Antimicrobial Susceptibilities of *Campylobacter* Strains Isolated from Finnish Subjects Infected Domestically or from Those Infected Abroad

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The in vitro susceptibilities of 678 *Campylobacter jejuni* and *Campylobacter coli* strains isolated from stool samples of the same number of Finnish subjects were studied. A total of 523 patients, representing inhabitants from throughout Finland, had not traveled abroad within the 2 weeks prior to becoming ill, whereas 155 persons had presumably acquired their infections abroad. The antimicrobial agents studied were erythromycin, ciprofloxacin, levofloxacin, trovafloxacin, and moxifloxacin. The MICs of these antimicrobial agents were determined by the agar dilution method. The growth of all domestic isolates was inhibited by erythromycin at concentrations of 4 µg/ml, and for these isolates the fluoroquinolone MICs at which 90% of isolates are inhibited (MIC₉₀s) ranged from 0.06 to 0.5 µg/ml. For the foreign isolates, the erythromycin MIC₉₀ was still low (4 µg/ml), but their susceptibilities to fluoroquinolones were clearly reduced (MIC₉₀s, 8 to 64 µg/ml). Of the four different fluoroquinolones studied, ciprofloxacin was the least active (MIC₉₀, 64 µg/ml).

Campylobacter are the most common bacterial enteropathogens in developed countries (4, 15), and the species most commonly identified is *Campylobacter jejuni*. The number of reported *Campylobacter* infections has clearly increased in recent years in most developed countries. Important risk factors for the acquisition of *Campylobacter* infections include the handling or eating of chicken, the consumption of unpasteurized milk or contaminated water, and travel abroad (4). *Campylobacter* enteritis is usually self-limited, but if antimicrobial treatment is needed, macrolide and fluoroquinolone antimicrobials have been the recommended treatments (21).

Since the 1990s, rapid increases in the rates of *campylobacter* resistance to fluoroquinolones have been recognized in many countries (3, 6, 19, 20, 23). For instance, in Finland ciprofloxacin-resistant *campylobacter* were not found at all in 1980, although 10 years later they composed 9% of the strains studied (16). Later, the rate of ciprofloxacin resistance clearly increased, and in 1999, the majority of strains isolated from Finnish patients after travel to Spain or Thailand were resistant (15). Resistance to fluoroquinolones has been suggested to be due to the use of quinolones in animal production industries such as the poultry industry (3, 23), and recently, rapid and persistent resistance was actually shown to develop in chickens treated with fluoroquinolones (9, 12). In Finland, quinolones are not used in the poultry production industry, and therefore, fluoroquinolone resistance has been extremely rare among domestically acquired *campylobacter* isolates (15).

During a seasonal peak of *Campylobacter* infections in Finland in 1999, human stool culture isolates of domestic origin were collected nationwide. The aim of the present study was to

test the in vitro susceptibilities of representative domestic *Campylobacter* isolates to fluoroquinolones and erythromycin. In addition, as we wanted to compare the activities of some newer fluoroquinolones against *campylobacter*, *Campylobacter* isolates of foreign origin (which are more likely to show reduced susceptibilities to fluoroquinolones) were included.

MATERIALS AND METHODS

***Campylobacter* strains.** All diagnostic clinical laboratories in Finland routinely culturing *campylobacter* were requested to collect human stool culture isolates of domestic origin during a 3-month period of a seasonal peak of *Campylobacter* infections in 1999 (1 July through 30 September) and to send the isolates to Helsinki University Central Hospital Laboratory Diagnostics. A total of 551 *Campylobacter* isolates (533 *C. jejuni* isolates and 18 *Campylobacter coli* isolates) were received. Isolates negative for hippurate hydrolysis, as tested with sodium hippurate tablets (Rosco Diagnostica, Taastrup, Denmark), were regarded as *C. coli*. Of these originally collected isolates, 511 *C. jejuni* isolates and 12 *C. coli* isolates were available for susceptibility testing. In addition, a total of 155 *Campylobacter* isolates (134 *C. jejuni* isolates and 21 *C. coli* isolates) presumably of foreign origin were included. The isolates of foreign origin were collected during the same seasonal peaks from 1999 to 2001 during which the domestic isolates were collected, but the isolates of foreign origin were collected only at the Helsinki University Central Hospital Laboratory Diagnostics. All isolates were stored in skim milk at –70°C before they were tested.

Patients. Patients whose *Campylobacter* infections were regarded as being of domestic origin had not been abroad for 2 weeks prior to becoming ill. The most common travel destinations of patients with infections of foreign origin were as follows (the travel destination information for the patients was given when the stool samples were sent to be cultured): Spain, 38 patients; Thailand, 17 patients; Greece, 13 patients; Portugal, 10 patients; and Estonia, 10 patients. All cases were sporadic, and consecutive isolates related to outbreaks were not included.

