) and *C. coli* (16S rRNA gene) (Inglis et al. 2019; Inglis and Kalischuk 2003; Klena et al. 2004; Linton et al. 1997). Where warranted, the near complete 16S rRNA and *cpn60* genes were sequenced, and sequences were compared to those in GenBank (National Center for Biotechnology Information, Bethesda, MD) using BlastN.
Susceptibility to antimicrobials

The minimum inhibitory concentrations (MIC) to ciprofloxacin, chloramphenicol, clindamycin, erythromycin, gentamicin, nalidixic acid, and tetracycline were determined using the agar dilution methodology according to Clinical and Laboratory Standards Institute standards (Clinical-and-Laboratory-Standards-Institute. 2006) with the exception that the Mueller-Hinton agar (Difco BD, Mississauga, ON) was not amended with 5% defibrinated horse blood. Campylobacter cells were harvested from the surface of the medium after 48 h growth in a microaerobic atmosphere (5% O₂, 3% H₂, 10% CO₂, and 82% N₂) at 37°C. Cells were suspended in sterile NaCl (0.075%), and the density of cells was adjusted to 0.5 McFarland standard using a spectrophotometer (Genesys 20, Thermo Scientific, Rockford, IL; 625nm). Aliquots (300 µl) of the saline suspension were pipetted into the seeding wells of a Cathra replicator (Oxoid, Inc.). Freshly prepared plates of Mueller-Hinton agar amended with antimicrobial agents were then inoculated using 1-mm pins in the inoculating head of the replicator. Cultures were incubated microaerobically at 37°C for 48 h, and the MIC was defined as the lowest concentration resulting in complete inhibition of visible growth on the medium. Campylobacter jejuni (ATCC 33560) was utilized as a quality control strain. The breakpoint value for resistance (intermediate resistance breakpoint in parentheses) was \( \geq 4 \) µg/mL (2 µg/mL) for ciprofloxacin, \( \geq 32 \) µg/mL (16 µg/mL) for chloramphenicol, \( \geq 8 \) µg/mL (4 µg/mL) for clindamycin, \( \geq 32 \) µg/mL (16 µg/mL) for erythromycin, \( \geq 8 \) µg/mL (4 µg/mL) for gentamicin, \( \geq 64 \) µg/mL (32 µg/mL) for nalidixic acid, and \( \geq 16 \) µg/mL (8 µg/mL) for tetracycline (National-Animal-Health-Monitoring-System 2005). MICs for all isolates exhibiting resistance to erythromycin and ciprofloxacin were repeated.

Genetics of tetracycline resistance

The presence of tetB, tetC, tetL, tetM, tetO, tetQ, and tetW in 692 arbitrarily-selected C. jejuni isolates resistant to tetracycline were examined by PCR. In addition, 21 C. jejuni isolates that were not resistant to tetracycline were included as control strains. Briefly, each 20 µl PCR reaction contained 2 µl of DNA (20-50 ng), 2 µl of 10X PCR buffer, 0.4 µl of 10 mM dNTP, 0.4 µl of 25 mM MgCl₂, 1 µl each of forward and reverse primers (10 µM), 2 µl of BSA (1 mg/mL; Life Technologies Inc., Burlington, ON), 0.1 µl of HotStar Taq polymerase (Qiagen Inc., Toronto, ON), and 11.1 µl of Optima water (Fisher Scientific, Ottawa, ON). Previously published primers and annealing temperatures (Ta) were used (Peak et al. 2007; Wu et al. 2010). Cycle conditions were one cycle at 95°C for 15 min; 35 cycles at 94°C for 30 s, at the appropriate Ta for 1 min, and at 72°C for 1 min; one cycle at 72°C for 5 min; and a hold at 4°C. Amplicons were visualized by capillary electrophoresis using a QIAxcel (Qiagen Inc.). For positive controls, plasmids were constructed by amplifying the target gene from a positive intestinal digesta sample, ligating the product into a pDRIVE Cloning Vector (Qiagen Inc.) and transforming it into QIAGEN EZ Competent Cells (Qiagen Inc.) according to manufacturers’ protocols (Inglis et al. 2020b). Plasmids were sequence verified for the insert.
Genetics of fluoroquinolone resistance

The genetics of resistance to FQs was examined in C. jejuni that were resistant to ciprofloxacin. Arbitrarily selected C. jejuni isolates that were not resistant to either nalidixic acid or ciprofloxacin were included as control strains. Amplification and sequencing of the quinolone resistance determining region (QRDR) of the gyrA gene was conducted using primers modified from those described by Jesse et al. (Jesse et al. 2006). Modifications to the primers were made to encompass more C. jejuni strains based on new sequence data in the National Center for Biotechnology Information; the software program Geneious (Biomatters, Inc., San Diego, CA) was used. The newly designed primers were GyrAFcj (5’-GCTCTTGTTTTAGCTGATGCA-3’) and GyrARcj (5’-TTGTCGCCCATACCTACAGCTA-3’). The PCR reaction chemistry was the same as above. The conditions for PCR amplification were 1 cycle at 95°C for 15 min; 35 cycles at 94°C for 30 s, 56°C for 60 s, and 72°C for 60 s; and extension for 10 min at 72°C. The resulting gyrA amplicons were sequenced in house using an ABI 3130 Genetic Analyzer (Life Technologies, Carlsbad, CA) with POP7 and BigDye 3.1 chemistry. Further sequencing was done off site by Macrogen USA (Rockville, MD). Sequence alignments and analyses, including the identifications of mutations that would result in amino acid substitutions (e.g. Thr86Ile), were completed using Geneious (Biomatters, Inc.).

