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gyrA Polymorphism in *Campylobacter jejuni*: Detection of *gyrA* Mutations in 162 *C. jejuni* Isolates by Single-Strand Conformation Polymorphism and DNA Sequencing

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Mutations in the quinolone resistance-determining region of the *gyrA* gene from 138 ciprofloxacin-resistant (MIC, ≥4 μg/ml) and 24 ciprofloxacin-susceptible (MIC, ≤1 μg/ml) clinical *Campylobacter jejuni* isolates were subjected to single-strand conformation polymorphism analysis and sequencing. All of the isolates could be assigned to three genotypic clusters based on silent mutations. All resistant isolates had a point mutation at codon 86.

Fluoroquinolones are the antimicrobials most commonly used for treatment of adults with *Campylobacter* infections. During the 1990s, however, resistance to this antimicrobial group has increased worldwide among *Campylobacter* spp. (3, 8, 9, 12–15, 18, 19, 21, 22). The primary target of the fluoroquinolones in *Campylobacter jejuni* has been shown to be DNA gyrase, a type II topoisomerase that is an essential enzyme for DNA replication (2, 20). DNA gyrase is composed of two A subunits and two B subunits encoded by the *gyrA* and *gyrB* genes, respectively. A point mutation in the quinolone resistance-determining region (QRDR) of the *gyrA* gene at codon 86 (ACA to ATA), substituting isoleucine for threonine, is the most common cause of class-wide fluoroquinolone resistance among *C. jejuni* isolates (17, 24, 27). There have also been anecdotal reports of mutations leading to additional amino acid changes, as well as silent nucleotide mutations (1, 24, 27).

The purpose of the present study was to explore the molecular epidemiology of the QRDR of *gyrA* among *C. jejuni* isolates. To this end, we analyzed the QRDR (codons 69 to 120) of *gyrA* from 138 ciprofloxacin-resistant and 24 ciprofloxacin-susceptible clinical *C. jejuni* isolates by single-strand conformation polymorphism (SSCP) analysis and DNA sequencing.

A total of 162 clinical *C. jejuni* isolates collected in Finland between 1995 and 2000 were included in this study. All isolates were from stool samples, and the majority (n = 158) were collected from Finnish travelers returning from 22 different countries. Four isolates were from Finnish patients without any travel history. One hundred thirty-eight of the isolates were resistant to ciprofloxacin (MIC, ≥4 μg/ml), and 24 were ciprofloxacin susceptible (MIC, ≤1 μg/ml). The ciprofloxacin MICs were 8 μg/ml for 10 of the ciprofloxacin-resistant isolates and ≥16 μg/ml for 128 of the ciprofloxacin-resistant isolates. The resistant isolates were from travelers to 20 different countries, the majority being from travelers to Spain (34%) and Thailand (21%). The susceptible isolates were from travelers to 12 countries, the number of isolates varying between one and three from each country.

Chromosomal DNA from each strain was prepared by boiling for 10 min and proteinase K (Promega, Madison, Wis.) digestion for 30 min. DNA was amplified by PCR for the *gyrA* gene as described by Wang et al. (24). One microliter of [α-^{32}P]dCTP (activity, 10 μCi/μl) was added to each PCR mixture for SSCP. SSCP was run in accordance with the manufacturer's instructions on nondenaturing MDE Gel (BioWhittaker Molecular Applications, Rockland, Maine) at 4°C. Autoradiograms were incubated overnight. The QRDR of the *gyrA* gene was sequenced in both directions with PCR primers from one or more representative isolates belonging to a different SSCP pattern (Table 1). Sequencing of a nonradioactive PCR product was performed with an ABI Prism BigDye Terminator Kit (Applied Biosystems, Foster City, Calif.). All of the *gyrA* sequences of the different SSCP patterns were confirmed by PCR and sequencing with two additional oligonucleotide primers, Cj-gyrA-393 (5′-CTTTGCCTGACGCAAGAG-3′) and Cj-gyrA-759r (5′-TCGCTTTCTGAACCATCA-3′). One representative isolate of every different SSCP pattern was confirmed to be *C. jejuni* by 16S rRNA gene sequencing (6). *C. jejuni* RH3583 was used as a control strain.

