Impact of mutations in DNA gyrase genes on quinolone resistance in *Campylobacter jejuni*

Ruchirada Changkwanyeun[a], Tomoyuki Yamaguchi[a], Siriporn Kongsoi[a], Kanjana Changkaew[a], Kazumasa Yokoyama[b], Hyun Kim[c], Orasa Suthienkul[d], Masaru Usui[e], Yutaka Tamura[e], Chie Nakajima[a, f], Yasuhiko Suzuki[a, f, *]

Division of Bioresources, Hokkaido University Research Center for Zoonosis Control, Sapporo, Japan[a]; Central Research Laboratory, Kissei Pharmaceutical Co., Ltd, Nagano Japan[b], Laboratory of Tuberculosis, Department of Bacteriology II, National Institute Infectious Diseases, Tokyo, Japan[c], Faculty of Public Health, Thammasat University, Rangsit, Thailand[d], School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan[e], Hokkaido University The Global station for Zoonosis Control, Sapporo, Japan[f,]

* Corresponding author: Mail address: Division of Bioresources, Hokkaido University Research Center for Zoonosis Control, Kita 20, Nishi 10, Kita-ku, Sapporo, 001-0020 Japan. Phone: 81-11-706-9503. Fax: 81-11-706-7310. E-mail: suzuki@czc.hokudai.ac.jp

Running Head: Quinolone resistance mechanism of *C. jejuni*

Abstract

Amino acid substitutions providing quinolone resistance to *Campyloabcter jejuni* have been found in the quinolone resistance-determining region of protein DNA gyrase subunit A (GyrA), with the highest frequency at position 86 followed by position 90. In this study, wild-type and mutant recombinant DNA gyrase subunits were expressed in *Escherichia coli* and purified using Ni-NTA agarose column chromatography. Soluble 97 kDa GyrA and 87 kDa DNA gyrase subunit B were shown to reconstitute ATP-dependent DNA supercoiling activity. A quinolone-inhibited supercoiling assay demonstrated the roles of Thr86Ile, Thr86Ala, Thr86Lys, Asp90Asn, and Asp90Tyr amino acid substitutions in reducing sensitivity to quinolones. The marked effect of Thr86Ile on all examined quinolones suggested the advantage of this substitution in concordance with recurring isolation of quinolone-resistant *C. jejuni*. An analysis of the structure-activity relationship showed the importance of the substituent at position 8 in quinolones to overcome the effect of Thr86Ile. Sitafloxacin (SIT), which has a fluorinate cyclopropyl ring at R-1 and a chloride substituent at R-8, a characteristic not found in other quinolones, showed the highest inhibitory activity against all mutant *C. jejuni* gyrases including ciprofloxacin-resistant mutants. The results suggest SIT as a promising drug for the treatment of campylobacteriosis caused by CIP-resistant *C. jejuni*.

Keyword; *Campylobacter jejuni*, quinolone resistance, DNA gyrase, mutation

1. Introduction

Campylobacter is an intestinal infection-causing bacterium that affects an estimated 400-500 million people globally each year [1, 2]. *Campylobacter jejuni*, commonly found in the gastrointestinal tract of animals, is considered one of the most important pathogenic bacteria for humans. Generally, most patients infected with *C. jejuni* do not require treatment, except for severely ill or immune-compromised patients [3]. Quinolones are the antimicrobials recommended as first-line drugs against *Campylobacter* infections in severe cases. Recently, *C. jejuni* has shown increased resistance to quinolones, which has become a major concern for public health [3 - 5]. A high percentage of *C. jejuni* resistant to quinolones has been reported in animal products, especially chicken meat [6], with the wide use of quinolones in poultry farming suggested as the cause [7].

Quinolones belong to a family of broad-spectrum synthetic antimicrobials. The bacterial targets of quinolones include the essential microbial enzymes DNA gyrase and DNA topoisomerase IV, which are classified as Type II topoisomerases[8]. DNA gyrase is unique in that it catalyses the negative supercoiling of DNA and is essential for DNA replication, transcription and recombination. By contrast, topoisomerase IV has a specialised role in chromosome segregation. Moreover, complete genome sequencing revealed that *C. jejuni* lacks the genes encoding topoisomerase IV [9 - 11] and that DNA gyrase is the sole target of quinolones in *Campylobacters*.

Quinolone resistance is mediated by a point mutation in the quinolone resistance-determining region (QRDR) of gene *gyrA*. An amino acid substitution from threonine to isoleucine at codon 86 (Thr86Ile) has been identified as the predominant mutation and shown to associate with high-level quinolone resistance, whilst other

substitutions, i.e., Thr86Ala, Thr86Lys, Asp90Asn, and Asp90Tyr, were found as minor mutations [10, 12 - 14]. Han *et al.* showed the association of these amino acid substitutions with quinolone resistance in *C. jejuni* DNA gyrase by *in vitro* DNA supercoiling assays with recombinant proteins [15]. They also showed a deteriorative effect by the Thr86Ile substitution on the gyrase function, which was inconsistent with an *in vivo* study that found mutated strains to be predominant in chickens [15]. To explain this disagreement, in the present study we investigated by *in vitro* DNA supercoiling assays the impact of amino acid substitutions at positions 86 and 90 in DNA gyrase subunit A (GyrA) of *C. jejuni* on quinolone resistance.