Determination of MICs. The antimicrobials studied were erythromycin (Sigma, St. Louis, Mo.), ciprofloxacin and moxifloxacin (Bayer, Leverkusen, Switzerland), levofloxacin (Aventis Pharma, S.A. Antony Cedex, France), and trovafloxacin (Pfizer, Groton, Conn.). Erythromycin was initially dissolved in a 50% solution of ethanol, and the fluoroquinolones were first dissolved in 0.1 N NaOH. Serial dilutions of all the agents studied were prepared with sterile water. Antibacterial agent-containing medium was prepared within 24 h of use and stored overnight at room temperature. MICs were determined by an agar dilution method with Mueller-Hinton agar plates (BBL, Cockeysville, Md.) supple-

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TABLE 1. MICs of five antimicrobial agents for *C. jejuni* and *C. coli* strains of domestic and foreign origin

Antimicrobial agent	Strain origin ^a	MIC ($\mu\text{g/ml}$)		
		Range	50%	90%
Erythromycin	Domestic	0.25–4	1	2
	Foreign	0.5–1,024	1	4
Ciprofloxacin	Domestic	0.06–64	0.25	0.5
	Foreign	0.03–128	1	64
Moxifloxacin	Domestic	0.015–4	0.125	0.125
	Foreign	0.015–32	0.25	8
Levofloxacin	Domestic	0.015–16	0.125	0.25
	Foreign	0.06–64	1	16
Trovafoxacin	Domestic	0.015–8	0.06	0.06
	Foreign	0.03–32	0.125	16

^a A total of 523 strains were isolated from patients with no foreign travel within 2 weeks prior to becoming ill. A total of 155 strains were isolated from patients with foreign travel prior to their illness.

mented with 5% horse blood. Isolates were inoculated into Mueller-Hinton broth and incubated at 42°C for 24 h. The bacterial suspensions were diluted to match the turbidity of a 0.5 McFarland standard, and a multipoint inoculator was used to apply approximately 10^5 CFU per spot. *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *C. jejuni* 143483 were included as control organisms. The plates were incubated in a microaerobic atmosphere for 48 h. The lowest concentration that completely inhibited the visible growth of the organism was recorded as the MIC.

RESULTS

The MICs of erythromycin and the four fluoroquinolones (ciprofloxacin, moxifloxacin, levofloxacin, and trovafoxacin) are shown in Table 1. Among the domestic isolates, growth was inhibited by erythromycin at 4 $\mu\text{g/ml}$ or less and the fluoroquinolone MICs at which 90% of isolates are inhibited (MIC_{90} s) ranged from 0.06 to 0.5 $\mu\text{g/ml}$. Of the few domestic isolates for which MICs were clearly higher (Fig. 1A), the ciprofloxacin MIC was greater than 16 $\mu\text{g/ml}$ for only one isolate. For the isolates presumably of foreign origin, erythromycin MICs were ≥ 8 $\mu\text{g/ml}$ for only 3% of the isolates, but the MICs of the fluoroquinolones for 34 to 49% of the strains were at or above the concentrations (2 to 8 $\mu\text{g/ml}$) suggested as breakpoints for aerobic bacteria (2, 5, 10).

The range of erythromycin MICs was narrow for the domestic isolates, but the erythromycin MICs were very high (1,024 $\mu\text{g/ml}$) for some isolates (all *C. coli*) of foreign origin. For the domestic isolates, the MIC_{50} s and MIC_{90} s of the fluoroquinolones were almost identical (Table 1). In general, among the foreign isolates, the MICs were either low or high, with intermediate values being quite rare (Fig. 1B).

Among the isolates of foreign origin, the levofloxacin MICs for 34% of the isolates were at or above the concentration (≥ 8 $\mu\text{g/ml}$) suggested as the breakpoint for aerobic bacteria. However, for 93 isolates (including both domestic isolates and isolates of foreign origin), ciprofloxacin MICs were ≥ 4 $\mu\text{g/ml}$ (breakpoint concentration for aerobic bacteria, 4 $\mu\text{g/ml}$). In the present study, the MICs of both erythromycin and ciprofloxacin for four isolates were at or above the breakpoint concentrations used for aerobic bacteria. None of the other fluoroquinolones studied was effective against these four strains.

FIG. 1. MICs of ciprofloxacin, levofloxacin, moxifloxacin, and trovafoxacin (the results for each drug are indicated by the bars from left to right, respectively, for each concentration) for human *Campylobacter* strains isolated from Finnish patients with no foreign travel ($n = 523$ strains) (A) and with foreign travel ($n = 155$ strains) (B) prior to becoming ill.

Spain and Thailand were the most common travel destinations of the Finnish patients in this study. The ciprofloxacin MICs for the majority of the campylobacter strains isolated from patients who had traveled to Spain (61%) and Thailand (94%) were ≥ 4 $\mu\text{g/ml}$. Strains for which ciprofloxacin MICs were high were also frequently isolated from patients after travel to Portugal (80%) but were less frequently isolated from patients after travel to Greece and Estonia (23 and 20%, respectively).