In isolates that did not exhibit mutations that would confer an amino acid substitution in the gyrA gene, other genetic resistance determinants were investigated. The presence of plasmid-mediated quinolone resistance (PMQR) was investigated with methods designed for non-Campylobacter bacteria (Cattoir et al. 2007; Guillard et al. 2011; Hansen et al. 2005; Kim et al. 2009; Park et al. 2006); slight modifications to primers were made to enhance comprehensiveness. In addition, potential mutations in the cmeABC operon were investigated (Lin et al. 2005).

Comparative genomic fingerprinting

All C. jejuni isolates were fingerprinted using the 40 locus comparative genomic fingerprinting (CGF40) method (Taboada et al. 2012). Briefly, eight PCR reactions were performed on each isolate; each five-plex reaction mix contained 1 U Fisherbrand Taq DNA polymerase (Fisher Scientific, Nepean, ON), 1X buffer, 2.5 mM MgCl₂, 0.2 mM of each deoxynucleoside triphosphate (dNTP), 0.12 to 0.74 µM of the 10 primers, and 1 µl of DNA template (20 to 100 ng) in a 25 µl reaction mix. An EP Gradient Mastercycler (Eppendorf, Mississauga, ON) was used, and PCR conditions were: an initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s; and a final extension step at 72°C for 5 min.

Amplicons were resolved using a QIAxcel high throughput capillary electrophoresis system with DNA screening cartridges (Qiagen Inc.) using the AM320 separation method and a 20 s injection time. The 15 to 3000 base pair alignment marker and a 100 to 2500 bp size ladder were used as size standards (Qiagen Inc.). Data were
analyzed and visualized using the BioCalculator v3.2 software (Qiagen Inc.), and converted to binary values (i.e. where a ‘0’ represented the absence of the ancillary gene locus, and a ‘1’ indicated its presence).

**Data analysis**

Temporal changes in the prevalence of resistance in *C. jejuni* were determined using a Fisher’s exact test (Kim 2017) in Statistical Analyses Software (SAS; Cary, NC). The dynamic fit wizard of SigmaPlot (Systat Software Inc., Chicago, IL) was used to plot and analyze linear relationships between year and the prevalence of resistance. Resistant and susceptible *C. jejuni* isolates were assigned to CGF subtype clusters at 100% and 95% levels of resolution using the simple matching analysis coefficient with unweighted pair group method with arithmetic mean (UPGMA) clustering in Bionumerics (version 6.6, Applied Maths, Austin, TX). Population structures were visualized as Minimum Spanning Trees (MSTs) using Bionumerics (version 6.6, Applied Maths). Venn diagrams of *C. jejuni* subtypes were generated using pivot tables at a 95% level of resolution, including subtypes resistant to ciprofloxacin, nalidixic acid, and tetracycline. Cluster richness and abundance were used to calculate the Shannon diversity index (i.e. for resistant subtypes), and Hutcheson’s t-test was used to test the significance of differences in subtype diversity (Hutcheson 1970). All *C. jejuni* isolates resistant to ciprofloxacin were queried against isolates within the Canadian *Campylobacter* Comparative Genomic Fingerprinting database (C3GFdb) to ascertain primary host associations, and frequency and geographical distribution of isolation (i.e. endemicity) in Canada. The C3GFdb, which is curated by the National Microbiology Laboratory (Public Health Agency of Canada) currently includes >30,000 *C. jejuni* isolates representing >5,000 subtypes. Metadata included with each isolate includes the location, date, and source of isolation; the C3GFdb contains data for >4,700 isolates from human beings, >6,000 isolates from cattle, >7,200 isolates from poultry, >4,300 isolates from water, and >3,400 isolates from other sources (e.g. swine, other miscellaneous livestock, wild birds, wild mammals, and miscellaneous environmental matrices). A significant number of *C. jejuni* isolates from SWA have been accessioned into the database. Whether infections were domestically or internationally acquired (e.g. “traveller’s diarrhea”) was determined from the follow up interview with patients conducted by AHS (i.e. information on travel history during the incubation period).

**Results**

*Campylobacter jejuni* was the most frequent species recovered from stools of diarrheic people in SWA. Thirteen hundred and fifty four *Campylobacter* isolates were recovered from stool samples submitted to the diagnostic facility located at the CRH in Lethbridge from 2004 to 2018. The most common species recovered was *C. jejuni* (n=1,291; 95.3%) followed by *C. coli* (n=57; 4.2%), *C. lari* (n=3; 0.2%), *C. fetus* (n=1; 0.1%), *C. subantarcticus* (n=1; 0.1%), and *C. upsaliensis* (n=1; 0.1%). The average number of people infected by *C. jejuni* per year was 86 (± 3.4), and ranged from 65 in 2011 to 109 in 2006 (Fig. 1A).
Resistance to tetracycline but not to clindamycin, chloramphenicol, erythromycin, or gentamicin was common in *Campylobacter jejuni*

Resistance to antimicrobial agents was assessed in 1,287 *C. jejuni* recovered from diarrheic people over a 15 year period from 2004 to 2018. Rates were determined using the agar dilution method; four isolates died in storage and resistance was not assessed. Annual rates of resistance to chloramphenicol, clindamycin, erythromycin, and gentamicin remained low (≤4.8%) throughout the examination period (Table S1-S4). In contrast, the prevalence of resistance to tetracycline did not change over time (P=0.242), and remained constantly high ranging from 41.6% in 2012 to 65.1% in 2016 (Fig. 1B; Table S5). Although tetracycline resistance rates did not change appreciably over the examination period, a shift in the distribution of isolates with a MIC of 128 and ≥256 µg/mL was observed after 2009 (Fig. S1). The majority of isolates resistant to tetracycline (96.2%) were positive for the tetO resistance determinant. None of the resistant isolates carried tetB, tetC, tetL, tetM, tetQ, or tetW. None of the tetracycline susceptible *C. jejuni* isolates examined carried tetB, tetC, tetL, tetM, tetO, tetQ, or tetW.