On the basis of the SSCP patterns of the QRDR of *gyrA*, all 138 ciprofloxacin-resistant *C. jejuni* isolates could be distinguished from the 24 susceptible isolates. Eight different SSCP patterns were found: five among the ciprofloxacin-resistant isolates and three among the ciprofloxacin-susceptible isolates. These patterns

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TABLE 1. SSCP patterns and QRDRs of *gyrA* of 138 ciprofloxacin-resistant and 24 ciprofloxacin-susceptible *C. jejuni* isolates

SSCP pattern	No. (%) of isolates analyzed[a]		Ciprofloxacin MIC range (μg/ml)	Nucleic acid codons and corresponding amino acids of *C. jejuni* QRDR of *gyrA*[b]													
	CIP-S	CIP-R		Codon	Amino acid	Codon	Amino acid	Codon	Amino acid	Codon	Amino acid	Codon	Amino acid	Codon	Amino acid	Codon	Amino acid
I_{CIP-S}	7 (29)		0.064–0.5	GGT	Gly-78	CAC	His-81	ACA	Thr-86	GAT	Asp-90	CCA	Pro-104	GGC	Gly-110	AGT	Ser-119	GCC	Ala-120
I_{CIP-R1}		21 (15)	16–>64	– – –	–	– – –	–	– T –	Ile-86	– – –	–	– – –	–	– – –	–	– – –	–	– – –	–
I_{CIP-R2}		1 (0.7)	>64	– – –	–	– – –	–	– T –	Ile-86	A – –	Asn-90	– – –	–	– – –	–	– – –	–	– – –	–
I_{CIP-R3}		1 (0.7)	16	– – –	–	– – –	–	– T –	Ile-86	– – –	–	T – –	Ser-104	– – –	–	– – –	–	– – –	–
II_{CIP-S}	2 (8)		0.25–0.5	– – C	–	– – –	–	–	–	– – –	–	– – –	–	– – C	–	– – –	–	– – –	–
III_{CIP-S}	15 (63)		0.125–1	– – –	–	– – T	–	–	–	– – –	–	– – –	–	– – C	–	– – T	–		
III_{CIP-R1}		113 (82)	8–>64	– – –	–	– – T	–	– T –	Ile-86	– – –	–	– – –	–	– – C	–	– – T	–		
III_{CIP-R2}		2 (1.4)	32	– – –	–	– – T	–	– T –	Ile-86	– – –	–	– – T	–	– – C	–	– – T	–		

[a] Number of isolates sequenced per SSCP pattern: I_{CIP-S}, 4; I_{CIP-R1}, 2; I_{CIP-R2}, 1; I_{CIP-R3}, 1; II_{CIP-S}, 2; III_{CIP-S}, 9; III_{CIP-R1}, 12; III_{CIP-R2}, 2. CIP-S, ciprofloxacin susceptible (MIC, ≤1 μg/ml); CIP-R, ciprofloxacin resistant (MIC, ≥4 μg/ml).

[b] –, no change.

were designated as follows. Roman numerals I to III were used to define the three susceptible SSCP patterns. The subscript CIP-S indicates ciprofloxacin susceptibility, and the subscript CIP-R indicates ciprofloxacin resistance. The numerals after the subscript CIP-R define resistant variants within each cluster.

The most common susceptible SSCP pattern was III_{CIP-S}, with 15 (63%) isolates, followed by patterns I_{CIP-S} and II_{CIP-S}, with 7 (29%) and 2 (8%) isolates, respectively (Fig. 1). The most common resistant SSCP pattern, III_{CIP-R1}, was exhibited by 113 (82%) resistant isolates. Pattern I_{CIP-R1} was exhibited by 21 (15%) resistant isolates, pattern III_{CIP-R2} was exhibited by 2 (1.4%) resistant isolates, and patterns I_{CIP-R2} and I_{CIP-R3} were exhibited by 1 resistant isolate each.

We sequenced the *gyrA* QRDRs of 1 to 12 representative *C. jejuni* isolates of all eight defined SSCP patterns (Table 1). Compared to ciprofloxacin-susceptible *C. jejuni* UA580 (24) and *C. jejuni* NCTC 11168 (11), all of the resistant isolates sequenced had a point mutation at codon 86 (ACA to ATA), substituting isoleucine for threonine. Two resistant isolates harbored one additional mutation leading to an amino acid change each. In addition, silent mutations were identified among isolates with SSCP patterns III_{CIP-R1} and III_{CIP-R2} (Table 1).

