Antimicrobial resistance and antimicrobial use associated with laboratory-confirmed cases of *Campylobacter* infection in two health units in Ontario

Anne E Deckert DVM MS1,2, Richard J Reid-Smith DVM DVSc1,2, Susan E Tamblyn MD3, Larry Morrell RN BScN4, Patrick Seliske PhD5, Frances B Jamieson MD FRCP6, Rebecca Irwin DVM MS2, Catherine E Dewey DVM PhD1, Patrick Boerlin DVM MSc7, Scott A McEwen DVM DVSc1

**AIM:** A population-based study was conducted over a two-year period in the Perth District (PD) and Wellington-Dufferin-Guelph (WDG) health units in Ontario to document antimicrobial resistance and antimicrobial use associated with clinical cases of laboratory-confirmed campylobacteriosis.

**METHODS:** Etest (bioMérieux SA, France) was used to determine the minimum inhibitory concentration of amoxicillin/clavulanic acid, ampicillin, chloramphenicol, ciprofloxacin (CIP), clindamycin, erythromycin (ERY), gentamicin, nalidixic acid and tetracycline. Data regarding antimicrobial use were collected from 250 cases.

**RESULTS:** Of the 250 cases, 165 (65.7%) reported staying home or being hospitalized due to campylobacteriosis. Fifty-four per cent of cases (135 of 249) reported taking antimicrobials to treat campylobacteriosis. In 115 cases (51.1%), fecal culture results were not used for treatment decisions because they were not available before the initiation of antimicrobial treatment and/or they were not available before the cessation of symptoms. Of the 250 cases, 124 (49.6%) had available Campylobacter isolates, of which 66 (53.2%) were resistant to at least one of the antimicrobials tested. No resistance to ampicillin, chloramphenicol or gentamicin was found in these isolates. Six isolates (4.8%) were resistant to CIP. Two isolates (1.6%) were resistant to ERY; however, no isolates were resistant to both CIP and ERY.

**CONCLUSION:** Prudent use practices should be promoted among physicians to reduce the use of antimicrobials for the treatment of gastroenteritis in general and campylobacteriosis in particular, as well as to minimize the future development of resistance to these antimicrobials in *Campylobacter* species.

**Key Words:** Antimicrobial resistance; Antimicrobial use; Campylobacter; Canada; Ontario

**Conclusions:**

*Campylobacter* species are an important human enteric pathogen that can cause severe, bloody diarrhea, fever and abdominal pain (1,2). In Canada, the National Notifiable Disease Program reported 31.8 isolations of *Campylobacter* per 100,000 people in the province of Ontario and 30.2 per 100,000 people nationally in 2004, which is the most recent year for which results are available (3). In 2004, there were 3945 and 9345 campylobacteriosis cases reported in Ontario and Canada, respectively (3). In 2009, the FoodNet Surveillance system in the United States (US) reported 6033 cases of campylobacteriosis and a rate of 13.02 per 100,000 people across their sites (4). Because gastrointestinal illness is significantly under-reported, the number of cases is likely to be substantially higher (5,6). In Canada, it was estimated that for every reported case of campylobacteriosis there are 23 to 49 cases that occur in the population, resulting in an estimated 8.9 to 18.8 cases per 100,000 people in 2001 (7).
The case fatality rate for Campylobacter infection was reported to be one in 1000 in the US in 1999 (8). It has been estimated that 9% to 14% of cases develop postinfectious irritable bowel syndrome (9,10) and approximately one in 1000 cases progress to Guillain-Barré syndrome (11).

Campylobacteriosis is usually a self-limiting infection and treatment is recommended only in vulnerable populations with severe or invasive disease (12,13). However, observational studies have shown that a substantial percentage of patients are prescribed antimicrobials to treat campylobacteriosis (14-17). There is currently no information regarding the types and prevalence of antimicrobial treatment for campylobacteriosis in Canada. Erythromycin (ERY) is commonly used in both adults and children, and fluoroquinolones, such as ciprofloxacin (CIP), are used in adult cases (12). There is also evidence in the literature that antimicrobial resistance in Campylobacter may be associated with increased virulence and adverse outcomes in human cases (18) as well as a prolonged duration of diarrhea (16,19,20). Therefore, resistance in Campylobacter organisms to antimicrobials in general, and these clinically important antimicrobials in particular, is of concern.