Resistance to ciprofloxacin and nalidixic acid increased in *Campylobacter jejuni* over the examination period

Over the 15-year study period, a substantive increase in resistance to ciprofloxacin (P<0.001) and to nalidixic acid (P=0.004) was observed in *C. jejuni* isolates recovered from diarrheic people in SWA (Fig. 1C-D). The linear equation fit to the ciprofloxacin resistance data (r²=0.71; y = -3075.32 + 1.5358x) showed a ten times increase in FQ resistance from 2.4% in 2004 to 23.9% in 2018, with a maximal resistance prevalence of 28.9% in 2016. Although a less robust linear relationship was observed, resistance to nalidixic acid mirrored that of resistance to ciprofloxacin, with the equation fit to the data (r²=0.49; y = -2607.83 + 1.3041x) showing an increase in resistance from 5.6% in 2004 to 23.8% in 2018. An examination of the ciprofloxacin MIC distributions by year revealed an upward shift in the frequency of isolates with a MIC of 16 µg/mL, and to a lesser extent of isolates with an MIC ≥32 µg/mL after 2009 (Fig. 2; Table S6). A temporal shift in the MIC distribution of *C. jejuni* isolates resistant to nalidixic acid after 2009 was similarly observed (Fig. S2; Table S7). Overall, 10.5% of the *C. jejuni* isolates resistant to ciprofloxacin were also resistant to nalidixic acid and tetracycline (Table S8). Multiple resistance to other antimicrobial agents was not frequently observed (≤0.5%).

The observed increase in resistance to ciprofloxacin was attributed to domestically acquired infections

Follow up interviews with people infected by *C. jejuni* that were resistant to ciprofloxacin indicated that rates attributed to internationally acquired infections (i.e. traveller’s diarrhea) remained constant, and the slope (0.125 ± 0.181) of the temporal relationship did not differ (P=0.507) from zero (Fig. 1C). Moreover, infections that could not be verified as either domestically or internationally acquired (e.g. due to an inability to contact the afflicted individual) remained consistently low. Thus, follow up interviews indicated that the
observed increase in infections with *C. jejuni* resistant to ciprofloxacin was due to infections that occurred within Canada.

**Resistance to ciprofloxacin corresponded to mutations in the *Campylobacter jejuni* gyrA gene**

The majority (n=155; 93.9%) of the 165 *C. jejuni* isolates resistant to ciprofloxacin carried the C257T mutation on the *gyrA* gene conferring an amino acid substitution (i.e. Thr86Ile) (Table 1). The 10 resistant *C. jejuni* isolates that did not carry the C257T mutation all carried other SNPs in the *gyrA* gene (n=2 for A21G; n=2 for A64G; n=4 for T72C; n=6 for C243T; n=9 for T357C; n=5 for C360T; n=1 for C408A; n=7 for C471T; and n=5 for T483C). None of the 12 *C. jejuni* isolates that possessed intermediate resistance to ciprofloxacin (i.e. MIC of 2 µg/mL) carried the C257T mutation, nor did any of the 46 *C. jejuni* isolates that were susceptible to ciprofloxacin (i.e. MIC ≤1 µg/mL). All the *C. jejuni* isolates that carried the Thr86Ile mutation were also resistant to nalidixic acid. There was no relationship between the number of mutations in the *gyrA* gene with subtype (Fig. S3). In resistant isolates not exhibiting the Thr86Ile mutation in the *gyrA* gene, plasmid-mediated quinolone resistance was not detected, and a mutation in the *cmeABC* operon indicative of an efflux pump mechanism of resistance was observed in only one *C. jejuni* strain isolated from a human in 2006 (data not shown). Nonetheless, the likely mechanism of FQ and/or quinolone resistance could be ascertained for the majority of resistant isolates, with only a small number of Thr86Ile mutation-negative isolates undefined.

**Considerable genotypic variation was observed in *Campylobacter jejuni* recovered from diarrheic people**

All of the *C. jejuni* isolates recovered from diarrheic people in SWA since 2004 were genotyped by comparative genomic fingerprinting (i.e. CGF40; a method that targets 40 accessory loci located throughout the *C. jejuni* chromosome), and queried against genotypic data within the C3GFdb. Considerable genotypic variation was observed among the isolates (Fig. 3). Four prominent CGF subtypes (i.e. CGF 0044.003.001, 0169.001.002, 0269.004.001, and 0695.006.001) comprised 20.0% of the isolates (Fig. 3; black stars). These four *C. jejuni* subtypes are common across Canada (ranked as the sixth, first, twenty-third, and second most common subtypes within the C3GFdb, respectively (Table 2). Although the CGF subtypes 0044.003.001, 0169.001.002, 0269.004.001, and 0695.006.001 are associated with a variety of hosts, cattle is their primary reservoir in Canada. An examination of subtype prevalence by year revealed the absence of a temporal bias (Fig. S4).