Among members of the ciprofloxacin-susceptible *C. jejuni* population, the QRDRs of *gyrA* from the representative isolates (*C. jejuni* IH 111169 and *C. jejuni* IH 111607; Fig. 1 and Table 1) showing SSCP pattern I_{CIP-S} were identical to those of *C. jejuni* UA580 (24) and *C. jejuni* NCTC 11168 (11). None of the susceptible *C. jejuni* isolates had mutations leading to amino acid changes. However, silent nucleic acid mutations were observed. The QRDR of *gyrA* from the representative isolates (*C. jejuni* IH 41670 and *C. jejuni* IH 111677; Fig. 1 and Table 1) showing SSCP pattern II_{CIP-S} and from those showing SSCP pattern III_{CIP-S} (*C. jejuni* RH 3583 and *C. jejuni* IH 41957; Fig. 1 and Table 1) had mutations in two and three codons, respectively.

Genetic variation of the QRDR of *gyrA* in *C. jejuni* was demonstrated here by the finding of eight SSCP patterns among our whole study population and, in particular, by identification of three defined SSCP patterns among members of the ciprofloxacin-susceptible population. Considering the relatively small number of susceptible isolates studied, it seems likely that additional variants of *C. jejuni* do exist. This polymorphism is surprising because bacterial topoisomerase genes are generally highly conserved even across the borders of the bacterial genera (4, 5, 7, 10, 16, 23, 24). However, the present findings are in line with the results of the *C. jejuni* genome sequencing project reported by Parkhill et al. (11), who found hypervariability in the sequence of *C. jejuni* NCTC 11168. They suspected that *C. jejuni* might be lacking in DNA repair functions. They also assumed that it may not be possible to produce a single definitive sequence for the *C. jejuni* genome (11).

Since fluoroquinolones are currently recommended as the first-choice empirical therapy for adult patients with suspected bacterial gastroenteritis, recognition of *C. jejuni* as the causative agent of a patient's disease is important, as is its susceptibility to antimicrobial agents. Many studies have shown that the Thr-86-Ile mutation in the QRDR of *gyrA* is the most common mechanism causing ciprofloxacin resistance in *C. je-*