A population-based study was conducted over a two-year period in the Perth District (PD) and Wellington-Dufferin-Guelph (WDG) health units of Ontario to document antimicrobial resistance and antimicrobial use associated with clinical cases of campylobacteriosis.

**METHODS**

The present research project was approved by the University of Guelph Research Ethics Board (Guelph, Ontario). Agreements with hospital and private laboratories were established that enabled the isolates obtained from clients living in these health unit areas to be forwarded for further characterization to the Central Public Health Laboratory (CPHL, Toronto, Ontario), Ontario Ministry of Health and Long-term Care (now the Public Health Ontario Laboratory, Toronto, Ontario). These agreements ensured the confidentiality of patient information. Case physicians were not provided with the results of the further characterization conducted for the present research project. The testing delays inherent in a research project precluded the results from being available in a clinically relevant time-frame.

**Cases**

Laboratory confirmed cases of Campylobacter infection occurring between February 2002 and February 2004 that resided in the PD and WDG health unit areas were eligible for inclusion in the study. Cases were excluded if the patient could not speak English or did not have a home telephone. Patients were informed of the purpose of the study and provided informed consent for participation at the time of questionnaire administration. Questionnaires were administered by health unit personnel via telephone.

**Study area**

The average population of the PD and WDG health unit areas during the time period of the study was 77,188 and 252,844, respectively. Both health unit areas have a combination of urban and rural populations.

**Isolation**

Campylobacter strains were confirmed and speciated at the CPHL using Gram-stain, the oxidase test, hippurate hydrolysis, susceptibility to nalidixic acid (NAL) (30 µg) and cephalothin (30 µg), microaerophilic growth at 25°C, 36°C and 42°C, aerobic growth at 25°C, catalase production and indoxyl acetate hydrolysis. Supplementary tests included urea hydrolysis, nitrate reduction, H₂S production, and growth on 1% glycine and on MacConkey agar.

**Minimum inhibitory concentration**

The Etest (bioMérieux SA, France) was used for the determination of the minimum inhibitory concentration (MIC) in the isolates of amoxicillin/clavulanic acid (AMC), ampicillin (AMP), chloramphenicol (CHL), CIP, clindamycin (CLI), ERY, gentamicin (GEN), NAL and tetracycline (TCY). Subcultures were grown on Mueller-Hinton blood agar plates for 24 h under microaerobic conditions at 42°C before determining the MICs using Mueller-Hinton blood agar plates with 5% laked horse blood and microaerobic conditions with incubation for 48 h at 37°C. Control strains were Campylobacter jejuni American Type Culture Collection (ATCC) 33562, Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 29213. Breakpoints from the Clinical and Laboratory Standards Institute (CLSI, Wayne, Pennsylvania) and the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) were used in the present study. CIPARS breakpoints are based on CLSI guidelines when available (21). The breakpoints used were AMC ≥32 µg/mL, AMP ≥32 µg/mL, CHL ≥32 µg/mL, CIP ≥4 µg/mL, CLI ≥4 µg/mL, ERY ≥32 µg/mL, GEN ≥16 µg/mL, NAL ≥32 µg/mL and TCY ≥16 µg/mL.

**Questionnaire data**

The dates of fecal collection and availability of culture results to the physician were reported to the health unit by the primary laboratory. Information regarding the use of antimicrobials during the course of Campylobacter infection was collected as part of a comprehensive case questionnaire, which also collected information on sex, age and severity of illness. Severity of illness was reported for cases according to the following definitions: Quite mild – feeling slightly unwell but able to do all normal activities; Fairly mild – feeling quite unwell but able to do most normal activities; Moderate – having to stay at home but able to get out of bed for limited activities; Fairly severe – confined to bed at home and unable to do any normal activities; Quite severe – hospitalized. Age was categorized according to the age distribution of Campylobacter cases reported in the WDG health unit from 1991 to 2000: <5 years of age, five to <17 years of age, 17 to <41 years of age, 41 to <61 years of age and ≥61 years of age (Figure 1).