**Campylobacter jejuni** subtypes containing isolates resistant to ciprofloxacin exhibited lower diversity than isolates resistant to nalidixic acid and tetracycline

Ten subtype clusters (95% resolution) contained the majority of *C. jejuni* isolates (68.3%) resistant to ciprofloxacin (Fig. 3). This contrasted with *C. jejuni* subtypes resistant to tetracycline, which were more widely distributed (Fig. 4). Although the subtype map of isolates resistant to nalidixic acid was similar to ciprofloxacin (Fig. S5), the diversity of subtypes was higher for nalidixic acid (H=4.61; P=0.010) and for tetracycline (H=5.23;
P<0.001) as compared to ciprofloxacin (H=4.24) (Fig. S6). In total, 656 CGF subtypes were identified (100% resolution), and 118 subtypes contained isolates that were resistant to ciprofloxacin (18.0%) (Fig. 5). One hundred and fourteen subtypes contained isolates that were resistant to both ciprofloxacin and nalidixic acid, and 92 subtypes contained isolates that were resistant to both ciprofloxacin and tetracycline.

The majority of Campylobacter jejuni isolates resistant to ciprofloxacin represent subtypes that are endemic in Canada

Most of the C. jejuni isolates resistant to ciprofloxacin (67.1%) were assigned to 51 subtypes that have been recovered previously in Canada (Table 2). Of these subtypes, 27 are common (i.e. C3GFdb rank ≤150), and contained 48.5% of the isolates resistant to ciprofloxacin. In total, 41 subtypes contained isolates that were concluded to be acquired during travel outside of North America through follow-up interviews. A majority of these subtypes (58.5%) clustered separately from subtypes commonly observed in Canada (Fig. 3; white asterisks). Moreover, 53.7% of the isolates deemed to be from internationally acquired infections by C. jejuni were novel to the C3GFdb.

A majority of domestically acquired Campylobacter jejuni isolates resistant to ciprofloxacin were associated with cattle reservoirs

Of the C. jejuni subtypes that contained isolates resistant to ciprofloxacin and are commonly circulating in Canada, all are pan-Canadian, and are associated with cattle and/or chicken reservoirs (Table 2). Some subtypes were prominently associated with cattle (e.g. 0044.003.001, 0092.001.003, 0169.001.002, 0695.006.001, 0269.004.001, 0949.001.002, 0982.001.002), whereas other were associated predominately with chickens (e.g. 0018.001.002, 0117.001.001, 0173.010.002, 0253.004.001). CGF subtypes 0044.003.001, 0169.001.002, 0269.004.001, and 0695.006.001 were of particular relevance as C. jejuni isolates within these subtypes were commonly recovered from diarrheic people in the current study. These three subtypes are common in Alberta (i.e. SWA). CGF subtypes 0169.001.002 and 0269.004.001 are associated with multilocus sequence typing (MLST) clonal complex (CC) CC-21, and subtype 0695.006.001 is associated with CC-61. CGF subtype 0044.003.001 has no corresponding CC, but is strongly associated with Sequence Type (ST) 922.

Discussion

Infection by C. jejuni is responsible for significant morbidity in people living in Canada and elsewhere, and the development of resistance to antimicrobial agents, which are administered as the primary treatment for severe cases of campylobacteriosis is an ever increasing concern (Platts-Mills et al. 2020). As a result, ad hoc and formal surveillance programs for antimicrobial resistance in C. jejuni are conducted in most countries, including Canada (FoodNet-Canada 2013; Otto et al. 2019; Public-Health-Agency-of-Canada 2020) and the United States (Centers-for-Disease-Control-and-Prevention 2020; Geissler et al. 2017). In the current study, we applied a model agroecosystem approach to monitor changes in resistance to antibiotics, and to identify
important reservoirs from which resistant *C. jejuni* are likely to originate, and to glean information on potential transmission mechanisms. The study location in Canada, possessed a number of positive attributes. These include high rates of campylobacteriosis (i.e. >100 diagnosed cases per 100K) (Inglis et al. 2019), and the presence of a single public diagnostic facility located at the CRH that services the region. Furthermore, there is a moderately sized human population (<200K), representing a mixture of urban and rural residents (i.e. a ≈60:40 urban: rural divide), and there are high densities of livestock (i.e. poultry, pigs, and cattle) (Alberta-Government 2014), which are a significant contributor to the economy of the region. Importantly, the modest human population size of SWA coupled with the comprehensive molecular surveillance undertaken in the current study would be expected to reduce data noise. The region also has an accumulation of researchers, clinicians, public health officials, and livestock producers/processors that have devoted >15 years employing a regional ‘One Health’ approach to elucidate the molecular epidemiology of campylobacteriosis (i.e. to identify important reservoirs and transmission routes of clinically relevant subtypes) towards mitigation of *C. jejuni* with the overarching goal of reducing morbidity in people. The establishment of the C3GFdb (Schleihauf et al. 2017; Taboada et al. 2012), which currently contains significant contributions from the region, is a foundation of the research initiative in SWA.

