Characterization of *Campylobacter jejuni* DNA gyrase as the target of quinolones

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Abstract

Quinolones have long been used as the first-line treatment for *Campylobacter* infections. However, an increased resistance to quinolones has raised public health concerns. The development of new quinolone-based antibiotics with high activity is critical for effective, as DNA gyrase, the target of quinolones, is an essential enzyme for bacterial growth in several mechanisms. The evaluation of antibiotic activity against *Campylobacter jejuni* largely relies on drug susceptibility tests, which require at least 2 days to produce results. Thus, an *in vitro* method for studying the activity of quinolones against the *C. jejuni* DNA gyrase is preferred. To identify potent quinolones, we investigated the interaction of *C. jejuni* DNA gyrase with a number of quinolones using recombinant subunits. The combination of purified subunits exhibited DNA supercoiling activity in an ATP dependent manner. Drug concentrations that inhibit DNA supercoiling by 50% (IC$_{50}$s) of 10 different quinolones were estimated to range from 0.4 (sitafloxacin) to >100 µg/mL (nalidixic acid). Sitafloxacin showed the highest inhibitory activity, and the analysis of the quinolone structure-activity relationship demonstrated that a fluorine atom at R-6 might play the important role in the inhibitory activity against *C. jejuni* gyrase. Measured quinolone IC$_{50}$s correlated well with minimum inhibitory concentrations ($R = 0.9943$). These suggest that the *in vitro* supercoiling inhibition assay on purified recombinant *C. jejuni* DNA gyrase is a useful and predictive technique to monitor the antibacterial potency of quinolones. And furthermore, these data suggested that sitafloxacin might be a good candidate for clinical trials on campylobacteriosis.

Key words: DNA gyrase/Quinolones/*Campylobacter jejuni*
Introduction

*Campylobacter jejuni* infection is one of the most commonly identified bacterial causes of gastroenteritis worldwide, and it occurs more frequently than infections caused by other enteric pathogens. *Campylobacter* infection causes diarrhea in approximately 400-500 million people globally each year [1, 2]. Most patients with *Campylobacter* infection develop a self-controlled condition that does not require antibiotics. Nevertheless, an antibiotic treatment is recommended when the *Campylobacter* infection is severe or affects an immunocompromised host [3]. Quinolones are a family of antimicrobials for the treatment of *Campylobacter* infections in human and animals [1, 2]. However, the introduction of quinolones in veterinary medicine has been reported to cause an emergence of quinolone-resistant *Campylobacter* [4, 5, 6]. In fact, quinolone resistance in *Campylobacter* from food animals is now recognized as an emerging public health problem [3, 7]. Testing for quinolone susceptibility is essential to provide guidance to physicians and veterinarians on the appropriate treatment for *Campylobacter* infections. *C. jejuni* is a slow-growing bacterium that requires microaerophilic conditions and supplemented growth media. Variations in the culture media and the incubation conditions, namely, atmosphere, temperature, and time of incubation, could affect the results of antimicrobial susceptibility tests. Therefore, it is highly desirable to develop a simple and rapid test for quinolone susceptibility in *Campylobacter*.

Quinolones belong to a family of broad-spectrum synthetic antimicrobials. In bacteria, the target of quinolones are the essential enzymes DNA gyrase and DNA topoisomerase IV, belonging to the bacterial type II topoisomerase. DNA gyrase is
unique in that it catalyzes the negative supercoiling of DNA and is essential for DNA replication, transcription and recombination [8]. On the contrary, topoisomerase IV has a specialized role in chromosome segregation. Complete genome sequencing of *C. jejuni* revealed the lack of genes encoding topoisomerase IV [9, 10, 11] and thus DNA gyrase turned out to be the sole target of quinolones in *Campylobacters*. Quinolone inhibit DNA supercoiling by stabilizing the complex between gyrase and the cleaved DNA, interrupting the propagation of the replication fork. When the DNA gyrase cleaves the DNA, the antibiotics prevent relegation of the broken strand, resulting in a quinolone-enzyme-DNA complex that leads to inhibition of DNA replication [12, 13]. However, because of increased quinolone resistance in *C. jejuni*, an *in vitro* method that would accelerate the identification of more potent quinolones against *C. jejuni* is needed for its treatment and effective control.