**Data analysis**

Test results and antimicrobial use information were analyzed using Stata Intercooled version 11 (Stata Corporation, USA). Dichotomous variables were analyzed using the χ² and Fisher's exact tests as appropriate. Differences between groups of continuous variables were evaluated using the Student’s t-test; P<0.05 was considered to be statistically significant.

**RESULTS**

There were 317 laboratory-confirmed cases during the study period, with 49.4 cases per 100,000 people in 2002 and 46.7 cases per 100,000 people in 2003. There was a marked difference in this rate between the two health units, with 84.5 cases per 100,000 people and 63.3 cases per 100,000 people in the PD health unit in 2002 and 2003, respectively, compared with 38.6 cases per 100,000 people and 41.7 cases per 100,000 people in the WDG health unit in 2002 and 2003, respectively (P<0.05). Seventy-nine per cent of the cases (n=252) were successfully contacted and agreed to participate. Two cases were excluded: one due to an inability to communicate in English and one did not reside in the study area. Therefore, questionnaire data were collected from 250 cases, 51 from the PD health unit area and 199 from the WDG health unit area.

**Figure 1** Percentage of cases according to age category in laboratory confirmed cases of Campylobacter infection in the Perth District (Ontario) and Wellington-Dufferin-Guelph (WDG) (Ontario) health units during the study period as well as in the WDG health unit area over a 10-year period.
TABLE 1

Antimicrobial resistance in Campylobacter isolates from human cases in the Perth District and Wellington-Dufferin-Guelph health units (Ontario)

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC50, μg/mL</th>
<th>MIC90, μg/mL</th>
<th>Resistant isolates, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin/clavulanic acid</td>
<td>0.19</td>
<td>0.38</td>
<td>0 (0.0–2.9)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1.0</td>
<td>4.0</td>
<td>3 (0.5–6.9)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.75</td>
<td>1.5</td>
<td>0 (0.0–2.9)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.047</td>
<td>0.094</td>
<td>6 (4.8–10.2)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.094</td>
<td>0.25</td>
<td>2 (1.6–2.5)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.25</td>
<td>0.75</td>
<td>2 (1.6–2.5)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.25</td>
<td>0.38</td>
<td>0 (0.0–2.9)</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>1.5</td>
<td>3.0</td>
<td>6 (4.8–10.2)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.19</td>
<td>&gt;256</td>
<td>60 (48.4–57.5)</td>
</tr>
</tbody>
</table>

MIC50 Minimum inhibitory concentration required to inhibit the growth of 50% of organisms; MIC90 MIC required to inhibit the growth of 90% of organisms.

The age of the cases ranged from four months to 85 years, with a median age of 27.4 years. The age of cases was not significantly different between the health units (P > 0.05). There were 140 (56.2%) male cases and 109 (43.8%) female cases, resulting in a male to female case ratio of 1.3:1. In both 2002 and 2003, more than one-half of all the cases reported that symptoms began between June and September (2002: 72 of 119 [61%]; 2003: 68 of 122 [56%]).

The age of the cases ranged from four months to 85 years, with a median age of 27.4 years. The age of cases was not significantly different between the health units (P > 0.05). There were 140 (56.2%) male cases and 109 (43.8%) female cases, resulting in a male to female case ratio of 1.3:1. In both 2002 and 2003, more than one-half of all the cases reported that symptoms began between June and September (2002: 72 of 119 [61%]; 2003: 68 of 122 [56%]).

The antimicrobials taken by 138 cases to treat campylobacteriosis included: AMC (n=2), cephalexin (n=1), CIP (n=45), azithromycin (n=32), clarithromycin (n=11), ERY (n=32), metronidazole (n=7), sulfonamides (n=5), doxycycline (n=1) and TCY (n=2) (Figure 2). Eight cases reported treatment using more than one antimicrobial. Of the cases which began antimicrobial treatment after fecal culture results were available, seven were treated using CIP, three with ERY, one with trimethoprim-sulfamethoxazole (SXT), one with azithromycin and one with clarithromycin.