Of the isolates of *C. jejuni* from ≈1,300 diarrheic people in SWA that we examined from 2004 to 2018, the prevalence of resistance to chloramphenicol, clindamycin, erythromycin, and gentamicin was consistently less than 5%. This is consistent with the low rates of resistance to these antimicrobials observed in clinical *C. jejuni* isolates in Northern Alberta from 1999 to 2002 (n=203) (Gibreel et al. 2004), in Saskatchewan from 1999 to 2006 (n=1200) (Otto et al. 2019), in Ontario from 2002 to 2004 (n=121) (Deckert et al. 2013), in Ontario from 2011 to 2013 (n=135) (Riley et al. 2015), in Quebec from 1998 to 2001 (n=245) (Gaudreau and Gilbert 2003), in Quebec from 2002 to 2013 (n=440) (Gaudreau et al. 2014), and in the United States from 1999 to 2020 (Centers-for-Disease-Control-and-Prevention 2020). Macrolides are a front line drug used to treat campylobacteriosis (Platts-Mills et al. 2020). The low rates of resistance to the macrolide, erythromycin in SWA is a positive finding, and consistent with low rates of resistance in other locations in North America. In contrast, we observed that the prevalence of resistance to tetracycline was consistently high, ranged from 42 to 65% in SWA. This is comparable to resistance rates to tetracycline observed elsewhere in Alberta (Gibreel et al. 2004), Canada (Otto et al. 2019), and the United States (Centers-for-Disease-Control-and-Prevention 2020; Whitehouse et al. 2018b). The majority of the *C. jejuni* isolates resistant to tetracycline in SWA were found to carry tetO (96.2%), which has been reported previously (Webb et al. 2018). TetO is a paralog of the translational GTPase that actively removes tetracycline from the ribosome small subunit in a GTP-hydrolysis-dependent manner (Burdett 1996; Connell et al. 2003; Li et al. 2013). This determinant is commonly carried on plasmids in *C. jejuni* (Taylor 1986), which can be horizontally transferred *in vivo* (Avrain et al. 2004), facilitating

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their rapid and widespread dissemination. None of the \textit{C. jejuni} infecting people in SWA carried \textit{tetB, tetC, tetL, tetM, tetQ}, or \textit{tetW}, and these determinants are not thought to be carried by \textit{C. jejuni}, although they are common within other enteric bacteria (Barbosa et al. 1999; Inglis et al. 2020b; Scott et al. 2000).

Fluoroquinolones are frontline antibiotics for treating campylobacteriosis (Platts-Mills et al. 2020), although they are typically considered to be a secondary therapy to macrolides as a result of resistance development. The WHO has identified resistance to FQs and need for replacement drugs to treat individuals infected with \textit{C. jejuni} as high priority (World-Health-Organization 2017). We observed that rates of resistance to the FQ, ciprofloxacin, conspicuously increased over the examination period in SWA, with relatively low rates in 2004 to 2008 (≤ 8%) increasing to a maximal resistance prevalence of 29% in 2016. This is much higher than historical rates observed in other jurisdictions in Canada. For example, rates of ciprofloxacin in Saskatchewan from 1999 to 2006 ranged from 16% to 13%, respectively; however the degree to which domestically acquired infections were responsible was not determined (Otto et al. 2019). Examination of fluoroquinolone resistance (FQR) rates in \textit{C. jejuni} in the United States from 2004 to 2012 (n=8,219 isolates) revealed that 23.4% of \textit{C. jejuni} isolates were resistant, but 49% of the cases of resistant infections were deemed to be internationally acquired (Wieczorek 2009). Thus, rates of FQR resulting from domestic infections only marginally increased over the examination period (i.e. from 13% in 2004-2009 to 16% in 2010-2012). Data presented on the National Antimicrobial Resistance Monitoring System (NARMS) website for enteric bacteria indicated that FQR in \textit{C. jejuni} has increased dramatically since 2012, with a prevalence of FQR of 29% in 2018 and 40% in 2019 (Centers-for-Disease-Control-and-Prevention 2020), although it is not known to what degree this increase was due to domestically acquired infections. In central Alberta (Calgary region), Johnson et al. (Johnson et al. 2008) reported that ≈29% of 196 \textit{C. jejuni} from diarrheic people from 2004 to 2005 (n=57 isolates) were resistant to FQs, but the majority of the FQR isolates examined (78%) were linked to foreign travel. The high degree of FQR from internationally acquired infections that was observed by Johnson et al. (Johnson et al. 2008) emphasized the importance of determining whether the high rates of FQR observed in SWA were similarly attributable to traveller’s diarrhea.

Rates of resistance to the quinolone, nalidixic acid in \textit{C. jejuni} in SWA were slightly higher than to ciprofloxacin in \textit{C. jejuni} isolates; however, temporal resistance trends were comparable among the two agents. This is consistent with observations in the United States (Centers-for-Disease-Control-and-Prevention 2020). Moreover, the mechanisms conferring resistance to quinolones and FQs in \textit{C. jejuni} are similar, namely single nucleotide polymorphism mutations in the QRDR of the \textit{gyrA} gene (Wieczorek et al. 2013). In this regard, the majority of \textit{C. jejuni} isolates that were resistant to ciprofloxacin and nalidixic acid in SWA (94%) contained a C257T mutation within the \textit{gyrA} chromosomal gene. Others have observed a similar correlation between the C257T mutation and FQR, and the presence of this mutation is being used to predict phenotypic resistance
from genomic information (Zhao et al. 2016). In addition to the C257T mutation, we observed a trend for a larger number of single nucleotide polymorphisms within the gyrA gene for C. jejuni isolates exhibiting a MIC of \( \geq 32 \mu g/mL \) ciprofloxacin (i.e. ranging from 3.8 to 5.2 SNP mutations). Of these SNP mutations, only one confers an amino acid substitution (i.e. A64G). Although the mechanisms are not well understood, additional mutations within the gyrA gene have been reported to be associated with increased FQR (Wieczorek and Osek 2013). We did not investigate this in FQR isolates, and a mutation in the intergenic cmeR-cmeABC intergenic region in conjunction with the C257T mutation within the gyrA gene has previously been linked to higher MIC values (Yang et al. 2017). An examination of the ten FQR C. jejuni isolates that did not possess the C257T mutation in the current study, revealed that none of these isolates exhibited PMQR or mutations in the cmeABC operon; CmeABC is a resistance-nodulation-division type of efflux pump that contributes to resistance to a variety of antimicrobial agents in Campylobacter (Lin et al. 2005). Thus, the mechanism conferring FQR in these isolates was not identified.

