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Severe diarrhoea caused by highly ciprofloxacin-susceptible *Campylobacter* isolates

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Abstract

The impact of fluoroquinolone resistance of *Campylobacter jejuni* and *Campylobacter coli* isolates on the outcome of the disease in sporadic *Campylobacter* infections of Finnish individuals was studied. Questionnaires were sent, during a 6-month study period, to patients who were stool culture-positive for *Campylobacter* spp. In total, 192 returned questionnaires were analysed and assessed, together with the susceptibility data of the respective bacterial isolates. Only one (2%) of the domestic, but half of the imported, *Campylobacter* isolates were resistant to ciprofloxacin. Ciprofloxacin resistance was not associated with particularly severe infection. Instead, ciprofloxacin-susceptible *Campylobacter* isolates, as compared to ciprofloxacin-resistant isolates, showed a tendency to cause more severe infections, characterized by bloody stools and hospitalization.

Keywords: *Campylobacter coli*, *Campylobacter* infections, *Campylobacter jejuni*, ciprofloxacin, diarrhoea, drug resistance, Finland, hospitalization, microbial, signs and symptoms

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Introduction

Campylobacter jejuni and *Campylobacter coli* cause bacterial enteritis worldwide [1]. In Finland, most of the *Campylobacter* cases are imported, and domestically acquired infections occur mainly during the seasonal peak in the summer [2]. Eating improperly cooked meat, especially chicken, is a commonly recognized risk factor for the infection [1]. Recently, associations were shown between campylobacteriosis in the peak season and drinking water from a dug well and swimming in natural sources of water [3]. Typical symptoms of *Campylobacter* infection include diarrhoea, sometimes with blood, in addition to fever, abdominal pain, nausea, and myalgia [4]. Bloody diarrhoea and vomiting are associated with a longer and more severe clinical course of the infection [5].

The need for antimicrobial treatment of *Campylobacter* infections has been discussed, and a recent meta-analysis including 11 randomized clinical trials indicated that early antimicrobial treatment shortens the duration of diarrhoea, although only marginally [6]. However, increased resistance of *Campylobacter* isolates to antimicrobial agents is alarming. In particular, resistance to ciprofloxacin has substantially increased since the early 1990s [7–10]. It is highly probable that the use of antimicrobial agents in veterinary medicine is mainly responsible for the decreasing susceptibility of *Campylobacter* to antimicrobials [9]. Whether fluoroquinolone-resistant *Campylobacter* isolates cause more severe infections than the susceptible ones has been debated, but the results have so far been contradictory [11–16]. The present aim was to study the possible impact of ciprofloxacin resistance of *C. jejuni* and *C. coli* isolates on the outcome of the disease in sporadic *Campylobacter* infections in Finnish individuals.

Materials and Methods

Stool samples of patients were studied for *Campylobacter*, *Salmonella*, *Shigella* and *Yersinia enterocolitica* using routine

culture methods at the Helsinki University Central Hospital Laboratory, the central clinical microbiology laboratory of the Helsinki metropolitan and surrounding areas. Stool cultures were taken according to routine clinical procedures. From 1 July to 31 December 2006, *Campylobacter* stool culture isolates from sporadic cases were collected and stored for further analysis. In order to obtain information concerning the clinical course of the infection, the patients' physicians who had referred samples for laboratory testing or coordinated the treatment were contacted by mail, and asked to forward the information about the study along with a questionnaire to the patient. Each patient with a stool culture positive for *C. jejuni* or *C. coli* was to receive a questionnaire including questions on general health condition, previously taken antimicrobial and antidiarrhoeal drugs, travel within 2 weeks preceding the illness, and the clinical course and possible antimicrobial treatment of the illness. The patients were asked to return the questionnaire in a pre-paid envelope to the investigators. The questionnaire and the respective bacterial isolate were linked by a referral number. Data obtained in response to specific questions in the questionnaire were included in the analyses only when the respective questions had been adequately answered. All data concerning symptoms, medication and travel were obtained from the questionnaires. The study was approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa.

The MIC values of ciprofloxacin (Bayer, Leverkusen, Germany) were determined by an agar dilution method according to the CLSI guidelines [17]. Mueller–Hinton agar (Oxoid, Basingstoke, UK) plates supplemented with defibrinated sheep blood (5%) were used. *C. jejuni* strain ATCC 33560 was included as a control. The plates were incubated in a microaerobic atmosphere at 36°C for 48 h. The MIC was defined as the lowest concentration of ciprofloxacin that completely inhibited visible growth. Isolates for which MICs of ≥4 mg/L were determined were considered to be resistant [18].