2. Materials and Methods

2.1 Drugs and kits

Ciprofloxacin (CIP) was purchased from LKT Laboratories, Inc. (St. Paul, MN, USA) and Sitafloxacin (SIT) was donated by Daiichi-Sankyo Pharmaceutical, Co., Ltd. (Tokyo, Japan). Moxifloxacin (MXF) was purchased from Toronto Research Chemicals Inc. (Toronto, Canada), oxolinic acid (OXO) and nalidixic acid (NAL) from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan), and ampicillin from Meiji Seika Pharma Co., Ltd. (Tokyo, Japan). The structures of the used quinolones are shown in Fig. 1. A Ni-nitrolotriacetic acid (Ni-NTA) protein purification kit was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Restriction enzymes, *Nde*I and *Sac*I, were obtained from New England BioLabs Inc. (Ipswich, MA, USA). Supercoiled DNA and relaxed pBR322 DNA were purchased from John Innes Enterprises Ltd. (Norwich, United Kingdom). Protease inhibitor cocktail tablet (Complete Mini, EDTA free) was purchased from Roche Applied Science (Mannheim, Germany).

2.2 Bacterial strains and plasmids

DNA was extracted from *C. jejuni* ATCC33560. *Escherichia coli* strain TOP-10 (Thermo Fisher Scientific Inc.) was used as the host for bacterial DNA cloning purposes. *E. coli* strain BL-21 (DE3)/pLysS (Merck KGaA, Darmstadt, Germany) was used for protein expression, and vector plasmid pET-20b (+) (Merck KGaA) was used to construct expression plasmids for GyrA and DNA gyrase subunit B (GyrB) of *C. jejuni*.

2.3 Construction of wild-type and mutant DNA gyrase expression vectors

Mutations corresponding to Thr86Ile, Thr86Ala, Thr86Lys, Asp90Asn, and Asp90Tyr were introduced in wild-type (WT) *gyr*A gene by PCR using pairs of complementary primers containing the mutation of interest (Table 1). The reaction mixture (25 µL) consisted of primeSTAR GXL buffer (Mg^{2+} plus), 200 µM of dATP, dCTP, dGTP, and dTTP each, 10 ng of WT GyrA expression plasmid, 2.0 units of PrimeSTAR GXL DNA polymerase (Takara Bio Inc., Shiga, Japan), and 0.4 µM of each primer. PCR was carried out in an iCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA) under the following amplification conditions: pre-denaturation at 98 ºC for 2 min, 35 cycles of denaturation at 98 ºC for 20 s, annealing at 55 ºC for 30 s, extension at 68 ºC for 4 min, and final extension at 68 ºC for 4 min. *gyr*A N-terminal cassettes with base substitutions were digested with *Nde*I and *Sac*I, ligated into WT *gyr*A similar to our previous studies [16 - 18], and digested with the same restriction endonucleases to obtain the mutant *gyr*A expression plasmid. Nucleotide sequences of the DNA gyrase genes in the plasmids were confirmed using the ABI Prism BigDye Terminator v3.1 cycle sequencing kit (Thermo Fisher Scientific Inc.). Sequencing reactions were carried out according to the manufacturer's instructions.

Cycle sequencing products were subsequently analysed by an ABI PRISM 3130x automated genetic analyser (Thermo Fisher Scientific Inc.).

2.4 Recombinant expression and purification of DNA gyrase

Recombinant DNA gyrase subunits were expressed and purified as previously described [16 - 18]. Briefly, expression vectors carrying the *gyr*A and *gyr*B genes of *C. jejuni* were transformed in *E. coli* BL 21(DE3)/pLysS. Transformants were grown to the log phase in Luria-Bertani (LB) medium with 100 µg/mL ampicillin, and the expression of GyrA and GyrB was induced by the addition of 1 mM of isopropyl β-D-thiogalactopyranoside (Wako Pure Chemicals Industries, Ltd.) and incubation at 18 °C for 16 h. Harvested *E. coli* were sonicated on ice at 30% duty cycle, with 10 cycles of 40 s on and 40 s off (Sonifier 250; Branson, Danbury, CT, USA). After centrifugation (10000 × g for 30 min), recombinant DNA gyrase subunits in supernatants were purified by column chromatography using Ni-NTA agarose resin and dialysed against DNA gyrase dilution buffer (50 mM Tris-HCl, pH 7.5; 100 mM KCl; 2 mM DTT; 1 mM EDTA). The protein fractions were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with Prestained Protein Marker, Broad Range (7-175 kDa) (New England Biolabs Inc.).

