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Quinolone-Resistant Mutants of *Escherichia coli* DNA Topoisomerase IV *parC* Gene

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Escherichia coli **quinolone-resistant strains with mutations of the *parC* gene, which codes for a subunit of topoisomerase IV, were isolated from a quinolone-resistant *gyrA* mutant of DNA gyrase. Quinolone-resistant *parC* mutants were also identified among the quinolone-resistant clinical strains. The *parC* mutants became susceptible to quinolones by introduction of a $parC^+$ plasmid. Introduction of the multicopy plasmids carrying the quinolone-resistant *parC* mutant gene resulted in an increase in MICs of quinolones for the $parC^+$ and quinolone-resistant *gyrA* strain. Nucleotide sequences of the quinolone-resistant *parC* mutant genes were determined, and missense mutations at position Gly-78, Ser-80, or Glu-84, corresponding to those in the quinolone-resistance-determining region of DNA gyrase, were identified. These results indicate that topoisomerase IV is a target of quinolones in *E. coli* and suggest that the susceptibility of *E. coli* cells to quinolones is determined by sensitivity of the targets, DNA gyrase and topoisomerase IV.**

Topoisomerase IV (Topo IV) is a type II topoisomerase composed of the ParC and ParE subunits, which are homologous to the A and B subunits of DNA gyrase, respectively (12, 14). The temperature-sensitive *parC* or *parE* mutants formed aggregated chromosomes at the nonpermissive temperature (12, 13). In vitro studies using purified ParC and ParE proteins showed that Topo IV has strong decatenating activity (14, 18) and that Topo IV resolves replicated plasmid molecules (19). Catenated plasmid molecules are accumulated in the Topo IV mutants (1). These results indicate that Topo IV plays a role as a decatenase.

The in vitro studies also showed that the activity of Topo IV is inhibited by quinolones (9, 14, 18). These results raised a question of whether Topo IV is responsible for the level of quinolone susceptibility of *Escherichia coli* cells. DNA gyrase is thought to be a primary target of quinolones, because its topoisomerase activity is more sensitive to quinolones than that of Topo IV (9, 14, 18) and most of the quinolone resistance mutations occur in DNA gyrase genes (6, 24). Recently, mutations have been shown to occur in Topo IV genes in *Staphylococcus aureus* and *Neisseria gonorrhoeae* (2, 5). In particular, Topo IV is suggested, but not proved, to be a primary target of fluoroquinolones in *S. aureus* (4).

To elucidate that Topo IV is a target of quinolones in *E. coli*, we have isolated quinolone-resistant *parC* mutants. In addition, quinolone-resistant *parC* mutants were also identified among quinolone-resistant clinical isolates. These results indicate the involvement of Topo IV in quinolone susceptibility of *E. coli* cells. The susceptibility of cells is suggested to be dependent upon quinolone sensitivity of DNA gyrase and Topo IV. The *parC* mutants were characterized and are discussed along with the quinolone-resistant DNA gyrase mutants.

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MATERIALS AND METHODS

Antibacterial agents. Ciprofloxacin, DU-6859a (21), levofloxacin (DR-3355) (8), nalidixic acid, and sparfloxacin were synthesized by Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). These compounds were used as described previously (9).

Bacterial strains, plasmids, and culture media. DNS5101 is a highly quinolone-resistant strain which has a *gyrA* mutation and was isolated spontaneously from HB101 (8a, 20). To construct DNS5101CR (*gyrA parC*::Cm *recA*::Tn*10*), the *parC* gene of DNS5101 was disrupted in the presence of pJK818 ($parC^+$) as described previously (13) and the *recA* gene was inactivated by P1 phage-mediated transduction with GL787L as a donor (3). C600 *parC*::Cm *recA*::Tn*10* was constructed from C600 (20) in the same manner. DH1 (20) and JM109 (20) were used for plasmid construction. The *E. coli* quinolone-resistant clinical isolates used are 891145, 900857, 900858, and 900859. These strains were isolated in Japan (for details, see elsewhere in text).

Plasmids used were a mini-F plasmid vector, pJK282 (13), pUC118, pUC119, and pBR322 (20). The $parC^+$ plasmid, pJK904, was constructed by inserting a kanamycin resistance (Km^r) marker (a *Pvu*II fragment) of pYS8 (13) into the *Sca*I site in the *bla* gene of the $parC^+$ plasmid, pJK901 (12). The *parC* deletion derivative of pJK904, pJK904Δ, was obtained by deleting the *Pst*I fragment of pJK904.