The DNA gyrase consists of two subunits A (GyrA) and B (GyrB) encoded by *gyr*A and *gyr*B, respectively. In order to measure inhibitory activity against *C. jejuni*, we reconstituted DNA gyrase *in vitro*, expressed both GyrA and GyrB in *Escherichia coli*, and used their purified forms to evaluate a group of 10 quinolones.

**Materials and Methods**

**Reagents**

Ciprofloxacin (CIP), gatifloxacin (GAT), levofloxacin (LVX), sparfloxacin (SPX), enrofloxacin (ENR) and ofloxacin (OFX) were purchased from LKT Laboratories, Inc. (St. Paul, MN). Oxolinic acid (OXO) and nalidixic acid (NAL) were purchased from Wako Pure Chemicals Ltd. (Tokyo, Japan). Moxifloxacin (MXF) was obtained from Toronto Research Chemicals Inc. (Toronto, Ontario, Canada). Sitafloxacin (SIT) was a
gift from Daiichi Sankyo Pharmaceutical, Co., Ltd. (Tokyo, Japan). Oligonucleotide primers were synthesized by Life Technologies (Carlsbad, CA). A Ni-nitrolotriacetic acid (Ni-NTA) protein purification kit was purchased from Life Technologies. Restriction enzymes were obtained from New England BioLabs, Inc. (Ipswich, MA). Supercoiled and relaxed pBR322 DNA were purchased from John Innes Enterprises Ltd. (Norwich, United Kingdom). A protease inhibitor cocktail (Complete Mini, EDTA free) was purchased from Roche Applied Science (Mannheim, Germany).

**Bacterial strains and plasmids**

*Escherichia coli* strain TOP-10 (Life Technologies Corp., Carlsbad, CA) was used as the cloning host. The pUC118-*HincII*/BAP plasmid (Takara Bio, Kyoto, Japan) was used to clone amplified DNA fragments. *Escherichia coli* strains BL-21 (DE3)/pLysS (Merck KGaA, Darmstadt, Germany) were used for protein expression. Vector plasmid pET-20b (+) (Merck KGaA) was used to construct expression plasmids for *C. jejuni* proteins GyrA and GyrB.

**Quinolones susceptibility testing**

*Campylobacter jejuni* ATCC33560 was grown on Mueller-Hinton agar (MHA; Oxoid Ltd., Basingstoke, UK), and the minimum inhibitory concentrations (MICs) for this strain were determined by a broth dilution method. Briefly, 1 µl of suspension was inoculated into Mueller-Hinton broth (MHB; Oxoid Ltd.) supplemented with 5% defibrinated sheep blood and containing 2-fold serial dilutions of the quinolone. The microdilution tray was incubated at 37 °C under microaerophilic conditions (10% CO₂, 5% O₂, and 85% N₂) for 48 hours. The MIC was predetermined as the lowest concentration of quinolone to cause a complete growth inhibition.

**Construction of DNA gyrase expression vectors**
DNA fragments encoding gyrA and gyrB were amplified by polymerase chain reaction using the primers listed in Table 1. The reaction mixture (25 µl) consisted of primeSTAR GXL buffer (Mg²⁺ plus); 200 µM each of dATP, dCTP, dGTP, and dTTP; 10 ng DNA from C. jejuni ATCC33560; 2.0 units of PrimeSTAR GXL DNA polymerase (Takara Bio Inc., Kyoto, Japan); and 0.4 µM of each primer. PCR was carried out in an iCycle Thermal Cycler (Bio-Rad Laboratories GmbH, California, US) under the following amplification conditions: pre-denaturation at 98 ºC for 2 min, 35 cycles of denaturation at 98 ºC for 10 sec, annealing 50 ºC for 10 sec, extension 72 ºC for 3 min, and a final extension step at 72 ºC for 2 min. The PCR products corresponding to the 2.5-kb gyrA and 2.3-kb gyrB fragments were ligated into the pUC118-HincII/BAP plasmid and transformed into E. coli Top10 competent cells, according to the manufacturer’s instructions. Recombinant plasmids were recovered from white colonies and digested with Nde I and Xho I, and the obtained DNA fragments were ligated into Nde I-Xho I-digested pET-20b and transformed into E. coli Top10. Recombinant clones were selected from the resistant colonies on Luria-Bertani (LB) agar containing ampicillin (100 µg/mL). The nucleotide sequences of the DNA gyrase genes in the plasmids were analyzed using the ABI Prism BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA). Cycle sequencing products were subsequently analyzed in an ABI PRISM 3130x automated genetic analyzer (Applied Biosystems). The sequences were compared with their respective wild-type sequences using BioEdit software (http://www.mbio.ncsu.edu/bioedit/bioedit.html).