To determine if the increasing FQR rates observed in SWA were due to internationally acquired infections, infected individuals were interviewed following the standard protocol used by AHS; in Canada campylobacteriosis is a reportable disease mandating follow up with afflicted individuals. Examination of travel information indicated that international travel did not disproportionately contribute to the increased FQR rates that we observed, and that increased resistance due to domestically acquired infections was responsible, accounting for 83% of infections. This contrasts conspicuously with the findings of Johnson et al. (Johnson et al. 2008) in an adjacent region in Alberta in 2004 to 2005. Campylobacter jejuni is a genetically diverse bacterium, and to further examine the origin of FQR C. jejuni infecting people in SWA, isolates were genotyped by CGF40 (Taboada et al. 2012) in order to place resistant genotypes in the context of the wider population structure. It is noteworthy, the CGF method was found to be more discriminatory than MLST for the detection of clusters of C. jejuni cases (Clark et al. 2012), and the CGF method has previously been used to elucidate the molecular epidemiology of the bacterium (Hodges et al. 2019; Inglis et al. 2019; Mutschall et al. 2020; Thepault et al. 2018). We observed that the majority of CGF subtypes deemed to be internationally acquired clustered separately from subtypes from cases that are endemic within the region, supporting the conclusion from disease questionnaire assessments that the majority of FQR C. jejuni isolated from diarrheic people in SWA were domestically acquired. Using the C3GFdb, we also examined the molecular epidemiology of C. jejuni isolates in an attempt to glean information on reservoirs and possible transmission mechanisms of FQR C. jejuni in SWA. A majority of the prevalent subtypes among FQR C. jejuni isolates from diarrheic people in SWA are predominantly associated with beef cattle. A prominent characteristic of SWA is the high density of beef cattle (\( \approx 1,166K \)) in the region (Alberta-Government 2014). Beef cattle are colonized by diverse subtypes of C. jejuni, including clinically relevant subtypes (Inglis et al. 2020a), and they chronically shed the bacterium in
large quantities in their feces (Inglis et al. 2004). The majority of beef cattle in North America, including in SWA, are finished in CFOs (Alberta-Government 2014). Before December 2018, the continuous administration of antimicrobial agents at low doses in the feed of beef cattle, including tetracyclines and macrolides was permitted (i.e. as antimicrobial growth promoters; AGPs) (Public-Health-Agency-of-Canada 2019). We have previously observed the rapid proliferation of resistance to tetracycline in beef cattle within CFOs, presumable due to selection pressure and the close proximity of cattle to one another, which facilitates inter-animal transmission (Inglis et al. 2020a; Inglis et al. 2020b; Webb et al. 2018). The administration of FQs to cattle in North America is limited to short-term therapeutic administration against bovine respiratory disease (BRD) (Plumb 2011). Relatively limited research has comprehensively examined FQR in C. jejuni associated with beef cattle in CFOs. Most evaluations conducted to date have observed low rates of FQR (Englen et al. 2005; Inglis et al. 2005; Rao et al. 2010). An exception is the snapshot study conducted by Tang et al. (Tang et al. 2017) in the United States who observed conspicuously higher rates of FQR in C. jejuni in a study conducted from 2012 to 2013 (36%). It is noteworthy that this study was conducted across five states with multiple CFOs per state, yet a relatively small number of C. jejuni isolates were examined in total (n=320; five randomly selected isolates per CFO). This may have biased the authors’ conclusion of elevated FQR. However, it is also possible that the therapeutic administration of enrofloxacin contributed to the proliferation of FQR C. jejuni observed, although this was not specifically ascertained in the study. In this regard, even short-term exposure to FQs can result in resistance development in C. jejuni (McDermott et al. 2002) and C. coli (Delsol et al. 2004). However, subcutaneous and oral administration of enrofloxacin (i.e. mimicking treatment of BRD in beef cattle) did not result in FQR in C. jejuni NCTC 11168 in a murine colonization model (Inglis et al. 2018). It is noteworthy that this study did not address FQR development in different subtypes, and limited research to date has examined resistance at a subtype level of resolution in C. jejuni. In this regard, a significant increase in the prevalence of FQR in C. jejuni subtypes longitudinally recovered from beef cattle independent of FQ was observed in a recent study (Webb et al. 2018), suggesting that non-FQ factors may select for FQR resistance. Notably, the two C. jejuni CGF subtypes isolated from beef cattle for which significant FQR developed were 0169.001.002 and 0695.006.001, which correspond to human clinical subtypes that exhibited high levels of FQR in the current study. This further supports beef cattle as an important reservoir of clinically relevant subtypes of FQR C. jejuni.