Bacteria were grown in Luria-Bertani (LB) broth or on LB agar plates (16). For determination of MICs, Mueller-Hinton broth and agar (Difco, Detroit, Mich.) were used. The SOC medium (2% tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose) was used for electroporation. Transformants were selected on plates containing an appropriate antibiotic at the following concentrations: 50 μg of ampicillin per ml and 50 μg of kanamycin per ml.

Preparation and manipulation of DNA. Plasmid DNA was prepared by a method (lysis by alkali) described elsewhere (20). The techniques for manipulation of DNA were as described elsewhere (20). Transformation was carried out by the $CaCl_2$ method (20) or by electroporation with a Gene Pulser (Bio-Rad, Hercules, Calif.).

Isolation of quinolone-resistant *parC* mutants. The *parC* mutants were isolated by plasmid shuffling with a mini-F plasmid as a vector (11) (Fig. 1). The *Eco*RI-*Bam*HI fragments with the mutagenized *parC* gene were prepared by PCR in the presence of 0.2 mM $MnCl_2$ as described elsewhere (27) with the oligonucleotides 5′-CCGAATTCATGCCTTTATCGCCGTCGGA-3′ (primer 54) and 5′-TAACCAGTACAACTGCCCGA-3′ (primer 6) as primers and pJK800 ($parC^+$) (13) as a template. The *Eco*RI-*Bam*HI fragments were cloned into a mini-F vector, pJK282 (Km^r), and the resultant plasmids were introduced by electroporation into the *parC* disruptant, DNS5101CR, harboring pJK818 (mini-F-$parC^+$ Ap^r). The transformants were incubated in SOC medium for 1 h and in LB broth containing kanamycin overnight, and the levofloxacin-resistant mutants were selected by plating on LB plates containing 5 μg of levofloxacin per ml.

Determination of MICs. Strains were cultivated overnight at 37°C in Mueller-Hinton broth, and the MICs were determined by an agar dilution technique. The inoculum size was approximately 10^5 CFU/ml. MICs were defined as the lowest

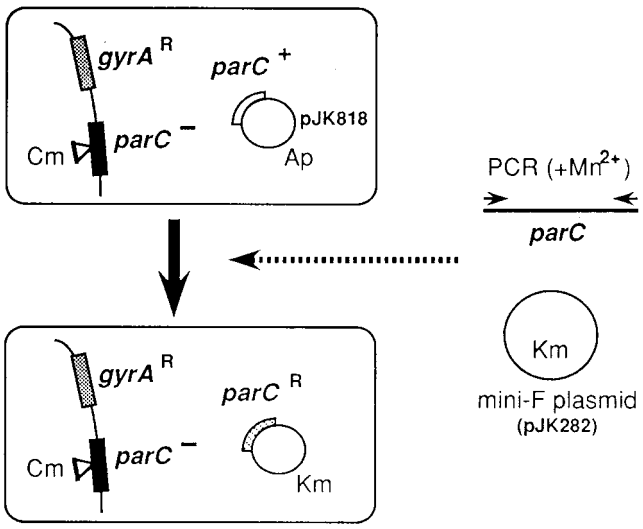

FIG. 1. Isolation of the *parC* mutants by plasmid shuffling. The *parC* DNA fragments were prepared by PCR in the presence of Mn^{2+} and cloned into a mini-F vector, pJK282 (Km^r). The resultant plasmids (Km^r) were introduced into the *gyrA* and *parC* disruptant carrying the *parC*$^+$ mini-F plasmid, pJK818 (Ap^r). The Km^r transformants, in which the Ap^r mini-F plasmid, pJK818, was replaced by the Km^r mini-F plasmid, were surveyed for levofloxacin-resistant mutants (for details, see Materials and Methods).

concentrations that completely prevented visible growth of the inoculum after incubation for 18 h at 37°C (23).

Cloning of *parC* genes from DNS5101 and clinical isolates. The *parC* genes were amplified by PCR with the chromosomal DNA of the strains as templates. The PCR primers used were primer 54 and primer 6 (see above) for the strains DNS5101 and 900857, and an oligonucleotide, 5′-CCGAATTCGAGGAATCA GAATTAATGAACG-3′ (primer 857), and primer 6 for the strains 891145, 900858, and 900859. The amplified fragments were digested with *Eco*RI and *Bam*HI and ligated with the *Eco*RI-*Bam*HI fragment of pUC118. The *Eco*RI-*Bam*HI fragments carrying the *parC* genes were subcloned from the plasmids into a mini-F vector, pJK282, for determination of MICs.