Recombinant expression and purification of DNA gyrase
DNA gyrase subunits were expressed and purified as previously described [14, 15, 16], with modifications. Briefly, expression vectors carrying \textit{C. jejuni gyrA} and \textit{gyrB} were transformed to \textit{E. coli} BL 21(DE3)/pLysS. The transformants were grown in LB medium in the presence of 100 µg/mL ampicillin to the log phase. Expression of GyrA and GyrB was induced with the addition of 1 mM isopropyl β-D-thiogalactopyranoside (Wako Pure Chemicals Industries Ltd.), and further incubation was conducted for 16 h at 18 °C. Harvested \textit{E. coli} was lysed by sonication at 30% duty cycle, 10 cycles of 40 sec on and 40 sec off using Sonifier 250 (Branson, Danbury, CT). After supernatants were centrifuged (10000×g) for 30 min, recombinant DNA gyrase subunits were purified by Ni-NTA resin column chromatography and dialyzed against DNA gyrase dilution buffer (50 mM Tris-HCl pH 7.5, 100 mM KCl, 2 mM DTT, 1 mM EDTA).

Protein fractions were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; Wako Pure Chemicals Industries Ltd.) with a protein molecular weight marker (New England Biolab).

**DNA supercoiling assay and inhibition by quinolones**

The DNA supercoiling activity of \textit{C. jejuni} DNA gyrase was assayed by monitoring the conversion of relaxed pBR322 to its supercoiled form. The DNA supercoiling activity was tested with a combination of purified \textit{C. jejuni} GyrA and GyrB. The reaction mixture consisted of DNA gyrase assay buffer (35 mM Tris-HCl pH 7.5, 24 mM KCl, 4 mM MgCl₂, 2 mM DTT, 1.8 mM spermidine, 1 mM ATP, 6.5% glycerol, 0.1 mg/mL of BSA), relaxed pBR322 DNA (0.3 µg) with 12.5 nM each of GyrA and GyrB for a total volume of 30 µl. Reactions were run for 30 min at 37 °C and stopped by the addition of 30 µl of chloroform-isoamyl alcohol (24:1 mixture, v/v) and 3 µl of 10× DNA loading dye. The total reaction mixtures were subjected to electrophoresis in 1% agarose gel in...
Tris-borate-EDTA (TBE) buffer. The gels were run for 1 h at 80 mA and stained with ethidium bromide (0.7 µg/mL). The extent of supercoiled DNA was quantified with ImageJ software (http://rsbweb.nih.gov/ij) by comparing with the titration curve drawn using various concentration of supercoiled pBR322 on the same agarose gel. The inhibitory effect of 10 quinolones on DNA gyrase was assessed by determining the drug concentrations required to inhibit the supercoiling activity of the enzyme by 50% (IC₅₀s).

Results

Construction and purification of recombinant WT DNA gyrase subunits

Full-length gyrA and gyrB of C. jejuni ATCC33560 were inserted downstream of the T7 promoter in expression vectors pET-20b (+) for expression as His-tagged recombinant protein, as His-tag has been previously shown not to interfere with the catalytic function of GyrA and GyrB (3). The expression of GyrA and GyrB in E. coli BL21 (λDE3)pLysS by induction with IPTG and subsequent purification by Ni-NTA resin resulted in 2.8 mg and 1.3 mg of soluble His-tagged 97-kDa protein of GyrA and 87-kDa protein of GyrB, respectively, from 500 mL cultures. The high purity (>95%) of recombinant protein was confirmed by SDS-PAGE (Figure 1C).

ATP-dependent DNA supercoiling activities of WT DNA gyrase

GyrA and GyrB were examined for DNA supercoiling activity with relaxed pBR322 DNA as the substrate in the presence and absence of ATP (Figure 1D). A combination of GyrA and GyrB at 50 ng each of GyrA and GyrB each was sufficient for the conversion of 0.3 µg of relaxed plasmid pBR322 DNA to its supercoiled form and was used for all DNA supercoiling experiments. The DNA supercoiling activities were observed in the
presence of ATP and both recombinant DNA gyrase subunits, which confirmed the
reconstitution of functional DNA gyrase. No subunit alone exhibited DNA supercoiling
activity and no supercoiling activity was observed when ATP was omitted.