Campylobacter isolates from 124 of 250 cases (49.6%) were received by the CPHL and, therefore, had antimicrobial susceptibility information. Of these, 121 (97.6%) were C. jejuni and three (2.4%) were Campylobacter coli. Isolates from the remaining cases were not available from the primary laboratory or were unable to be matched using case information. There were no significant differences in age category, sex or severity of illness between cases with and without accompanying isolates (P > 0.05). There were also no significant differences between health units in the proportion of cases with accompanying isolates (P > 0.05). Among cases with isolate susceptibility results that were treated using CIP, ERY, clarithromycin and/or azithromycin, only 5.3% (3 of 57) were resistant to the antimicrobial class used.

Sixty-six isolates (53.2%) were resistant to at least one of the nine antimicrobials tested, including all three C. coli isolates. Six isolates (4.8%; five C. jejuni, one C. coli) were resistant to CIP (Table 1) and all isolates resistant to NAL were also resistant to CIP. Two isolates (1.6%, one C. jejuni, one C. coli) were resistant to ERY; however, no isolates were resistant to both CIP and ERY (Table 2).

The three cases with a C. coli isolate did not appear to be substantially different than the cases with a C. jejuni isolate with respect to the parameters described in the present study. All three cases were adult men; one was from the WDG health unit and two were from the PD health unit. Two of these cases had blood in the stool and one reported fever. One of these cases had travelled internationally. Severity was evenly distributed among the Fairly mild, Moderate and Fairly severe categories. Two of the cases used CIP to treat their illness and the use began before the availability of fecal culture results. The median duration of illness was four days (range four to seven days) for these three cases.
DISCUSSION

Campylobacteriosis is usually a self-limiting disease and antimicrobial treatment is not recommended in uncomplicated cases; however, macrolides and fluoroquinolones are the primary antimicrobials used for the treatment of campylobacteriosis in vulnerable populations (12,13,22). Antimicrobials may also be used in conjunction with infectious control practices in daycare or other group settings to decrease the duration of excretion and minimize person-to-person transmission; however, adequate hygiene and infection control practices are the preferred control measures (23-28).

Although only limited data are available, the percentage of campylobacteriosis cases treated using antimicrobials varied in published studies: 16% in a study conducted in Norway (14), 54% in the present study, 74% in a study conducted in Finland (17) and 81% to 85% in a study conducted in the US (15,16).

The mean and median number of days in the present study from the onset of symptoms to antimicrobial treatment (mean 9.8 days; median eight days) were slightly lower than what was reported in a study conducted in Norway (mean 11.3 days; median 10 days) (14). Also, the mean and median number of days from the onset of symptoms to the submission of a fecal sample (mean six days, median four days) were somewhat lower than reported in a previously published study (mean nine days; median six days) (14) but similar to the time period between the onset of symptoms and seeking of medical attention in other published studies (24-26).

In the majority of cases, fecal culture results were not used for treatment decisions because they were not available before the initiation of antimicrobial treatment and/or they were not available before the cessation of symptoms. For 78.9% of cases, fecal culture results were not available while they were symptomatic. This was due, in part, to the delay in seeking medical treatment, the relatively short duration of symptoms in the typical patient (mean 10.0 days) and the fastidious nature of Campylobacter, which can require up to five days to culture (2,14,29). Therefore, the primary value of fecal culture in clinical campylobacteriosis may be to properly manage complicated or prolonged cases by adjusting therapy based on susceptibility results, as well as to meet public health needs (13). Information on the number, demographics and risk factors of cases is essential for ongoing public health programs (13).

Ontario has a universal health care system; therefore, access to physicians and submission of stool samples should not be influenced by the willingness or ability to pay for these services. However, the cost of antimicrobials is not universally covered and, therefore, the use of antimicrobials may be affected by cost. Because antimicrobials for oral therapy in humans are not available over the counter in Canada, and because all of the cases in the present study accessed the health care system, it was assumed that the antimicrobials taken were prescribed for this illness by a physician. Further research is required to determine the factors used by physicians when determining whether to prescribe antimicrobials for acute-onset diarrhea.