In a number of countries, FQ use is common in livestock production, and relatively high rates of FQR are observed in C. jejuni recovered from animals and meat (Whitehouse et al. 2018a). It is thought that transmission of FQR C. jejuni originating from animals to people contributes to the high rates of clinical FQR observed in these countries (Whitehouse et al. 2018b). Moreover, infection of people by FQR bacteria during travel to these countries with subsequent diagnosis upon return to Canada or the United States contributes to the high rates of FQR infections in individuals with traveller’s diarrhea (Dalhoff 2012). In the United States, FQ
use in poultry was banned in 2005, but rates of FQR C. jejuni associated with poultry have not decreased appreciably, ranging from 10 to 30% (Whitehouse et al. 2018b). In Canada, FQ use in poultry is restricted to therapeutic use with a prescription from a licensed veterinarian (Chicken Farmers of Canada; https://www.chickenfarmers.ca/faq/are-category-1-antibiotics-completely-banned/). Examination of FQR rates in Campylobacter from poultry in Canada via on-going surveillance programs has consistently shown resistance to be low, although some regional differences in FQR are observed (Drame et al. 2020; Public-Health-Agency-of-Canada 2020). It is now recognized that not all subtypes of C. jejuni are clinically relevant; for example, 80 of 1,199 chicken-related CGF subtypes (6.7%) account for 47% of human clinical cases in Canada (our unpublished data). As indicated above, many of the FQR C. jejuni infecting people in SWA were associated primarily with beef cattle in SWA, and mounting evidence is implicating cattle as an important reservoir of clinically relevant C. jejuni (Inglis et al. 2019). However, the epidemiology cattle-borne C. jejuni remains enigmatic, although direct transmission via occupational contact has been identified as a risk factor in SWA (Hasselback 2002). Evidence indicates that the infection risk posed by consumption of beef contaminated with C. jejuni is low in SWA (Inglis et al. 2020a; Inglis et al. 2020b), suggesting that alternate transmission mechanisms are responsible. It is recognized that certain clinically relevant subtypes of C. jejuni are associated with multiple non-human reservoirs, including beef cattle and chickens, and these subtypes (e.g. CC-21) have been designated as “generalists” (Dearlove et al. 2016; Gripp et al. 2011). Although clinically relevant subtypes of C. jejuni resistant to FQs were predominately associated with beef cattle in the current study, including CGF subtypes that correspond to CC-21, they have also been observed in broiler chickens suggesting a possible transmission route linking cattle to human beings. Outbreaks of C. jejuni in broiler barns are a relatively rare event, and our research has shown that a single or restricted number of C. jejuni subtypes are responsible for outbreaks within individual barns (our unpublished data). Furthermore, we observed that subtypes associated with outbreaks in broiler barns are predominately associated with beef cattle, and it is possible that the diverse subtypes of C. jejuni excreted into the environment in high densities by cattle are transmitted to broilers. Under this scenario, beef cattle would serve as the primary reservoir of clinically relevant subtypes of C. jejuni, and the transmission route would be cattle to chickens to people via consumption of contaminated poultry as a vehicle. Evidence for this transmission pathway is a current line of active investigation by our research team. In this regard, we are currently applying large-scale whole genome sequencing of target subtypes to elucidate mechanisms of resistance development, to ascertain risk, to develop diagnostic methods (i.e. for high risk subtypes), and to identify primary transmission mechanisms.

In summary, resistance to antimicrobial agents was evaluated in C. jejuni isolated from ≈1,300 diarrheic people over a 15 year period in a model location in Canada employing a regional ‘One Health’ approach. Although, rates of resistance to the majority of antimicrobials tested remained stable during the study period,
FQR rates increased substantially over the examination period with a maximal resistance prevalence of 29%.

The vast majority of FQR *C. jejuni* isolates contained a C257T mutation within the *gyrA* chromosomal gene. The observed increase in FQR was attributed to domestically acquired infections primarily linked to the cattle reservoir. Study findings thus implicate beef cattle as a potentially important reservoir of clinically relevant *C. jejuni* subtypes, including those resistant to FQs. Moreover, evidence obtained in the study emphasizes the need to comparatively monitor FQR in *C. jejuni* from human, cattle, and chicken reservoirs at the subtype level of resolution to elucidate mechanisms of resistance development and transmission.
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Figure Legends

**Fig. 1.** (A) Number of people infected with *Campylobacter jejuni* in Southwestern Alberta from 2004 to 2018. Temporal resistance (%) in *C. jejuni* to antimicrobial agents. (B) Tetracycline. (C) Ciprofloxacin. (D) Nalidixic acid. Black histogram bars indicate resistant isolates, and grey histogram bars indicate isolates possessing intermediate resistance. Grey circles indicate *C. jejuni* isolates recovered from individuals who were infected outside of Canada and the USA (i.e. traveler’s diarrhea), and white squares indicate *C. jejuni* isolates that could not be designated as either domestically or internationally acquired. Histogram bars denoted with an asterisk were higher (P≤0.050) than the prevalence of resistance observed in 2004. Solid lines indicate the linear relationship between time and resistance, and dotted lines are 95% confidence limits.

**Fig. 2.** Relative sensitivities of *Campylobacter jejuni* isolates recovered from diarrheic people in Southwestern Alberta to ciprofloxacin during three 5-year time periods (2004-2008, 2009-2013, and 2014-2018). The arrow indicates the breakpoint minimum inhibitory concentration, and asterisks indicate conspicuous increases in resistance.