**Inhibition of DNA supercoiling by quinolones**

The inhibitory effect of 10 quinolones on DNA gyrase was investigated by the
quinolone-inhibited DNA supercoiling assay. Each quinolone showed a dose-dependent
inhibition and their IC\textsubscript{50}s ranged from 0.4 (SIT) to 106.8 µg/mL (NAL), as summarized
in Table 2. Two representative data are shown in Figure 1E, and the results for other
eight quinolones are presented in the supplementary figure. The MICs of 10 quinolones
ranged from 0.0078 to 4 µg/mL (Table 2). Furthermore, the IC\textsubscript{50}s and the MICs
correlated significantly ($R = 0.9943$), as shown in Figure 2.

**Discussion**

Quinolones, a family of drugs for treating *C. jejuni* infection, are used in
veterinary medicine for both prophylactic and therapeutic purposes [1, 2]. However,
there is concern about the use of quinolones in animal production, as their use
selectively enriches quinolone-resistant *Campylobacter*, which can then be transmitted
to humans via the food chain [17]. Consequently, CIP- and ENR-resistant
*Campylobacter* strains are widely found in humans and animals, respectively. Hence,
the development of new quinolone-based antibiotics with higher activity than CIP and
ENR has become critical, as DNA gyrase, the target of quinolones, is an essential
enzyme required for bacterial growth in several strains. Efforts have been made to find
alternative quinolones for the treatment of *C. jejuni* infections. However, the
complicated culturing of *C. jejuni* has been a serious impediment for the study of this
pathogen, particularly the screening of new drugs. Studying the interaction of *C. jejuni* DNA gyrase with quinolones is an important step to understand the quinolone structure-activity relationship and to select those quinolones with good antibacterial activity.

Based on findings of previous studies [14, 16], His-tagged recombinant GyrA and GyrB of *C. jejuni* were expressed and purified to obtain a functional DNA gyrase after reconstitution. The reconstituted DNA gyrase allowed us to examine and compare the inhibitory effect of ten quinolones. The inhibition of DNA supercoiling activities by quinolone was observed in a dose-dependent manner, as previously found in other bacterial DNA gyrase [18, 19, 20]. In the present study, eight quinolones (SIT, CIP, LVX, SPX, GAT, MXF, ENR, and OFX) showed a high activity against *C. jejuni* DNA gyrase, with IC$_{50}$s of 0.4 to 2.3 µg/mL. In contrast, the remaining two quinolones, OXO and NAL, showed a lower inhibitory activity, with IC$_{50}$s of 15.6 and 106.8 µg/mL, respectively (Table 2). Similar results were observed during the quinolone-inhibited DNA supercoiling assay with DNA gyrase from various bacterial species. Indeed, quinolones OXO and NAL weakly inhibited the DNA supercoiling activities of *Mycobacterium tuberculosis* [21], *M. leprae* [22] and *E. coli* [23]. Interestingly, the addition of a fluorine atom at position 6 was the earliest common modification in old quinolones. This single alteration causes an increase in inhibitory activity of more than 10 fold against DNA gyrase and an improvement in MIC also up to 10 fold [24]. The analysis of the quinolone structure-activity relationship showed that the other eight quinolones shared an additional structural feature: a substituent that consisted of three carbon atoms at R-1. Both of these structures exist in many quinolones.