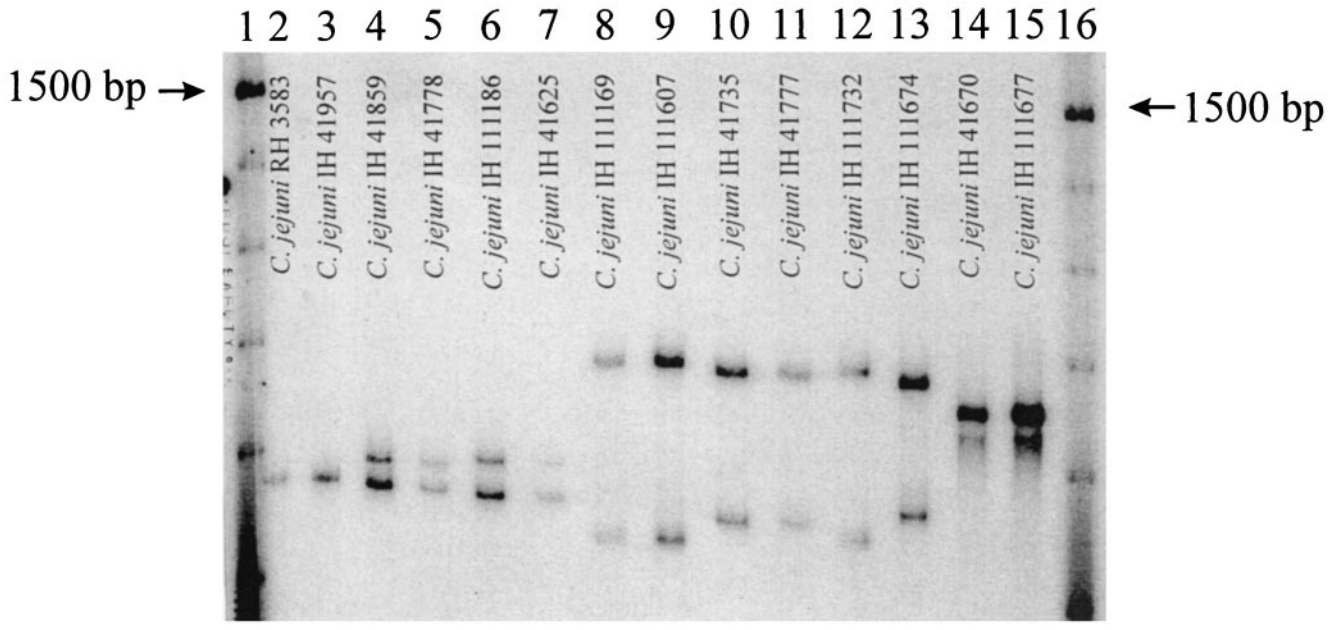

FIG. 1. Autoradiogram of SSCP patterns of the QRDR of *gyrA* of *C. jejuni* isolates. SSCP patterns are designated as explained in the text and Table 1. Lanes: 1 and 16, 100-bp DNA ladder (Gibco BRL, Life Technologies, Inc., Gaithersburg, Md.) labeled with [γ-^{32}P]ATP by using T4 polynucleotide kinase; 2 and 3, ciprofloxacin-susceptible isolates showing pattern $III_{CIP\text{-}S}$; 4 and 5, ciprofloxacin-resistant isolates showing pattern $III_{CIP\text{-}R1}$; 6 and 7, ciprofloxacin-resistant isolates showing pattern $III_{CIP\text{-}R2}$; 8 and 9, ciprofloxacin-susceptible isolates showing pattern $I_{CIP\text{-}S}$; 10 and 11, ciprofloxacin-resistant isolates showing pattern $I_{CIP\text{-}R1}$; 12, ciprofloxacin-resistant isolate showing pattern $I_{CIP\text{-}R2}$; 13, ciprofloxacin-resistant isolate showing pattern $I_{CIP\text{-}R3}$; 14 and 15, ciprofloxacin-susceptible isolates showing pattern $II_{CIP\text{-}S}$.

juni (17, 24, 27). Consequently, several molecular techniques have been developed to detect this particular mutation (25–27). If specific primers or probes are used, the nucleic acid variation on the areas described in this report or in previous reports (1, 24, 27) might confuse the primer-target interaction and cause false results. This should be considered in clinical laboratories if traditional susceptibility testing methods are replaced with modern PCR- or hybridization-based methods to detect the mutation at codon 86 (Thr to Ile).

A total of 130 ciprofloxacin-resistant and 23 ciprofloxacin-susceptible *C. jejuni* isolates from patients with an identified travel history were evaluated for their SSCP pattern profiles (Table 2). Of the five different resistant genotypic variants, two SSCP patterns were dominant while the other three were exhibited by merely one or two resistant isolates. The main pattern, defined as $III_{CIP\text{-}R1}$, which was presented by 113 (82%) resistant isolates, has also prevailed in earlier studies (27). This pattern seems to have spread all over the world, whereas pattern $I_{CIP\text{-}R1}$ was found mainly in Europe. Unexpectedly, pattern $I_{CIP\text{-}S}$, which is identical to the *C. jejuni* wild-type sequence described by Wang et al. (24), was not our leading susceptible variant. Pattern $III_{CIP\text{-}S}$ exceeded this wild-type pattern in frequency, with a percentages of 63 versus 29%.

In conclusion, consistent with earlier findings, all of the resistant *C. jejuni* isolates sequenced here had the Thr-86-Ile mutation in the QRDR of *gyrA*. Only this mutation distinguished the two main resistant genotypic variants from the two main susceptible variants, suggesting a common origin. Considering that three different SSCP patterns were identified among the relatively small number of susceptible isolates studied, it seems likely that additional variants of *C. jejuni* exist. Polymorphism of the QRDR of *gyrA* should be considered when any PCR- or hybridization-based method is used to detect the Thr-86-Ile mutation in *gyrA* as an indication of *C. jejuni* fluoroquinolone resistance.

TABLE 2. Geographical distribution of the SSCP patterns of *gyrA* of 130 ciprofloxacin-resistant and 23 ciprofloxacin-susceptible *C. jejuni* isolates

Continent (no. of countries)	No. of isolates[a]		No. of isolates with SSCP pattern:[b]							
	CIP-S	CIP-R	$I_{CIP\text{-}S}$	$I_{CIP\text{-}R1}$	$I_{CIP\text{-}R2}$	$I_{CIP\text{-}R3}$	$II_{CIP\text{-}S}$	$III_{CIP\text{-}S}$	$III_{CIP\text{-}R1}$	$III_{CIP\text{-}R2}$
Asia (10)	7	59	1	3		1		6	54	1
Africa (3)	4	5						4	5	
Central America (1)	0	1							1	
Europe (9)	12	65	6	16	1		2	4	48	

[a] CIP-S, ciprofloxacin susceptible (MIC, ≤1 mg/ml); CIP-R, ciprofloxacin-resistant (MIC, ≥4 mg/ml).
[b] SSCP patterns are designated as explained in the text and Table 1.

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