2.5 DNA supercoiling assay and inhibition by quinolones

The DNA supercoiling activity of recombinant DNA gyrase was assayed by monitoring the conversion of relaxed pBR322 to its supercoiled form. DNA supercoiling activity was tested with a combination of purified GyrA and GyrB. The reaction mixture consisted of DNA gyrase assay buffer (35 mM Tris-HCl pH 7.5, 24 mM KCl, 4 mM $MgCl_2$, 2 mM DTT, 1.8 mM spermidine, 1 mM ATP, 6.5% glycerol, and 0.1 mg/mL of BSA), 0.3 µg of relaxed pBR322 DNA, and 32 nM of GyrA and GyrB in a total volume of 30 µL. Reactions

were run at 37 °C for 30 min and stopped by the addition of 30 µL of chloroform-isoamyl alcohol (24:1 mixture) and 3 µL of 10× DNA loading dye (10mM Tris-HCl pH 7.6, 0.03% bromophenol blue, 0.03% xylene cyanol FF, 60% glycerol, and 60 mM EDTA). 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). The inhibitory effects of quinolones on DNA gyrase were assessed by determining the drug concentrations required to inhibit the supercoiling activity of the enzyme by 50% (IC_{50}) in the presence or absence of serial 2-fold increases in the concentrations of CIP, MXF, SIT, OXO, and NAL. Distinct ranges of quinolone concentrations were used for WT and mutant DNA gyrases. Namely, 0.08 – 20, 0.08 – 20, 0.08 – 20, 20 – 5120, and 20 – 5120 µg/mL were employed for CIP, MFX, SIT, NAL, and OXO, respectively, against WT DNA gyrase. In contrast, 5 – 128, 5 – 128, 5 – 128, 31.3 – 8000, and 31.3 – 8000 µg/mL were employed for CIP, MFX, SIT, NAL, and OXO, respectively, against mutant DNA gyrases. Enzymatic assays were conducted at least three times to confirm reproducibility.

3. Results

3.1 Expression and purification of recombinant WT and mutant DNA gyrase subunits

DNA fragments with mutations causing amino acid substitutions at position 86 or 90 in GyrA were successfully amplified from WT *gyr*A gene and inserted in-frame downstream of a T7 promoter in the expression vector pET-20b (+). Recombinant mutant DNA gyrases were expressed as N-terminus hexa histidine-tagged (His-tag™, Roche Applied

Science) proteins, as His-tag™ has been previously shown not to interfere with the catalytic function of GyrA or GyrB [15, 17, 19 - 22]. WT and mutant DNA gyrase subunits were successfully expressed and purified. The amount of purified GyrA-WT, Thr86Ile, Thr86Ala, Thr86Lys, Asp90Asn, Asp90Tyr, and GyrB-WT obtained from 500 mL cultures was 2.8, 1.5, 1.1, 1.2, 0.8, 1.7 and 1.3 mg, respectively. The high purity (>95%) of recombinant proteins was confirmed by SDS-PAGE (Figure 2).

3.2 ATP-dependent DNA supercoiling activities of WT and mutant DNA gyrases

The combination of GyrA-WT or mutant GyrA (Thr86Ile, Thr86Ala, Thr86Lys, Asp90Asn, and Asp90Tyr) with GyrB-WT was examined for DNA supercoiling activity with relaxed pBR322 DNA as the substrate in the presence or absence of ATP (Figure 3). DNA supercoiling activities were observed in the presence of ATP and recombinant DNA gyrase subunits, which demonstrated that they reconstituted functional DNA gyrase. No subunit alone exhibited DNA supercoiling activity. In addition, no supercoiling was observed when ATP was omitted. Consequently, ATP-dependent DNA supercoiling activities were confirmed in WT and the five mutant DNA gyrases. WT DNA gyrase showed incomplete supercoiling of DNA as a second band above the supercoiled DNA is visible. The reason of this phenomenon should further be investigated in our future study. DNA gyrase with Thr86Ile, Asp90Asn, and Asp90Tyr amino acid substitution showed similar activities to WT whereas DNA gyrase with Thr86Lys and Thr86Ala showed significantly lower activities than WT (Figure 4).