In this study, SIT showed the highest inhibitory activity against *C. jejuni* DNA gyrase with an IC$_{50}$ of 0.4 µg/mL. Analysis of the quinolone structure-activity
relationship showed that SIT has a fluorinate cyclopropyl ring at R-1 while other fluoroquinolones have a cyclopropyl ring or a N1-C8 bridge. Furthermore, the addition of a chloride substituent at R-8 may potentially provide an active compound. A number of previous studies have focused on the in vitro efficacy of SIT on microbes other than Campylobacters. SIT has better activity than other available fluoroquinolones against several enterobacterial species, including CIP-resistant strains [25]. Yokoyama et al. [16] conducted an in vitro assay using recombinant mutant DNA gyrases of *M. leprae* and showed that SIT has an improved activity against quinolone-resistant *M. leprae* bearing mutant DNA gyrase. Furthermore, SIT showed higher activity against the recombinant DNA gyrase of quinolone-resistant *Enterococcus faecalis* with alteration in GyrA and ParC than did other tested fluoroquinolones [26]. SIT belongs to the 4th generation of quinolones that are more effective than CIP against *C. jejuni* strains [27]. Thus, SIT might be a promising candidate for the treatment of campylobacteriosis caused by CIP-resistant *C. jejuni*. Although SIT, now only available in Japan, has been reported to cause mild gastrointestinal disorders as an adverse reaction, our data might encourage its use for *C. jejuni* infections.

A good correlation (*R* = 0.9943) between MICs and IC₅₀s of quinolones against *C. jejuni* and its DNA gyrase, respectively, was observed (Figure 2). This result strongly suggested that DNA gyrase inhibition is the major factor in quinolone inhibition of *C. jejuni*. Based on MIC values, the quinolones were classified into the same categories as by IC₅₀ values. However, the IC₅₀/MIC diversity ratio ranged from 8 to 51 among quinolones. This proportional diversity between the MICs and the IC₅₀s has been found in *E. coli* [23], *Streptococcus pneumoniae* [28], *Mycoplasma pneumoniae* [29], *M. tuberculosis* [21] and *M. leprae* [22], and might be due to differences in cell-permeating
properties and accumulation in distinct quinolones [30]. As shown in Table 3, IC$_{50}$s of quinolones against *C. jejuni* and *E. coli* DNA gyrase were lower [23] than those against *S. pneumoniae* [28], *M. pneumoniae* [29], *M. tuberculosis* [21], and *M. leprae* [22]. These data suggested a stronger interaction of quinolones with *C. jejuni* and *E. coli* DNA gyrase than with DNA gyrase from other bacteria. Moreover, the analysis of QRDR sequences showed that *E. coli* has highly identical QRDR sequences to *C. jejuni*. In *E. coli*, substitutions of several amino acids in the GyrA have been shown to be important with regard to quinolone resistance. The most frequently occurring substitution was the replacement of serine 83. Substitution of serine 83 with leucine or tryptophan has been shown to confer a high-level quinolone resistance in both clinical isolates and laboratory-derived *E. coli* resistant mutants [31]. The alignment of 40 amino acid sequences of *C. jejuni* GyrA QRDR with analogous sequences from *E. coli*, *S. pneumoniae*, *M. pneumoniae*, *M. tuberculosis*, and *M. leprae* is shown in Figure 3, in which threonine 86 (indicated by arrow head) in *C. jejuni* is analogous to amino acid 83 in *E. coli* GyrA, while those at an equivalent position in GyrA of *S. pneumoniae*, *M. pneumoniae*, *M. tuberculosis*, and *M. leprae* were serine, methionine, alanine, and alanine, respectively. From these results it can be inferred that amino acid residues at an equivalent position to threonine 86 in *C. jejuni* may be the cause of the increased quinolone resistance of mycobacterial DNA gyrase. In contrast, *S. pneumoniae* DNA gyrase with serine at the same position showed intrinsic resistance to quinolone. The amino acids in QRDR that are distinct between bacteria species may contribute to various intrinsic susceptibilities.

An early study by Han *et al.* [32] demonstrated the inhibitory activity of a fluoroquinolone, CIP, against *C. jejuni* DNA gyrase and showed the minimum effective
concentration (MEC) as 32 µg/mL. The extremely high concentration of DNA gyrase used in their study, 300 nM, comparing to our study (12.5 nM) might be the cause of the discrepancy with our result (IC₅₀ = 1.0 µg/mL). Further, the quality or specific activity of the DNA gyrase used in our study might have been much higher than that used in theirs. These data clearly exhibited the advantage of the usage of recombinant DNA gyrase with high activity as produced in our study.

In conclusion, we have succeeded in producing and purifying *C. jejuni* DNA gyrase, which is the sole target of quinolones. Based on the determination of the inhibition activity of quinolones on recombinant *C. jejuni* DNA gyrase, we identified SIT as a promising quinolone for the treatment of campylobacteriosis. The results of this study suggested that the *in vitro* supercoiling inhibition assay may be a useful and predictive technique to monitor the antibacterial potency of quinolones.