DNA sequencing. Nucleotide sequences of the whole regions of *parC1*, *parC15*, and *parC48* genes were determined as follows. The *Eco*RI-*Bam*HI fragments containing the *parC* genes were purified from the mini-F *parC* plasmids. These *Eco*RI-*Bam*HI fragments were digested with *Pvu*II and *Pst*I, since there are two *Pvu*II sites [*Pvu*II$_{(1)}$ and *Pvu*II$_{(2)}$] and one *Pst*I site in the *parC* gene. The *Eco*RI-*Pvu*II, *Pvu*II$_{(1)}$-*Pst*I, *Pst*I-*Pvu*II$_{(2)}$, and *Pvu*II-*Bam*HI fragments were ligated with *Eco*RI-*Hin*cII, *Hin*cII-*Pst*I, *Pst*I-*Hin*cII, and *Hin*cII-*Bam*HI fragments of pUC118 or pUC119, respectively, and the nucleotide sequences were determined with pUC primers. The nucleotide sequences around the active site region, the region corresponding to the quinolone-resistance-determining region of the *gyrA* gene, of the *parC101*, *parC145*, *parC857*, *parC858*, and *parC859* genes were determined with pUC plasmids carrying these genes as templates and the oligonucleotide 5′-AGCGCCGCATTGTGTATGCG-3′ (primer 817) and the pUC universal primer M4 (Takara, Kyoto, Japan), 5′-GTTTTCCCAGTCAC-GAC, as primers. The nucleotide sequences of the mutant genes were determined not always by sequencing of both DNA strands but by simultaneous sequencing of the corresponding parts of the wild-type *parC* gene as standards.

RESULTS

Isolation of quinolone-resistant *parC* mutants. Since DNA gyrase is thought to be a primary target of quinolones in wild-type *E. coli*, we have tried to isolate the Topo IV mutants from a quinolone-resistant DNA gyrase mutant. We have isolated the ParC subunit mutants (the ParC subunit is homologous to GyrA protein), because most of the quinolone-resistant DNA gyrase mutants have mutations in the A subunit gene (*gyrA*) of DNA gyrase.

To isolate the *parC* mutants efficiently, we used the method of plasmid shuffling (11) (Fig. 1). The *parC* DNA fragments prepared by PCR in the presence of Mn^{2+} were cloned into a mini-F vector, pJK282 (Km^r), and the resultant plasmids were introduced into the *parC* disruptant DNS5101CR carrying pJK818 (mini-F-*parC*$^+$ Ap^r). Because of incompatibility of mini-F plasmids, selection of Km^r cells leads to isolated cells that carry the pJK282 (mini-F Km^r)-*parC* plasmid but not the original plasmid, pJK818. Among the Km^r transformants, three levofloxacin-resistant mutants (*parC12*, *parC15*, and *parC48*) were isolated (Table 1). Another levofloxacin-resistant mutant (*parC1*) was isolated by plasmid shuffling of the *parC* fragment amplified by PCR in the absence of Mn^{2+} (Table 1).

Recloning of the *Eco*RI-*Bam*HI *parC* fragments of the mini-F plasmids into a mini-F vector, pJK282 (Km^r), confirmed that the levofloxacin resistance of the mutants is ascribed to the *parC* mutant genes on the introduced mini-F plasmids (data not shown). In this experiment, the mutant *parC* plasmid was selected by kanamycin resistance alone but was not selected by quinolone resistance. The *parC* gene cloned from DNS5101 was also found to confer resistance to levofloxacin in a *parC* disruptant (Table 1), indicating that the strain is a *parC* (*parC101*) mutant.

When the *parC* mini-F (Km^r) plasmids were introduced into the *gyrA*$^+$ and *parC* disruptant (C600*parC*::Cm *recA*::Tn*10* carrying pJK818 [mini-F-*parC*$^+$ Ap^r]), MICs of levofloxacin in the Km^r Ap^s transformants were the same as that in the control strain, the *parC* disruptant carrying the *parC*$^+$ mini-F (Km^r) plasmid (0.05 μg/ml). With the isogenic *gyrA* strain, the same result as with DNS5101CR (described above) was obtained. These results indicate that DNA gyrase is a primary target in a *gyrA*$^+$ strain but that Topo IV is a primary target in a quinolone-resistant DNA gyrase mutant.