Only 22.2% of cases receiving antimicrobials in the present study began treatment after fecal test results were available. Although 10 different antimicrobials were used in the present study, those used after fecal results were available were primarily ERY, CIP and SXT, which was similar to previously published studies (15,30). It should be noted that antimicrobials are not indicated for acute undifferentiated diarrhea in children due to the high proportion of cases with a viral etiology and the self-limiting nature of bacterial gastroenteritis in children (31). However, in the present study, 26 of 42 (61.9%) cases who were <5 years of age were treated using antimicrobials. The three cases in this age group with reported daycare exposure were all treated using antimicrobials. With regard to severity of illness, none of the cases who were <5 years of age were hospitalized or were in the Fairly severe category; however, 46% were in the Quite mild category. This was similar to the results for all the cases, where those who were treated using antimicrobials did not differ from those that were not treated using antimicrobials with regard to severity of illness. However, cases in this dataset may be biased toward an increased level of severity due to the requirement for submission of a fecal sample. Empirical treatment of gastroenteritis in adults is only recommended in specific populations, such as international travellers (22,32), yet, there was no significant difference in the proportion treated in cases with and without histories of international travel (ie, P=0.05). With respect to fluoroquinolones, their use was of concern because it is a risk factor for fluoroquinolone-resistant Clostridium difficile-associated diarrhea (CDAD) (33). CDAD is an increasing public health concern in both hospital and community settings, illustrating the importance of the judicious use of fluoroquinolones (33). AMC, cephalxin and metronidazole were used for treatment in 11 cases in the present study, although these drugs are not indicated for undifferentiated gastroenteritis (22,32). Although metronidazole is recommended for the treatment of Giardia infections, one of the distinguishing clinical features of Giardia infection is persistent diarrhea (>7 days); however, six of eight patients treated using metronidazole initiated treatment at <7 days. The use of SXT for the clinical treatment of laboratory-confirmed campylobacteriosis was unexpected due to the high proportion of Campylobacter isolates with resistance to SXT and the demonstrated lack of clinical effect (34,35). SXT is recommended for the treatment of other enteric pathogens but not Campylobacter or undifferentiated diarrhea in international travellers (13,22).

The prevalence of CIP and NAL resistance in the isolates from the present study (<5%) was consistent with previously published research but was low compared with more recent Canadian and international data (15,19,29,34,36-39). In a 1992/1993 hospital-based Montreal (Quebec) study (36), only 3.5% of isolates were resistant to CIP, but the prevalence of resistance increased to 12.7% by 1997. A study conducted in the US (15) found that in 2001, 18% of C jejuni isolates were resistant and the adjusted OR for a Campylobacter isolate to be resistant to CIP was 2.5 times higher than in 1997. Similar increases in Campylobacter resistance to fluoroquinolones have been observed in other countries (34,40). The only previous study using clinical cases from Ontario, conducted almost 20 years earlier (35), did not evaluate the resistance to CIP but found no resistance to NAL. The relatively low prevalence of resistance to CIP in the present study may be due to regional differences in specific risk factors including antimicrobial prescribing practices, antimicrobial access, travel, water supply and the prevalence of CIP resistance in Campylobacter isolated from retail poultry (19,37,39). Case-control studies have consistently identified chicken consumption, in particular, as a major risk factor for campylobacteriosis (40-45). Additional research has estimated that the proportion of human campylobacteriosis cases that can be attributed to the consumption of chicken ranges from 20% to 80% (46-48). Therefore, CIP resistance in Campylobacter isolated from retail chicken may play an important role in CIP resistance in human campylobacteriosis. The prevalence of CIP resistance in Campylobacter isolated from retail chicken was sampled as an additional component to the current study and was also found to be quite low (1.9%) (49). Antimicrobial use practices in poultry production in Canada are unknown but may impact antimicrobial resistance in Campylobacter from retail chicken (50). These results suggest that fluoroquinolone use in domestic poultry production during the time period of the present study may have been uncommon.

Differences in the populations studied may also have played a role in the observed disparity in resistance, because many studies only included isolates from hospitals or referral laboratories (36,37,51). Hospitalized patients are more likely to have been treated using antimicrobials, including fluoroquinolones, and isolates from referral laboratories may not be representative.