**Conflict of Interest:** There is no conflict of interest to be declared.

**Acknowledgements**

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References


Figure legends

**Figure 1.** Expression and characterization of recombinant DNA gyrase.

Pannel A; Schematic representation of the construction of expression plasmids for GyrA and B. Pannel B; Expression of gyrA and B with His-Tag are controlled by T7 promoter in the expression vector pET20b. Pannel C; His-tagged proteins were overexpressed by an *E. coli* expression system and purified by nickel resin chromatography. Approximately 300 ng of each protein was loaded on a 5-20% gradient polyacrylamide gel. Following electrophoresis, proteins were stained with Quick CBB. Lanes: M, protein marker (sizes are indicated at the left in kilo-Daltons); 1, GyrA; 2, GyrB. Pannel D; Reconstitution of ATP-dependent DNA supercoiling activity was confirmed. Relaxed pBR322 DNA (0.3 µg) was incubated with DNA gyrase reconstituted with 12.5 µM each of GyrA and GyrB in the presence or absence of 1 mM ATP. The reactions were stopped, and the DNA products were analysed by electrophoresis in 1% agarose gels. Lanes: 1, relaxed pBR322 DNA; 2, relaxed pBR322 and both recombinant GyrA and GyrB; 3, relaxed pBR322 and GyrB only; 4, relaxed pBR322 and GyrA only; 5, absence of ATP. R and SC, relaxed and supercoiled pBR322 DNA, respectively. Pannel E; Inhibitory activity of (A) SIT and (B) NAL on the supercoiling activities of *C. jejuni* DNA gyrase were investigated. Relaxed pBR322 DNA (0.3 µg) was incubated with 12.5 µM each of GyrA and GyrB in the presence of the indicated amounts (µg/ml) of both quinolones. The reactions were stopped and the DNA products were analyzed by electrophoresis in 1% agarose gels. R and SC denote relaxed and supercoiled pBR322 DNA, respectively.
Figure 2. Correlation between quinolone inhibition of *C. jejuni* gyrase (IC$_{50}$s for DNA supercoiling) and quinolone MICs for *C. jejuni*. $R$ is the correlation coefficient. SIT, sitafloxacin; GAT, gatifloxacin; MXF, moxifloxacin; SPX,sparfloxacin; ENR, enrofloxacin; CIP, ciprofloxacin; LVX, levofloxacin; OFX, ofloxacin; OXO, oxolinic acid; NAL, nalidixic acid.

Figure 3. Alignment of amino acid sequences of GyrA QRDR. The identity with the sequence from *C. jejuni* is represented. An asterisk, a colon and a dot indicates positions which have a single, fully conserved residue, conservation between groups of strongly similar properties and conservation between groups of weakly similar properties, respectively. The numbering system used is that for *C. jejuni gyrA*.
Changkwayenun et al. Fig. 1

A) Primers R-5 & R-36

C. jejuni DNA → PCR

pUC118-HincII/BAP → Blunt end ligation

B) T7 promoter

Nde I

Xho I

C. jejuni gyrA or gyrB

His-Tag

B)

Primers R-5 & R-36

C) kDa

M 1 2

175

80

58

46

D) R

SC

GyrA - + - + +

GyrB - + + - +

ATP - + + + -

DNA + + + + +

E) (a) SIT conc. (µg/ml)

0 0.08 0.16 0.31 0.62 1.25 2.5 5 10 20

R

SC

(b) NAL conc. (µg/ml)

0 5 10 20 40 80 160 320 640 1280

R

SC
MIC ($\mu$g/ml) vs. IC$_{50}$ ($\mu$g/ml)

$y = 0.0371x + 0.084$

$R = 0.9943$

Changkwanyenun et al. Fig. 2
Changkwanyenun et al. Figure 3

C. jejuni  
70  ARIVGAVIGRYHPHGDTAVYDALVRMAQDFSMRYPSTGQ 109

E. coli  
68  ARVVGDVIGKYHPHGDSAVYDTIVRMAQPFLRYMLVGDQ 106

S. pneumoniae  
65  ARITGDVMGKYHPHGDSIYEAMVRMAQWWSYRYMLVGDH 104

M. pneumoniae  
79  ARIVGDMKSFPHGDMAIYDTRMAQDFSLRYYLIDGH 118

M. tuberculosis  
74  ARSVAETMGNYHPHDAMSYDSLVRMAQPWSLRYPLVGDQ 113

M. leprae  
75  ARSVAETMGNYHPHDAMSYDTLVRMAQPWSLRYPLVGDQ 114

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## Table 1. Oligonucleotide sequences of primers used for PCR