Statistical analyses were performed with Graphpad Prism version 4.03 (GraphPad Software, San Diego, CA, USA) and SPSS version 15.0 (SPSS, Chicago, IL, USA). The chi-square test, Fisher's exact test and the Mantel–Haenszel test were used for comparison of categorical variables. The Mann–Whitney test was used for comparison of age and MIC distributions between different groups. All tests were two-sided, and a p-value <0.05 was considered to be statistically significant.

Results

During the 6-month study period, 373 patients had stool samples positive for *C. jejuni* or *C. coli*. The questionnaire was sent to 363 (97%) patients. The ten patients to whom no questionnaires were sent included four patients whose physicians could not be reached, three whose isolates were not stored for analysis, two temporary foreign visitors, and one patient co-infected with *Salmonella typhi*. In total, 206 of the 363 patients (57%) returned an adequate response to the questionnaire. The MIC values for eight of the 206 *Campylobacter* isolates could not be determined, because the isolates were lost, and six further patients were excluded because of co-infection (with *Salmonella* spp., *Vibrio cholerae* or *Y. enterocolitica*); thus, 192 of the 363 patients (53%) were included in the final analysis. The median delay in responding to the questionnaire after giving the stool sample was 22 days (range, 8–99 days). Almost half (46%) of the included cases were diagnosed during the seasonal peak of July and August.

Patient characteristics are summarized in Table 1. One hundred and forty-eight patients (77%) had been abroad within 2 weeks prior to the onset of symptoms. The most frequently visited countries were Spain (21 patients), Bulgaria (21 patients), India (13 patients), Turkey (11 patients) and Thailand (ten patients). More than half of the patients who had been abroad had visited one of these five countries. The median age of patients who had been abroad within 2 weeks prior to the onset of symptoms was 37 years, and the median age of the 44 patients who had not been abroad was 46 years (p 0.03; Mann–Whitney test).

All but one of the 192 patients included in the analysis reported having had diarrhoea, and 160 patients reported

TABLE 1. Characteristics of the 192 *Campylobacter*-positive patients included in the final analysis

	Included patients			All patients
	Infection acquired			
Characteristics	In Finland	Abroad	Σ (*N* = 192)	(% of *N* = 363)
Gender				
Female	23 (52%)	88 (59%)	111 (58%)	52%
Male	21 (48%)	60 (41%)	81 (42%)	48%
Age (years)				
0–9	4 (9%)	4 (3%)	8 (4%)	6%
10–19	0	12 (8%)	12 (6%)	9%
20–29	6 (14%)	35 (24%)	41 (21%)	25%
30–39	7 (16%)	31 (21%)	38 (20%)	18%
40–49	9 (20%)	28 (19%)	37 (19%)	17%
50–59	10 (23%)	20 (14%)	30 (16%)	13%
60 and older	8 (18%)	18 (12%)	26 (14%)	10%
Median age (years)	46	37	39	35
Antimicrobial treatment				
Yes	34 (77%)	106 (72%)	140 (73%)	
No	10 (23%)	41 (28%)	51 (27%)	
Uncertain		1	1	

For comparison, the age and gender distribution of the patients to whom the questionnaires were originally sent is also shown.

having had fever. Characteristics indicating a more severe disease were reported by the patients as follows: 48 of 179 (27%) reported vomiting, 47 of 186 (25%) had had diarrhoea for at least 10 days, 24 of 133 (18%) reported having had bloody stools, and 31 of 189 (16%) reported having received treatment at a hospital ward for at least 2 days. The proportion of patients with no underlying disease did not significantly differ between those treated at hospital for at least 2 days (20 of 29; 69%) and those treated at hospital for <2 days or not at all (122 of 155; 79%) (p 0.25; chi-square test). No significant difference in the prevalence of any underlying disease was observed between domestically infected patients (13 of 44; 30%) and those presumably infected abroad (30 of 143; 21%) (p 0.24; chi-square test). The prevalence of underlying diseases did not significantly differ between those with severe symptoms (bloody stools, vomiting or diarrhoea lasting ≥10 days) and those who reported no such symptoms.

A large number of patients were treated with antimicrobials; 106 of 148 (72%) of patients presumably infected abroad and 34 of 44 (77%) of domestically infected patients had received antimicrobial drugs (Table 1). Macrolides (54%) and fluoroquinolones (49%) were the most commonly prescribed antimicrobial drugs. Multiple antimicrobials were prescribed for 19% of the patients. Among the patients with ciprofloxacin-susceptible isolates, 69 of 95 (73%) were treated with antimicrobials, whereas the figure for those infected with ciprofloxacin-resistant isolates was 71 of 97 (73%). Among those patients who reported having had bloody stools, 19 of 24 (79%) had been treated with antimicrobials, as compared to 77 of 109 (71%) of those who reported no blood in stools (p 0.46; Fisher's exact test).

