Long-Lasting Outbreak of Erythromycin- and Ciprofloxacin-Resistant Campylobacter jejuni Subspecies jejuni From 2003 to 2013 in Men Who Have Sex With Men, Quebec, Canada

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From January 2003 to December 2013, sexual transmission of 2 clades of Campylobacter jejuni subspecies jejuni isolates resulted in a prolonged outbreak among men who have sex with men living in Quebec, Canada. The outbreak isolates were acquired locally and were resistant to erythromycin and ciprofloxacin.

Keywords. Campylobacter; multidrug resistance; Quebec; homosexuality.

Campylobacter jejuni subspecies jejuni (C. jejuni) is the most frequently isolated Campylobacter species in many countries [1–3]. When antimicrobial treatment is indicated, macrolides and fluoroquinolones are first- and second-choice agents [1–3]. Whereas sexual transmission of Shigella species has been widely demonstrated [4], few studies have documented sexually transmitted infections caused by Campylobacter in men who have sex with men (MSM) [5, 6]. At the Centre Hospitalier de l’Université de Montréal (CHUM)—Saint-Luc, C. jejuni isolates are routinely tested for susceptibility to erythromycin, tetracycline, and ciprofloxacin, and have been stored in a biobank as frozen specimens since 1982. These procedures allowed us to uncover an outbreak caused by an erythromycin-resistant, tetracycline-susceptible, C. jejuni fla type 1 that lasted from 1999 to 2001 [5]. The C. jejuni isolates from this outbreak were acquired locally in Quebec, Canada, by sexual transmission. Nine of the 11 isolates were resistant to ciprofloxacin. We here describe the persistence over >10 years of this outbreak in MSM caused by 2 related clades of C. jejuni. This outbreak is made even more challenging to control due to the resistance of the isolates to first- and second-line therapies.

METHODS

After approval by the CHUM Ethics committee, hospital charts and public health files of individuals infected with the outbreak C. jejuni clades A and B strains were reviewed retrospectively. Phenotypic identification of C. jejuni isolates at the genus and species levels was confirmed by cpn60 gene sequencing at the Laboratoire de Santé Publique du Québec [2, 7]. Susceptibilities to erythromycin, tetracycline, and ciprofloxacin were assessed by disk diffusion and confirmed by Etest (AB Biodisk, Solna, Sweden) and/or agar dilution, whereas susceptibilities to azithromycin, clarithromycin, clindamycin, ampicillin, gentamicin, and imipenem were obtained with the latter 2 techniques. Susceptibility testing for nalidixic acid was performed using disk diffusion only. Susceptibility breakpoints and the presence of β-lactamase were determined as described elsewhere [2, 6, 8].

Pulsed-field gel electrophoresis (PFGE), with Smal and KpnI restriction enzymes, was performed according to international standards (http://cdc.gov/pulsenet/PDF/campylobacter-pfge-protocol-598c.pdf).

Typing of the flagellin A gene (fla) with polymerase chain reaction (PCR) sequencing was applied on 45 C. jejuni isolates: 35 were suspected to be from the current outbreak, 4 from the previous 1999–2001 outbreak (included to study the evolutionary relationship with isolates obtained after 2001), the C. jejuni ATCC 33560 and 29428 strains, and 4 erythromycin-susceptible isolates from our bacterial collection (2003–2013 except for 1 isolate from 1999). DNA was extracted from bacterial suspensions with Master pure (Epicentre, Madison, Wisconsin) [9] and resuspended in 300 µL of 20 mmol/L Tris buffer (pH 8.3) with 0.1 mM ethylenediaminetetraacetic acid. Extracted DNA (5 µL) was amplified with 50 pmol of each primer