3.3 Inhibition of DNA supercoiling by quinolones

Quinolone-inhibited DNA supercoiling activities were assessed to determine IC_{50}. Figure 5 shows a representative result of the inhibitory effect of CIP; the results for the other quinolones are presented in Figure S1. The results showed a dose-dependent inhibition of five

quinolones against WT and mutant DNA gyrases, as summarized in Table 2. The three fluoroquinolones (FQs) investigated, CIP, MXF and SIT, inhibited the DNA supercoiling activities of WT DNA gyrase at low concentrations (IC_{50} < 1 μg/mL), whereas IC_{50} values for customary quinolones, such as OXO and NAL, were significantly higher at 19.7 and 145 μg/mL, respectively. Amongst the five amino acid substitutions, Thr86Ile showed the highest IC_{50} values for all five quinolones, suggesting a higher resistance potential to quinolones compared with the other mutants. This effect was clearly seen when the degrees of resistance were shown as fold increases of IC_{50} to WT, e.g., the inhibitory concentrations of CIP and OXO were 200 to 300-fold higher for Thr86Ile than they were for WT (Figure 6). In contrast, the degree of resistance was lower in Thr86Ala compared with other mutants. Amongst the quinolones, SIT showed the lowest IC_{50} for all mutants, as even its highest IC_{50} was significantly lower than that of other quinolones.

4. Discussion

Amino acid substitutions at positions 86 and 90 in GyrA of *C. jejuni* have been linked to quinolone resistance [3, 10]. Our data suggested a strong contribution of Thr86Ile to the resistance of DNA gyrase to CIP. Han *et al*. reported that whilst *C. jejuni* strain with Thr86Ile mutation showed dominance in chicken infection model, this amino acid substitution had a destructive effect on DNA gyrase function both *in vivo* and *in vitro*, which disagree with our data. The possible reason for this discrepancy may be that Han *et al.* used an extremely high concentration of recombinant proteins for the supercoiling assays, which involved non-functional protein components that caused low activity of the recombinant DNA gyrase.

Thus, the results of the present study suggest that our system more effectively assayed the contribution of Thr86Ile to quinolone resistance in DNA gyrase.

Most of the mutations causing quinolone resistance were found at position 86 in GyrA of the field isolates. Our results suggest that Thr86Ile provides some advantage for the acquisition of quinolone resistance. This claim is supported by the fact that Thr86Ile was the most commonly observed mutation in quinolone-resistant *Campylobacter* isolates and provided high-level (CIP minimum inhibitory concentration [MIC] ≥16 μg/mL) resistance to quinolones. In contrast, the other four mutations studied in the present study were less common and associated with intermediate level resistance to quinolones [10, 13]. Regarding these mutations, Thr86Lys and Thr86Ala had different effects on DNA gyrase resistance despite sharing the same location with Thr86Ile. Threonine and lysine are both hydrophilic amino acids, whereas isoleucine and alanine are hydrophobic residues. The substitution of a hydrophilic with a hydrophobic amino acid may weaken the interaction between DNA gyrase and quinolones via hydrogen bonding to cause resistance. Moreover, the low prevalence of Thr86Lys in the field isolates may be due to the cationic characteristics of lysine, which may affect the function of DNA gyrase. This was proved by the analysis of relative activity to WT DNA gyrase (Figure 4). IC_{50} values of all five quinolones against Thr86Ala were significantly lower than those against Thr86Ile and Thr86Lys. This observation may be due to an exclusion effect that depends on the size of the amino acids. Alanine is a small amino acid with only one methyl group as the side chain, whereas isoleucine and lysine carry side chains consisting of at least 4 carbon atoms. The exclusion effect might have been smaller due to the smaller size of alanine.

In this study, the IC_{50} values of CIP were 186.8-, 5.6-, 44.5-, 31.3-, and 35-fold

223 higher for Thr86Ile, Thr86Ala, Thr86Lys, Asp90Asn, and Asp90Tyr, respectively, than those
224 for WT DNA gyrase (Figure 6). Interestingly, Thr86Ile provided significantly high resistance
225 to mutant gyrases. A very similar pattern was observed for the IC_{50} values of OXO, but less
226 so in the case of MXF. The existence of a substituent at position 8 in quinolones is a common
227 feature in both CIP and OXO (Figure 1). A substituent at this position seems to be important
228 for reducing the drastic effect of Thr86Ile on the acquisition of quinolone resistance.

229 Amongst the five quinolones studied, SIT was shown to be the most effective
230 inhibitor of WT and mutant DNA gyrases. An analysis of the structure-activity relationship
231 showed that SIT has a fluorinated cyclopropyl ring at R-1, whilst the other FQs have a
232 cyclopropyl or N1-C8 bridge (Figure 1), which may explain the stronger activity of SIT [15].
233 Furthermore, the presence of a chloride substituent at R-8 may also contribute to this effect.

234 A number of previous studies have focused on the *in vitro* efficacy of SIT on
235 microbes other than campylobacters. SIT exhibits stronger activity than other available FQs
236 against several enterobacterial species, including CIP-resistant strains [23]. Deguchi *et al*.
237 reported that SIT exhibited stronger activity against quinolone-resistant *Klebsiella*
238 *pneumoniae* and *Enterobacter cloacae* isolates with alterations in GyrA and ParC [24]. Our
239 results along with previous data suggest that SIT may be a promising candidate for the
240 treatment of campylobacteriosis caused by CIP-resistant bacteria with mutated gyrases,
241 including *C. jejuni*.