The distribution of MIC values for ciprofloxacin for all 192 *Campylobacter* isolates is shown in Fig. 1. All domestic isolates except one were susceptible to ciprofloxacin, whereas isolates presumably obtained abroad were frequently resistant.

C. coli was isolated from 26 of 192 (14%) patients. Infection with *C. coli* was not associated with more severe symptoms or longer duration of illness than infection with *C. jejuni*. The proportion of isolates resistant to ciprofloxacin was 13 of 26 (50%) for *C. coli* isolates, as compared to 84 of 166 (51%) for *C. jejuni* isolates.

Bloody stools were significantly more often reported by patients with diarrhoea lasting 10 days or more (p 0.03; chi-square test) and by those who had not travelled abroad within 2 weeks prior to becoming ill (p 0.01; chi-square test). When analysed separately according to different age groups, bloody stools were associated with domestic infections only within the age group of 30–59 years (p 0.01; Mantel–Haenszel test), whereas an association between bloody stools and longer duration of diarrhoea was only observed in the youngest age group, 0–29 years (p 0.02; Mantel–Haenszel test). In the oldest-age group, 60–89 years, nobody reported having had bloody stools.

Of all the 192 *Campylobacter* isolates included, 97 (51%) were resistant to ciprofloxacin (MIC: ≥4 mg/L), whereas among the *Campylobacter* isolates causing bloody diarrhoea, 16 (67%) were highly susceptible to ciprofloxacin (MIC: 0.06–0.25 mg/L), as shown in Fig. 2. The distribution of ciprofloxacin MICs of the isolates differed significantly between the patients who reported bloody stools and those who did not (p 0.04; Mann–Whitney test). Hospitalization was not associated with any particular symptom or duration of symptoms. Among the patients treated at hospital for 2 days or more, 20 (65%) had ciprofloxacin-susceptible isolates. The distribution of ciprofloxacin MICs for the isolates from patients who received hospital treatment for at least 2 days was significantly different from the respective distribution for patients who had been treated at hospital for <2 days or not at all (p 0.01; Mann–Whitney test).

Discussion

Ciprofloxacin-resistant *C. jejuni* and *C. coli* isolates were not associated with severe disease, i.e. disease with bloody stools

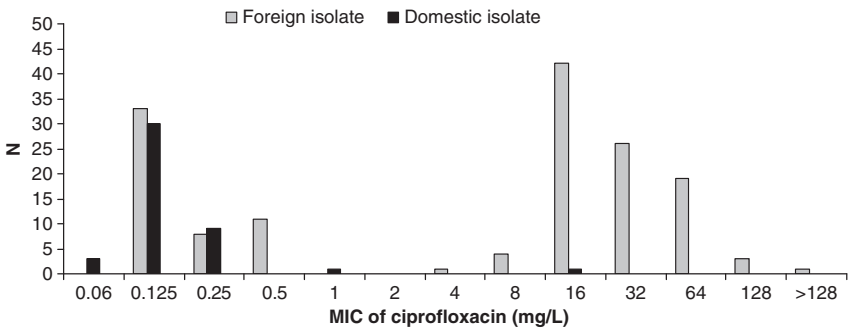

FIG. 1. The distribution of ciprofloxacin MICs for 192 *Campylobacter jejuni* and *Campylobacter coli* isolates. MIC values are shown separately for isolates acquired domestically and abroad.

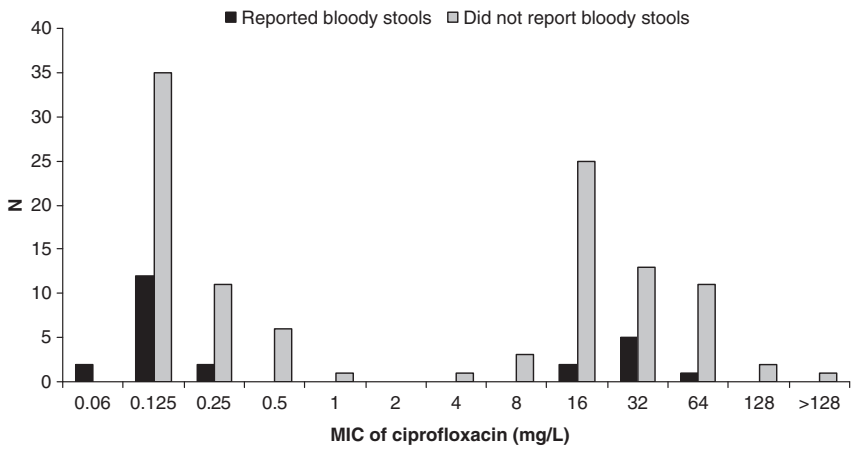

FIG. 2. The distribution of ciprofloxacin MICs for *Campylobacter* isolates from 24 patients reporting bloody stools and 109 patients who did not report bloody stools.

or diarrhoea lasting for ≥10 days or leading to hospital treatment.