In 2005, the breakpoint established by the CLSI for ERY was changed from ≥28 µg/mL to ≥32 µg/mL; as a result, many relevant studies had described levels of resistance to ERY based on the lower breakpoint. In the present study, there were two isolates (1.6%) that were resistant to ERY and both had an MIC of >256 µg/mL. Therefore, these isolates would be categorized as resistant at both the ≥28 µg/mL and ≥32 µg/mL levels.
breakpoints. Although differences in antimicrobial access may play a role in the high level of resistance to CIP in human isolates from some countries, this does not appear to result in high levels of resistance to ERY (37,39,51,52). The prevalence of resistance to ERY at the current breakpoint was between 0% and 2.9% in other published studies (35-37,39,51-54). The prevalence of resistance to CLI was also low, both in the present study and in previous studies, with the exception of the Guévermont study in Quebec where 10.3% of isolates were resistant to CLI (15,35,53,55,56). The two isolates in the present study that were resistant to ERY with an MIC of >256 µg/mL were also resistant to CLI, which is consistent with a previously published Canadian study (35).

Differences in the panel of antimicrobials tested makes comparisons of multidrug resistance difficult between studies, and differences in isolation methodologies also make comparisons of individual resistance results difficult. In addition, the lack of standardized breakpoints used for Campylobacter makes comparisons problematic, unless MIC data are provided in a form that enables the recalculations of the proportion of resistant isolates (39,51).

The incidence of campylobacteriosis per 100,000 people in the WDG health unit was similar to that found in the neighbouring Waterloo (Ontario) region (49.69 per 100,000 people) (57), in the province of Ontario (42.3 per 100,000 people, 1997 to 2001) (58) and nationally (43.9 cases per 100,000 people, 2001 to 2004) (59). The incidence of campylobacteriosis in the PD health unit was substantially higher than in the WDG health unit, which was expected because of the historically high reported incidence in this health unit. This may be related to differences in exposure to risk factors, which may include the presence of a chicken abattoir as a major employer in this region, because Campylobacter has been associated with occupational exposure to chicken processing (60). Moreover, there may be an increased proportion of rural properties with chickens and exposure to raw milk due to the presence of a farm-based religious community in the PD health unit area. The PD and WDG health units are located in different watersheds, which may result in a different level of exposure due to water supply. Differences in physician awareness of zoonoses and subsequent differences in levels of stool testing may also play a role.

The age distribution of the cases involved in the present study was consistent with the previously observed age distributions in these health units, and with other literature, although the substantial peak in children <5 years of age that was reported in some studies was not observed (Figure 1) (29,59,61,62). It has been suggested that this peak may be due to an increased sampling frequency in young children with gastrointestinal illness compared with older children and adults (14,61,62). It is possible that this increased sampling frequency in young children does not occur in these health units, possibly due to the length of time required to obtain fecal culture results and the shorter duration of clinical signs that have been found in children <10 years of age (26). Alternatively, the true incidence of Campylobacter infection in young children in this region may be lower than reported elsewhere.

Data from the present study were collected between 2002 and 2004. Antimicrobial use and antimicrobial resistance in these two health unit areas may have changed since that time.

CONCLUSIONS

Because antimicrobials are not indicated for undifferentiated gastroenteritis or campylobacteriosis in most cases that involve children, the high proportion of cases in the present study that were treated using antimicrobials (54% overall, 62% of children) is concerning. However, among cases that were treated using an antimicrobial considered efficacious against Campylobacter, approximately 95% of tested isolates were susceptible to that antimicrobial. This is not surprising considering the low levels of resistance found in the present study to ERY and CIP; although, in the majority of cases, treatment decisions were made before the availability of fecal culture results. Therefore, although the use of antimicrobials appeared to be quite high, the majority of cases were treated appropriately from a microbiological perspective. However, the value of this treatment is questionable considering the minimal impact expected on the illness, the potential increased risk for CDAD and the increased opportunity for the development of antimicrobial resistance. The low levels of antimicrobial resistance to CIP, ERY and NAL in Campylobacter from this region are encouraging. Prudent use practices should be promoted among physicians in family practice, walk-in clinics and emergency rooms to reduce the use of antimicrobials in the treatment of gastroenteritis in general and Campylobacter infection in particular, as well as to minimize the future development of resistance to these antimicrobials in Campylobacter species.

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