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (nucleotide position), underlined element(s)</th>
<th>Remarks</th>
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</thead>
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<tr>
<td>R-5</td>
<td>5'-CCCATATGGAGAATATTTTTTAGCAAAG-3' (1-22), \textit{Nde I} site</td>
<td>\textit{gyr A} Forward</td>
</tr>
<tr>
<td>R-36</td>
<td>5'-GGCTCGAGTTAATGATGATGATGATGATGCAAATCTAAACAAAAGTTTCATC- ( (1455-1510) ), \textit{Xho I} site and 6-histidine tag, respectively</td>
<td>\textit{gyr A} Reverse</td>
</tr>
<tr>
<td>R-1</td>
<td>5'-CCCATATGCAAGAAAATTACGCTGC-3' (1-25), \textit{Nde I} site</td>
<td>\textit{gyr B} Forward</td>
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<tr>
<td>R-47</td>
<td>5'-GGCTCGAGTTAATGATGATGATGATGATGCACATCCAAATGCTTTACATC-3' ( (2177-2230) ), \textit{Xho I} site and 6-histidine tag, respectively</td>
<td>\textit{gyr B} Reverse</td>
</tr>
</tbody>
</table>

* Single underlines denote the restriction endonuclease recognition sequences.

** Double underlines denote the coding sequences for His hexamer.
Table 2. Structure features, IC$_{50}$s and MICs of quinolones against *C. jejuni*

<table>
<thead>
<tr>
<th>Quinolones</th>
<th>Substituents</th>
<th>IC$_{50}$ (µg/ml)</th>
<th>MIC (µg/ml)</th>
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<td></td>
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<td>R-5$^a$</td>
<td>R-6$^a$</td>
</tr>
<tr>
<td>SIT</td>
<td>fluorocyclopropyl</td>
<td>H</td>
<td>F</td>
</tr>
<tr>
<td>CIP</td>
<td>Cyclopropyl</td>
<td>H</td>
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<td>Bridge N1-C8</td>
<td>H</td>
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</tr>
<tr>
<td>OXO</td>
<td>Ethyl</td>
<td>H</td>
<td>Bridge C6-C7</td>
</tr>
<tr>
<td>NAL</td>
<td>Ethyl</td>
<td>H</td>
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$^a$Basic structure of quinolones and position of substitution
Table 3. IC<sub>50</sub>s and MICs (µg/ml) of quinolones inhibiting various DNA gyrase

<table>
<thead>
<tr>
<th></th>
<th>C. jejuni</th>
<th>E. coli&lt;sup&gt;a&lt;/sup&gt;</th>
<th>S. pneumoniae&lt;sup&gt;b&lt;/sup&gt;</th>
<th>M. pneumoniae&lt;sup&gt;c&lt;/sup&gt;</th>
<th>M. tuberculosis&lt;sup&gt;d&lt;/sup&gt;</th>
<th>M. leprae&lt;sup&gt;e&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC</td>
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<td>0.0078</td>
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<td>ND</td>
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<td>0.25</td>
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<td>0.125</td>
<td>ND</td>
<td>ND</td>
<td>3.5</td>
<td>0.5</td>
</tr>
<tr>
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<td>0.125</td>
<td>ND</td>
<td>ND</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
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<td>0.0312</td>
<td>0.39</td>
<td>0.0125</td>
<td>114</td>
<td>0.15</td>
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<tr>
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<td>5.71</td>
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<td>4</td>
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</tr>
</tbody>
</table>

<sup>a</sup> Data from Yoshida et al. (23)
<sup>b</sup> Data from Morrissey et al. (28)
<sup>c</sup> Data from Nakatani et al. (29)
<sup>d</sup> Data from Aubry et al. (21)
<sup>e</sup> Data from Matrat et al. (22)
<sup>f</sup> Data from animal experiments using a mouse model