**Fig. 3.** Minimum spanning tree of *Campylobacter jejuni* comparative genomic fingerprinting subtypes recovered from diarrheic people (2004-2018) showing subtypes possessing isolates resistant to ciprofloxacin. Black fill denotes resistant isolates, grey fill indicates isolated possessing intermediate resistance, and white fill denotes susceptible isolates. The thickness of lines connecting subtypes represent mismatched loci (i.e. one to three loci), and subtypes with no line represent ≥ four mismatched loci between respective subtypes. Stars indicate major subtypes recovered from diarrheic people in Southwestern Alberta that are associated livestock reservoirs, and white asterisks within markers indicate subtypes attributed to internationally acquired infections. Highlighted clusters A to J illustrate prominent clusters (95% level of resolution) that contain subtypes resistant to ciprofloxacin, where orange highlighting indicates clusters containing resistant subtypes primarily associated with cattle reservoirs, blue highlighting indicates clusters containing resistant subtypes associated with both cattle and chickens, and green highlighting indicates clusters with an accumulation of subtypes associated with traveler’s diarrhea (see Table 2 for subtype-specific information).

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**Fig. 5.** Three-way Venn diagram of *Campylobacter jejuni* comparative genomic fingerprinting subtypes (100% level of resolution) that contained isolates resistant to ciprofloxacin (A), nalidixic acid (B), and/or tetracycline (C). The total number of subtypes identified was 656.
Table 1. Single nucleotide polymorphism (SNP) mutations (%) in the GyrA gene of 165 Campylobacter jejuni isolates recovered from diarrheic people in Southwestern Alberta that were resistant to ciprofloxacin\(^a\)

| MIC | \(n^b\) | Avg | A21G\(^c\) | A64G\(^d\) | T72C | C243T | C257T\(^e\) | C330T | T357C | C360T | G408A | C471T | T483C |
|-----|--------|-----|-----------|-----------|------|-------|-----------|-------|-------|-------|-------|-------|-------|
| 4-8 | 15     | 3.8 | 6.7       | 26.7      | 33.3 | 40.0  | 66.7      | 0.0   | 60.0  | 33.3  | 26.7  | 53.3  | 33.3  |
| 16  | 77     | 3.8 | 2.6       | 18.2      | 23.4 | 36.4  | 96.1      | 7.8   | 57.1  | 31.2  | 22.1  | 54.5  | 32.5  |
| 32  | 43     | 5.4 | 14.0      | 32.6      | 58.1 | 62.8  | 100.0     | 2.3   | 69.8  | 58.1  | 11.6  | 74.4  | 58.1  |
| >32 | 30     | 5.2 | 3.3       | 40.0      | 56.7 | 63.3  | 93.3      | 6.7   | 66.7  | 63.3  | 0.0   | 63.3  | 63.3  |

\(^a\)MIC, minimum inhibitory concentration determined by dilution plating (\(\mu\)g/mL); \(n\), number of isolates; Avg, average number of SNP mutations.

\(^b\)Two ciprofloxacin resistant isolates were not characterized for SNP mutations due to poor amplification of the gryA gene.

\(^c\)For SNP nomenclature the first letter represents the non-mutated nucleotide, the number represents the nucleotide position in the GyrA gene, and the second letter represents the mutated nucleotide.

\(^d\)Resulted in an amino acid substitution (Ser22Gly), where Ser is serine, 22 is the amino acid position, and Gly is glycine.

\(^e\)Resulted in an amino acid substitution (Thr86Ile), where Thr is tyrosine, 86 is the amino acid position, and Ile is isoleucine.
Table 2. Comparative genomic fingerprint (CGF) subtypes containing *Campylobacter jejuni* isolates recovered from diarrheic people in Southwestern Alberta (SWA) from 2004 to 2018 that were resistant to ciprofloxacin (CipR) with corresponding exact match subtype data within the Canadian *Campylobacter* Comparative Genomic Fingerprint database (C3Gdb)*a*

| Subtype Identifier | SCb | Size | CipRb | Rank | C3Gdb identifier | % of isolates of the same CGF subtype from the following: |
|-------------------|-----|------|-------|------|-------------------|-------------------------------------------------------------|
| 316** A 101 35 1 | 0169.001.002 | 30.8 | *47.6 | 13.9 | 2.2 | 76.5 | 0.0 | 0.0 | 17.4 | 1.8 | 0.0 | 0.3 |
| 317 A 1 1 1160 | 0171.002.003 | 100.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 323 A 1 1 751 | 0169.006.002 | 66.7 | *33.3 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 324 A 10 2 229 | 0169.011.002 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 325 A 1 1 3121 | 0169.011.003 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 326 A 6 1 28 | 0173.004.001 | 30.1 | 29.2 | 28.3 | 1.8 | 46.0 | 0.0 | 0.0 | 41.6 | 5.3 | 0.0 | 0.9 |
| 333 A 3 2 281 | 0173.008.002 | 66.7 | *33.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 22.2 |
| 348 A 4 4 24 | 0173.010.002 | 11.5 | 0.0 | *79.1 | 60.4 | 5.8 | 0.0 | 0.0 | 17.3 | 1.4 | 0.7 | 9.4 |

*Hu*, human beings; *Ca*, cattle; *Ch*, chickens; *BC*, British Columbia; *AB*, Alberta; *SK*, Saskatchewan; *MB*, Manitoba; *ON*, Ontario; *QC*, Quebec; *AL*, Atlantic provinces; *UK*, unknown province.

*bSC*, subtype clusters presented in Fig. 3; *CipR* size, number of isolates within the same CGF subtype resistant to ciprofloxacin.

**Primary animal reservoir.

**Primary subtypes infecting people in SWA (primary subtypes are designated with stars in Fig. 1).
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