DISCUSSION

The present study shows that the activities of fluoroquinolones against *C. jejuni* and *C. coli* strains of domestic origin in Finland are still good. Furthermore, the susceptibilities of both domestic and foreign *Campylobacter* isolates to erythromycin have remained high. Ciprofloxacin MICs were ≥ 4 $\mu\text{g/ml}$ for almost half of the campylobacter strains isolated from patients after foreign travel. Of the four different fluoroquinolones studied, the campylobacter strains were least susceptible to ciprofloxacin (MIC_{90} , 64 $\mu\text{g/ml}$).

Several reports on fluoroquinolone resistance among campylobacter isolates appeared at the beginning of the 1990s (3, 18). In Finland, fluoroquinolone resistance was very rare among isolates cultured before 1981, but as early as 10 years later, 9% of isolates showed high levels of resistance to ciprofloxacin (16). In that study, fluoroquinolone resistance was mainly demonstrated in campylobacter strains isolated from patients who had traveled abroad, but resistance among domestically acquired isolates was rare, as we also demonstrated later in other studies (8, 15). The results of the present study further support these findings; fluoroquinolone MICs were high for only 2 to 3% of all domestic isolates. In the present study, isolates for which fluoroquinolone MICs were high were especially frequently recovered after travel to Thailand, supporting the findings that others published earlier (13, 22). About half of the campylobacter strains isolated from patients in Spain were previously reported to be resistant to ciprofloxacin (7), and this was also reflected in our study, as the ciprofloxacin MICs were ≥ 4 $\mu\text{g/ml}$ for 61% of the strains isolated from patients after travel to Spain.

The emergence of fluoroquinolone resistance among campylobacters was previously proposed to be due to the frequent use of quinolones in the poultry production industry (3). Later, in the United States, fluoroquinolone resistance first appeared among domestically acquired *Campylobacter* isolates after the use of these antimicrobial agents in the poultry production industry (23). It has now also been shown that the development of fluoroquinolone resistance develops rapidly in campylobacter-infected broilers treated with quinolones (9, 12). High rates of resistance to fluoroquinolones have been demonstrated in campylobacter strains isolated from food animals (27), whereas on farms where quinolones have not been used, the levels of resistance have been lower (14). In Finland, quinolones are not used in the animal production industry, which is thus reflected in the low MIC₉₀s of fluoroquinolones for domestic campylobacters, as shown in the present study.

Despite the rapid increase in the MICs of fluoroquinolones, the activity of erythromycin has remained good (7, 23), as was also shown in the present study. Although the newer macrolides, like azithromycin, show cross-resistance with erythromycin and thus offer no advantage where erythromycin-resistant strains are concerned (17), their more favorable pharmacological properties may speak for their use. However, increasing levels of resistance to macrolides have also been reported (13), and the high MICs of both macrolides and fluoroquinolones for isolates pose a problem. *C. coli* strains have been shown to be less susceptible to erythromycin more often (27), and the MICs for these strains may be especially high (26), as was also the case for a few strains in the present study. The source for the high-level resistance to erythromycin in *C. coli* is not known but could be associated with the use of macrolides (such as tylosin) in the treatment of dysentery in pigs (25).

In the present study, four different fluoroquinolones were studied. Cross-resistance between the different fluoroquinolones was expected, as their modes of action (inhibition of DNA gyrase) are similar. Therefore, it was not surprising that most of the *Campylobacter* strains for which ciprofloxacin MICs were high were also not inhibited by low concentrations of other fluoroquinolones. In one earlier study with only a few ciprofloxacin-resistant *C. jejuni* strains, the activity of moxi-

floxacin was found to be superior to that of ciprofloxacin (24). In the present study, the MIC₉₀ of moxifloxacin (8 $\mu\text{g/ml}$) was lower than that of ciprofloxacin (64 $\mu\text{g/ml}$) for foreign isolates.

Early treatment with fluoroquinolones or macrolides has been shown to shorten the duration of campylobacteriosis and the excretion of bacteria (21), but treatment failures among patients infected with fluoroquinolone-resistant isolates have been reported (11). Although azithromycin has even been suggested as an alternative for the empirical treatment of traveler's diarrhea, which is often caused by campylobacters (1), the disease is self-limited in most cases and antimicrobial treatment is not needed at all. In severe cases, if antimicrobial therapy is needed, the drug of choice still remains a macrolide. Furthermore, on the basis of the in vitro results of the present study, it is unlikely that the newer fluoroquinolones would offer any significant advantage over ciprofloxacin if infections caused by isolates for which the MICs of both macrolides and ciprofloxacin are high need to be treated.

In summary, for the 523 domestic *Campylobacter* isolates collected throughout Finland, the erythromycin MIC₉₀ was 2 $\mu\text{g/ml}$ and the MIC₉₀s of the fluoroquinolones ranged from 0.06 to 0.5 $\mu\text{g/ml}$. For the 155 campylobacter strains isolated from patients after foreign travel, the MIC₉₀s of the fluoroquinolones were 8 to 64 $\mu\text{g/ml}$, but the MIC₉₀ of erythromycin was only 4 $\mu\text{g/ml}$. Among the four different fluoroquinolones studied, ciprofloxacin was found to be the least active.

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