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FLA242FU (5′-CTATGGATGAGCAATTWAAAAT-3′) and FLA625RU (CAAGWCCTGTTCCWACTGAAG) [10, 11] in 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 units of AmpliTaq Gold (Roche Diagnostic, Laval, Québec) and 2.5 mM MgCl2. Amplification was done after an initial activation step at 95°C for 9 minutes, with 35 cycles at 94°C for 30 seconds, at 55°C for 1.5 minutes, and at 72°C for 2.5 minutes, followed by a final step at 72°C for 5 minutes. Campylobacter amplicons were purified with the QIAquick gel extraction protocol (Qia-gen, Mississauga, Ontario, Canada). Direct double-stranded PCR sequencing was performed with the same primers using the fluorescent cycle-sequencing method (BigDye terminator ready reaction kit; Perkin-Elmer) on 10 ng of purified amplicons. Cycling parameters were 94°C for 1 minute followed by 25 cycles for 10 seconds at 96°C, 5 seconds at 50°C, and 4 minutes at 60°C. Campylobacter sequences were aligned with CLUSTAL W (version 1.8) [12]. A phylogenetic tree was constructed based on the neighbor-joining algorithm by using Mega, version 3.1 [13]. The significance of differences was analyzed by the χ² test with Epi Info software, version 6.

RESULTS

From 1 January 2003 to 31 December 2013, 35 isolates of C. jejuni were shown to be resistant to erythromycin and ciprofloxacin but susceptible to tetracycline. Of these 35 isolates, 32 were tested by PFGE and all were untypable by this method. The evolutionary relationship among the 45 C. jejuni isolates described in the Methods was explored by subjecting to phylogenetic analysis fla gene sequence data obtained with PCR sequencing. Isolates clustered into 2 related clades, with clade A preceding in time clade B (Figure 1). Seven C. jejuni clade A isolates collected from 2003 to 2008 were identical to the 4 C. jejuni fla type I isolates obtained from 1999 to 2001. The 24 C. jejuni clade B isolates had been collected from 2004 to 2013. Thus, 31 C. jejuni fla clade A or B isolates collected from 2003 to 2013, untypable by PFGE, resistant to erythromycin and ciprofloxacin but susceptible to tetracycline, were considered to be part of the current outbreak.

The 31 individuals from the current 2003–2013 C. jejuni outbreak were all men, aged 21–64 years; the C. jejuni infection was limited to the gastrointestinal tract for all individuals. They had not traveled recently outside Quebec. During this period, investigations by public health authorities did not uncover contaminated food as a possible source of infection in those patients. Twenty-eight men reported having had sex with men. Information on sexual activity was unavailable for 3 men. Nineteen of the 26 men for whom human immunodeficiency virus (HIV) testing results were available were HIV seropositive. However, we could not determine if 3 of the latter individuals were already HIV seropositive at the time of C. jejuni infection. CD4 counts were <150 × 10⁶ cells/L (10, 50, 140 × 10⁶ cells/L) for 3 men, 230–1090 × 10⁶ cells/L (median, 415 × 10⁶ cells/L) for 12 men, and unknown for 1 man. HIV RNA load was <50 copies/mL.

Figure 1. Phylogenetic tree of 45 Campylobacter jejuni subspecies jejuni isolates based on fla gene sequences. The sequences were aligned and a phylogenetic tree was constructed based on the neighbor-joining algorithm. Eleven C. jejuni isolates belonged to clade A; 4 C. jejuni type I isolates from the 1999–2001 outbreak are marked with *, and 7 C. jejuni clade A isolates from 2003–2008 are marked with **. Twenty-four C. jejuni clade B isolates from 2004–2013 are marked with **. The C. jejuni isolates 2054, 2596, 2641, and 1770, which were erythromycin and ciprofloxacin resistant and tetracycline susceptible, served as control isolates different from isolates from clade A or B. The other control isolates were C. jejuni subspecies jejuni ATCC 33560 and 29428 and erythromycin-susceptible C. jejuni 2071, 940, 1700, and 1677 from other outbreaks. The bar represents 0.02 substitution per 100 bases.
for 6 men and 92–886 420 copies/mL (median, 64 452 copies/mL) for 10 men. Information on opportunistic infection prophylaxis was not available. A diagnosis of sexually transmitted diseases (STDs) other than HIV infection was documented 69 times in 29 individuals before, simultaneously, or after the Campylobacter jejuni fla clade A or B infection: 59% (16/27) were infected with Treponema pallidum, 40% (8/20) with Neisseria gonorrhoeae, and 55% (11/20) with Chlamydia trachomatis. Not all participants were systematically tested for STDs during the study period. Nineteen episodes of sexually transmitted enteric infections were documented for 39% (9/23) of men (9 Shigella species, 8 Giardia lamblia, 2 Campylobacter species). Four men were evaluated in the emergency room, 24 were evaluated in outpatient clinics, and 3 required hospitalization for 2–17 days. Information on antimicrobial treatment was unavailable for 28 men.