Instead, highly ciprofloxacin-susceptible isolates seemed to cause a more invasive disease, as characterized by bloody stools, and reflected in a higher number of patients reporting hospitalization.

This study showed that 'domestic' *Campylobacter* isolates in Finland are still almost exclusively susceptible to ciprofloxacin, as only one of the isolates (2%) from the patients considered to have acquired infection domestically was resistant to ciprofloxacin. Bloody stools were significantly associated with domestically acquired infections within the age group of 30–59 years. This particular age group included a total of 105 patients (26 patients with domestically acquired infections and 79 with infections probably acquired abroad). None of the patients in the oldest age group reported bloody stools. In a Swedish study that provided no susceptibility data, a higher mortality rate was observed among patients with domestically acquired *Campylobacter* infections, which was speculated to be due to better health conditions of those who had been able to travel [19]. In the present study, patients with domestically acquired infections, although older in general, did not have underlying diseases significantly more often than those who had travelled abroad prior to becoming ill. Furthermore, bloody stools were only reported by young and middle-aged persons. Thus, neither older age nor impaired general health conditions explain the more unfavourable outcome of the domestically obtained infections characterized by bloody stools. This finding indicates either that domestic infections have a tendency to be more severe, or that the patients with a severe disease and infected in Finland may have been more active in answering and returning the questionnaires, compared to others.

The results of studies on whether resistance to fluoroquinolones increases the severity of *Campylobacter* infections have been taken to support and to reject that hypothesis. In an English study with a large number of patients, no differences in hospital admission or mean length of illness were observed when patients with ciprofloxacin-susceptible isolates and patients with resistant isolates were compared [11]. However, Nelson *et al.* [12] reported that persons with ciprofloxacin-resistant *Campylobacter* isolates had longer-lasting diarrhoea. In two separate Danish studies, quinolone resistance was associated with a longer duration of disease [13], and a greater risk of adverse events [14]. In contrast, in a recent re-analysis by Wassenaar *et al.* [16], no association between fluoroquinolone-resistant *Campylobacter* isolates and prolonged duration of diarrhoea could be observed. The current study supports the results of the re-analysis, and suggests that infections of domestic origin may actually cause a more severe disease, e.g. with bloody diarrhoea, which, on the other hand, is mostly attributed to isolates highly susceptible to ciprofloxacin.

The majority of the patients infected in Finland and of the patients presumably infected abroad were treated with antimicrobials. However, the impact of the administration of antibiotics on the course of the disease could not be analysed, because of scarce information on the timing and length of treatments. Although patients with infections caused by domestic ciprofloxacin-susceptible strains, and those with infections caused by highly ciprofloxacin-susceptible strains in general, were more likely to have been treated with effective antimicrobials, the disease in these cases was actually more severe, as concluded from more frequently reported bloody stools.

This finding is consistent with the conclusion of a previous meta-analysis that the overall effect of antimicrobial treatment on *Campylobacter* infection was only marginal [6]. The effect of antidiarrhoeal medication on the severity of symptoms was not considered in the present study. However, it would have been difficult to analyse the independent impact of antidiarrhoeal medicines on the outcome of the disease,

because the majority of the patients had been treated with antimicrobials.

The present study is based on information reported by the patients themselves, which has both advantages and limitations. The patients were able to describe the symptoms, treatment and other aspects of their illness in detail, and the information was easily interpreted and linked to the corresponding *Campylobacter* isolates. Only one case was excluded from this study due to a questionnaire that could not be interpreted, indicating that the questions were rather easy to understand. However, probably not all patients were reached, and reminders were not possible, because the information had to be sent to the patients' physicians first. Furthermore, some of the returned questionnaires were only partly answered. The patient cohort may, however, be regarded as quite representative, because over 50% of all patients diagnosed at the Helsinki University Central Hospital Laboratory as *Campylobacter*-positive during the 6-month period participated.

In conclusion, ciprofloxacin-susceptible isolates showed a tendency to cause bloody stools and lead to hospital treatment more often than ciprofloxacin-resistant isolates. This suggests that ciprofloxacin-susceptible isolates may be associated with a less favourable outcome of *Campylobacter* infection.

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Transparency Declaration

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