242 In conclusion, our study showed the importance of mutations causing amino acid
243 substitutions in GyrA, especially Thr86Ile, on the acquisition of quinolone resistance. In
244 addition, we elucidated the association between the structure of quinolones and amino acid
245 substitutions in GyrA that exert an effect on the level of resistance. Finally, our results suggest

246 that SIT may be a promising drug for CIP resistant *C. jejuni*.

247

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Figure legends

Fig. 1. Structure of quinolones.

A: Basic structure of quinolones, B: sitafloxacine, C: ciprofloxacine, D: moxifloxacine, E: oxolinic acid, and F: nalidixic acid. Numbers in A denote the position of substituents.

Fig. 2. SDS-PAGE analysis of recombination DNA gyrase subunits.

The His-tagged proteins were overexpressed by an *E. coli* expression system and purified by nickel resin chromatography, and approximately 3 μM of each protein was loaded onto a 5-20% gradient polyacrylamind gel. Following electrophoresis, proteins were stained with Quick CBB. Lanes (sizes in kilodaltons are indicated on the left): M: protein marker, 1: WT GyrA, 2: WTGyrB, 3: GyrA-Thr86Ile, 4: GyrA-Thr86Ala, 5: GyrA-Thr86Lys, 6: GyrA-Asp90Asn, and 7: GyrA-Asp90Tyr.

Fig. 3. DNA supercoiling assay.

WT, wild-type. Supercoiling activity of (A) WT DNA gyrase, (B) DNA gyrase bearing GyrA-Thr86Ile, (C) GyrA-Thr86Ala, (D) GyrA-Thr86Lys, (E) GyrA-Asp90Asn, and (F) GyrA-Asp90Tyr. Relaxed pBR322 DNA (0.3 μg) was incubated with DNA gyrase reconstituted with GyrA (32 nM), GyrB (32 nM) or both. Lanes: 1: relaxed pBR322 DNA, 2: relaxed pBR322 and both recombinant GyrA and GyrB, 3: relaxed pBR322 and only GyrB, 4: relaxed pBR322 and only GyrA, and 5: absence of ATP.

Fig. 4. Comparison of supercoiling activities of WT and mutant DNA gyrases. WT, wild-type. Amino acid substitutions in protein DNA gyrase subunit A: Thr86Ile; from threonine to

isoleucine at codon 86, Thr86Ala; from threonine to alanine, Thr86Lys from threonine to lysine, D90N, Asp90Asn; from aspartic acid to asparagine at codon 90, Asp90Tyr; from asparagine to tyrosine. Y-axis denotes the % activity of mutant DNA gyrases comparing to WT DNA gyrase.

Fig. 5. CIP-inhibited DNA supercoiling assay.

WT, wild-type. Relaxed pBR322 (0.3 μg) was incubated with 32 nM of GyrA and GyrB in the presence of the indicated concentration of CIP. Quinolone-inhibited supercoiling activity was performed with combinations consisting of (A) WT GyrB-WT GyrA, (B) GyrA-Thr86Ile, (C) GyrA-Thr86Ala, (D) GyrA-Thr86Lys, (E) GyrA-Asp90Asn, and (F) GyrA-Asp90Tyr. R: relaxed pBR322 DNA, SC: supercoiled pBR322 DNA.

Fig. 6. Fold increases of IC_{50} of quinolones for mutant DNA gyrase.

WT, wild-type. Amino acid substitutions in protein DNA gyrase subunit A: Thr86Ile; from threonine to isoleucine at codon 86, Thr86Ala; from threonine to alanine, Thr86Lys from threonine to lysine, D90N, Asp90Asn; from aspartic acid to asparagine at codon 90, Asp90Tyr; from asparagine to tyrosine. Drugs: CIP, Ciprofloxacin; SIT, Sitafloxacin; MXF, Moxifloxacin; OXO, oxolinic acid; NAL, nalidixic acid. Drug concentrations required to inhibit the supercoiling activity of DNA gyrase by 50% (IC_{50}) were calculated by the quinolone-inhibited supercoiling assay. The IC_{50} of NAL against WT DNA gyrases are not shown because they were considered too high for clinical use