Over the 11 years of the outbreak, 1–5 patients per year were infected with the epidemic Campylobacter jejuni isolate. From 2003 to 2013 at CHUM–Saint-Luc, Campylobacter jejuni clade A or B isolates were documented in 24 of 245 men and 0 of 156 women with Campylobacter jejuni infection (P = .0001), and from 24 of 288 Campylobacter jejuni infections acquired locally and 0 of 113 Campylobacter jejuni infections acquired abroad (P = .003).

The 31 outbreak isolates were resistant to erythromycin, azithromycin, clarithromycin, and clindamycin (minimum inhibitory concentrations [MICs] >256 mg/L for the 4 agents), to ciprofloxacin (MICs 8–16 mg/L), and to nalidixic acid (no zone around the disk), but were susceptible to tetracycline, ampicillin, gentamicin, and imipenem (MICs of 0.06–0.25, 0.5–1, 0.125–0.25, and 0.03 mg/L, respectively) and were β-lactamase negative. As the isolates were resistant to macrolides, quinolones, and clindamycin, they were considered to be multidrug resistant.

**DISCUSSION**

From 1999 to 2001, an erythromycin-resistant and tetracycline-susceptible Campylobacter jejuni fla type I outbreak was documented in 11 MSM and was considered to be sexually transmitted [5]. Nine of these isolates were also resistant to ciprofloxacin. The epidemic Campylobacter jejuni strain was not detected in 2002. From 2003 to 2013, 31 patients were infected with erythromycin- and ciprofloxacin-resistant, tetracycline-susceptible, Campylobacter jejuni isolates that initially clustered in fla clade A and later in the related clade B. The current cluster thus represents a prolonged outbreak acquired locally in MSM and caused by Campylobacter jejuni strains resistant to first- and second-line antibiotics. Epidemiologic data strongly suggest that the outbreak Campylobacter jejuni strain was transmitted sexually: Individuals involved were men, mostly MSM, a majority of whom were HIV seropositive with a history of STDs including 9 enteric Shigella infections, with persistence of the cluster for 11 years and the absence of food identified as the possible source of the outbreak. The majority of HIV-seropositive men had a detectable HIV RNA load. MSM should be counseled on methods to prevent sexually transmitted infections including enteric infections [4–6].

Whereas only a few Campylobacter jejuni isolates are untypable by PFGE using 2 enzymes [5] (unpublished data), all the outbreak isolates were untypable and clustered in 2 different clades detected sequentially over time. Erythromycin, tetracycline, and ciprofloxacin susceptibility patterns were epidemiologic markers in the current and in previous Campylobacter studies [3, 5, 6]. Erythromycin and ciprofloxacin testing is recommended for all Campylobacter jejuni isolates [8]. New oral antimicrobial agents are needed to treat Campylobacter jejuni enterocolitis considering the susceptibility profile of the outbreak strains [3] (present study). To the best of our knowledge, this is the first report of a persistent Campylobacter jejuni outbreak in MSM that last >10 years.

**Notes**

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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