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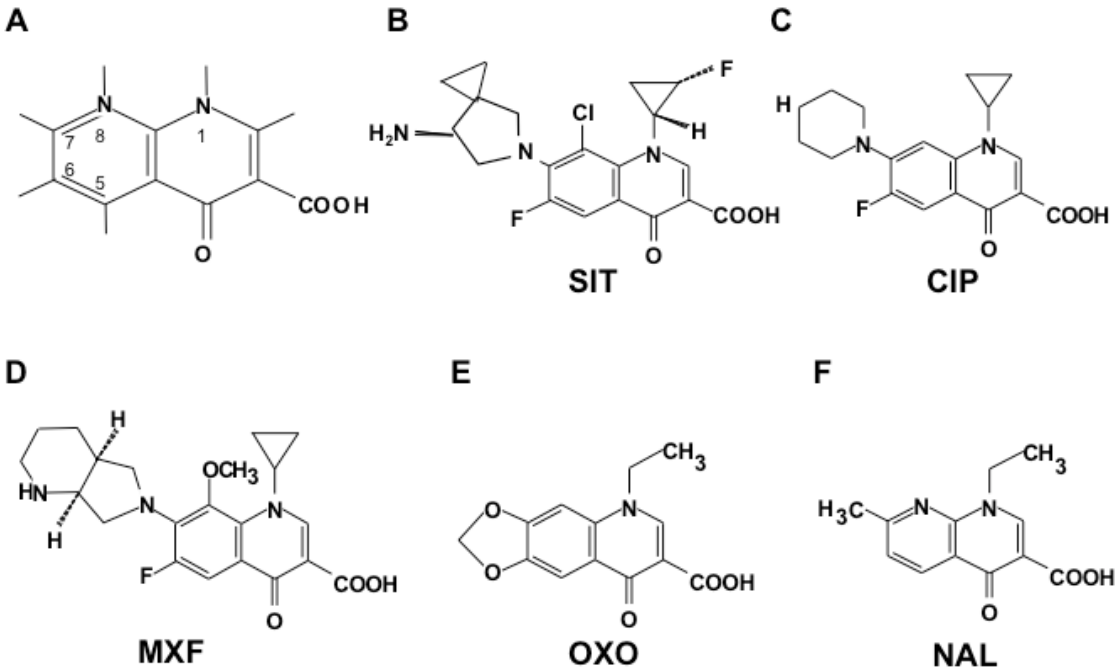
Changkwanyeun et al. Fig. 1.



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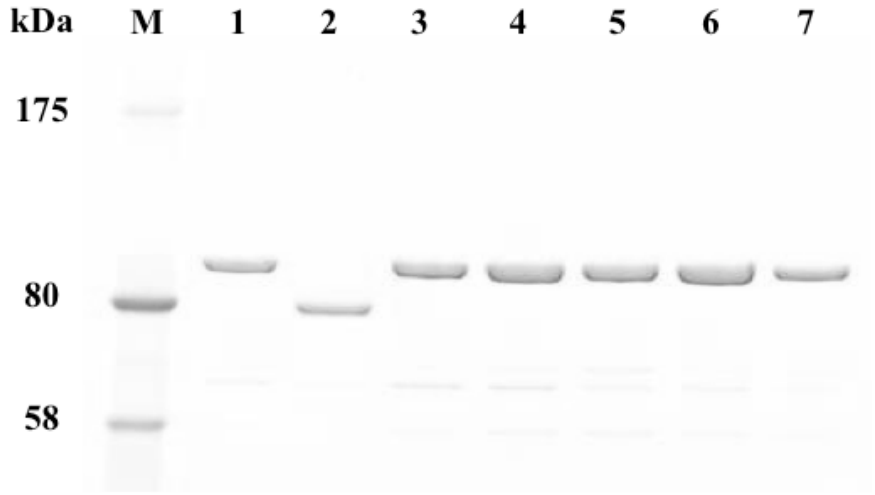
Changkwanyeun et al. Fig. 2.



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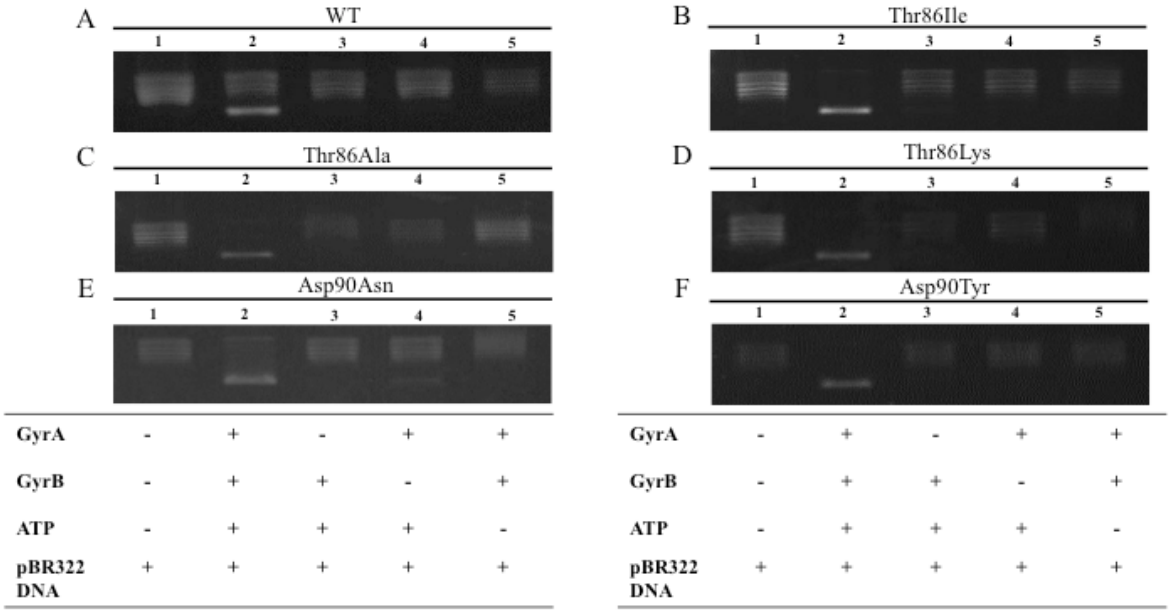
Changkwanyeun et al. Fig. 3.



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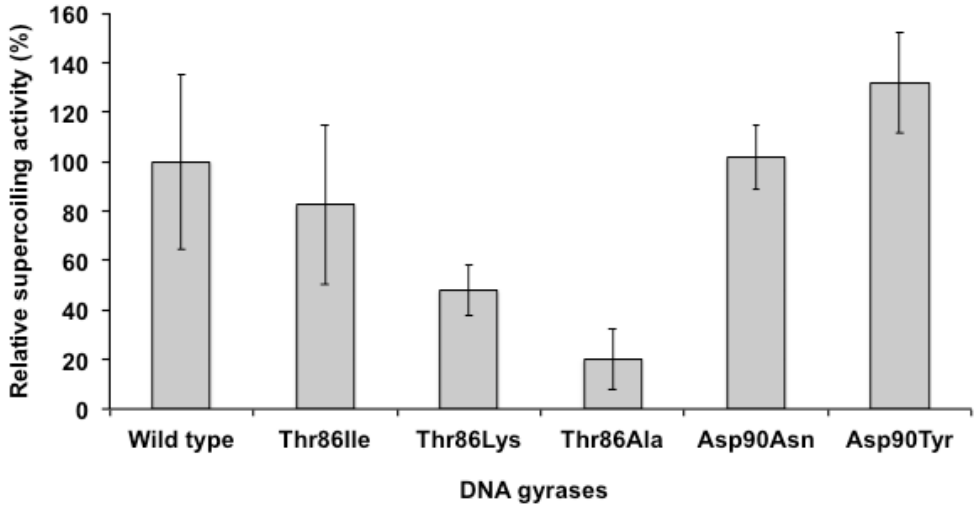
Changkwanyeun et al. Figure 4



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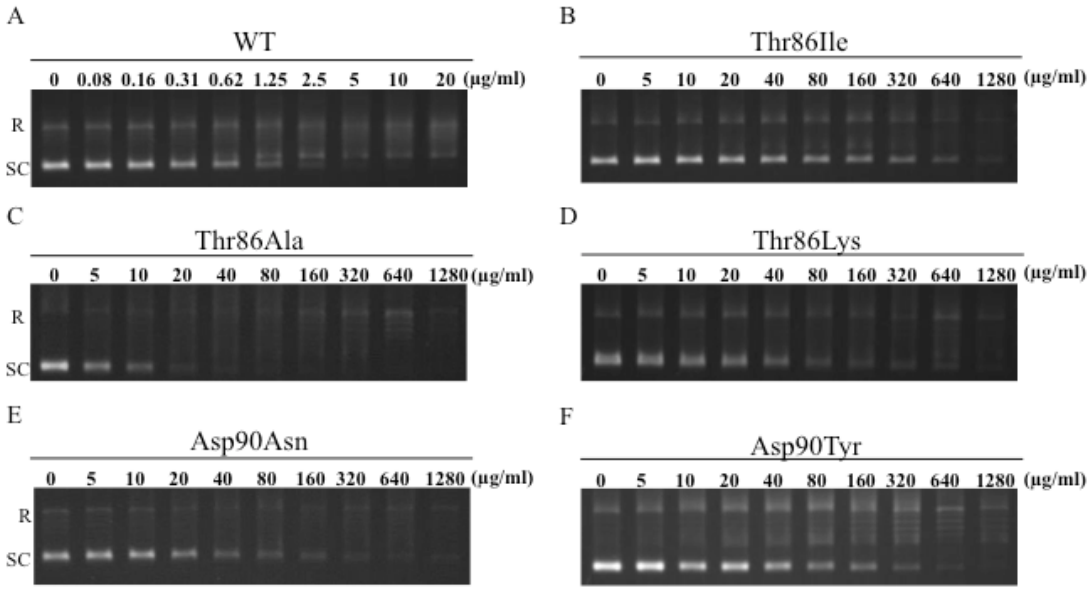
Changkwanyeun et al. Figure 5



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Changkwanyeun et al. Figure 6

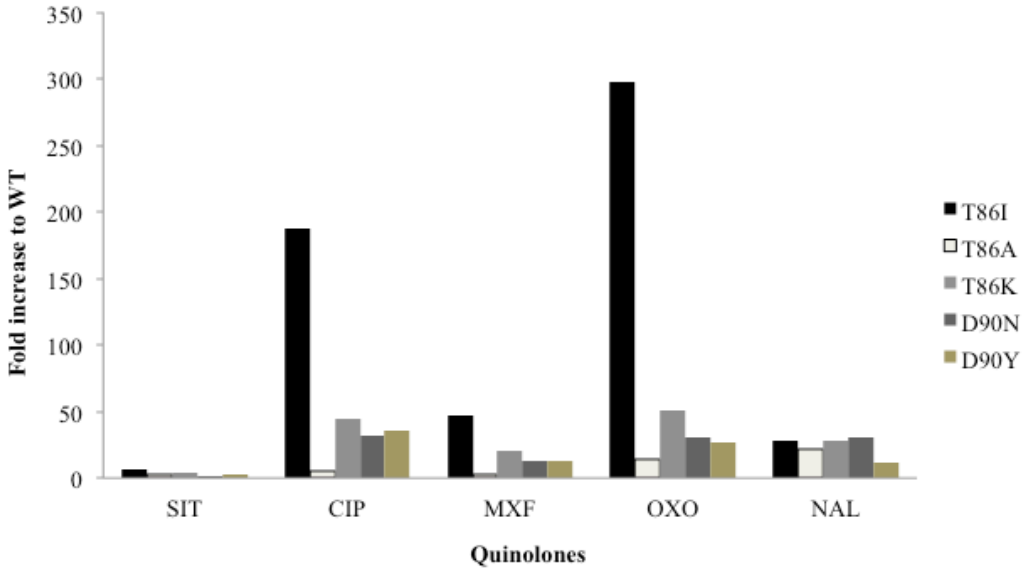

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TABLE 1. Nucleotide sequences of primers used in this study

Primer name	Primer sequence (Nucleotide position)	Proteins and genes
R-5	5'-CCCATATGGAGAATATTTTTAGCAAAG, *NdeI* site	WT GyrA
R-46	5'-CTAAAATATGAGCTCTTGCTCTTG, *SacI* site	WT GyrA
T86I_Fw	5'-CACATGGAGAT**ATA**GCAGTTTATG	Mutant *gyr*A
T86I_Rv	5'-CATAAACTGC**TAT**ATCTCCATGTG	Mutant *gyr*A
T86A_Fw	5'-CACATGGAGAT**GCA**GCAGTTTATG	Mutant *gyr*A
T86A_Rv	5-CATAAACTGC**TGC**ATCTCCATGTG	Mutant *gyr*A
T86K_Fw	5-CACATGGAGAT**AAA**GCAGTTTATG	Mutant *gyr*A
T86K_Rv	5-CATAAACTGC**TTT**ATCTCCATGTG	Mutant *gyr*A
D90N_Fw	5-CAGCAGTTTAT**AAT**GCTTTGGTTAG	Mutant *gyr*A
D90N_Rv	5-CTAACCAAAGC**ATT**ATAAACTGCTG	Mutant *gyr*A
D90Y_Fw	5-CAGCAGTTTAT**TAT**GCTTTGGTTAG	Mutant *gyr*A
D90Y_Rv	5-CTAACCAAAGC**ATA**ATAAACTGCTG	Mutant *gyr*A

WT, wild-type. Restriction endonuclease recognition sequences are underlined.
Mutated codons are indicated in bold.

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Table 2. IC_{50} of quinolones against wild-type and mutant DNA gyrases

Drug	IC_{50} (µg/ml)					
	Wild-type	**Thr 86 Ile**	**Thr 86 Ala**	**Thr 86 Lys**	**Asp 90 Asn**	**Asp 90 Tyr**
SIT	0.21 ± 0.11	1.25 ± 0.28	0.46 ± 0.05	0.93 ± 0.07	0.38 ± 0.16	0.60 ± 0.16
CIP	0.68 ± 0.21	127 ± 1.70	3.82 ± 0.93	30.3 ± 6.51	21.3 ± 2.96	23.8 ± 1.83
MXF	0.76 ± 0.03	36.0 ± 6.63	1.77 ± 1.01	15.3 ± 2.74	9.51 ± 1.94	9.65 ± 0.69
OXO	19.7 ± 4.52	5850 ± 607	274 ± 68.1	1010 ± 267	611 ± 229	536 ± 198
NAL	145 ± 7.40	4140 ± 464	3080 ± 418	4080 ± 810	4480 ± 473	1700 ± 107

IC_{50}, Drug concentration required to inhibit the supercoiling activity of DNA gyrase by 50%.

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