Nucleotide sequences of the three *parC* mutant genes (*parC1*, *parC15*, and *parC48*) and that of the region around the active site (300 bp from the initiation codon) of the *parC101* gene were determined (Table 2). There are missense mutations in all of the mutant genes. In the *parC1* mutant gene, there is a point mutation upstream of the coding region. The *parC1* DNA fragment without the upstream mutation was prepared by PCR with a set of primers (primer 857 and primer 6; see Materials and Methods) and the *parC1* mini-F plasmid as a template and cloned into a mini-F vector, pJK282 (Km^r). Since strain DNS5101CR carrying the resultant plasmid was as resistant to levofloxacin as was the *parC1* mutant (data not

TABLE 1. Susceptibility of *parC* mutants to quinolones[a]

Mutation	MIC (μg/ml) of drug				
	LVFX	Du-6859a	CPFX	SPFX	NA
parC$^+$	0.78	0.2	0.39	0.39	>200
parC48	6.25	1.56	12.5	12.5	>200
parC15	12.5	1.56	12.5	12.5	>200
parC1	25	1.56	25	12.5	>200
parC12	25	1.56	25	12.5	>200
parC101	25	1.56	25	12.5	>200
parC145	3.13	0.78	3.13	6.25	>200
parC857	6.25	1.56	12.5	12.5	>200
parC858	25	1.56	12.5	12.5	50
parC859	25	1.56	25	12.5	50

[a] Strains used are DNS5101CR (*gyrA parC*::Cm *recA*) carrying pJK282 (mini-F Km^r) plasmid derivatives with the *parC*$^+$ gene or the *parC* mutant genes. The *parC1*, *parC12*, *parC15*, *parC48*, *parC101*, and *parC857* genes were amplified by PCR with primer 54 and primer 6, and the *parC145*, *parC858*, and *parC859* genes were amplified with primer 857 and primer 6 (see Materials and Methods). The mini-F plasmids carrying the *parC*$^+$ genes amplified with these two sets of primers confer the same level of susceptibility to quinolones tested. LVFX, levofloxacin; CPFX, ciprofloxacin; SPFX, sparfloxacin; NA, nalidixic acid.

TABLE 2. Mutations of the *parC* genes[a]

Mutation	Nucleotide		Amino acid	
	Position	Change	Position	Change
parC1	−90	T→C		
	233	GGC→GAC	78	Gly→Asp
	757	GTT→ATT	253	Val→Ile
parC15	239	AGC→ATC	80	Ser→Ile
	1304	GGT→GCT	435	Gly→Ala
	1750	CAT→TAT	584	His→Tyr
parC48	238	AGC→CGC	80	Ser→Arg
	682	ATC→GTC	228	Ile→Val
	1748	GAC→GGC	583	Asp→Gly
parC101	233	GGC→GAC	78	Gly→Asp
parC145	240	AGC→AGA	80	Ser→Arg
parC857	239	AGT*→ATT*	80	Ser→Ile
parC858	250	GAA→AAA	84	Glu→Lys
parC859	250	GAA→AAA	84	Glu→Lys

[a] The nucleotide sequences determined are those of the whole regions of *parC1*, *parC15*, and *parC48* mutant genes and those of the regions around the active sites of the *parC101*, *parC145*, *parC857*, *parC858*, and *parC859* mutant genes. T* represents a silent mutation of *parC857*.

shown), the mutation upstream of the coding region is not responsible for quinolone resistance. It is not known which of the missense mutations is responsible for quinolone resistance.

Effects of the *parC*$^+$ or *parC* plasmids on quinolone resistance. To know whether the *parC* mutations were dominant, the *parC*$^+$ plasmid was introduced into the *parC* mutants. As shown in Table 3, introduction of the *parC*$^+$ plasmid made the mutants susceptible to levofloxacin. This effect was observed with another strain, DNS5101 (Table 4). The strain DNS5101 was transformed by either of the *parC*$^+$ multicopy plasmids, pUC118 *parC*$^+$ and pJK904 (pBR322-*parC*$^+$), or the *parC*$^+$ mini-F plasmid (pJK282-*parC*$^+$). All of the transformants showed similar levels of susceptibility irrespective of gene dosage (Table 4). Therefore, one copy of the *parC*$^+$ gene is enough for this effect, at least with regard to the *parC101* mutation of DNS5101.

To examine the effect of the *parC* plasmid, the *parC* mutant genes were cloned into a multicopy plasmid and the plasmids were introduced into the strain DNS5101CR carrying the *parC*$^+$ mini-F plasmid. As shown in Table 5, the transformants became more resistant than the control strains. This effect was also seen with the other *parC*$^+$ *gyrA* strain, C600*gyrA* (Table 5).

TABLE 3. Effect of introduction of the *parC*$^+$ plasmid on resistance of highly resistant mutants to levofloxacin[a]

Mutation	Plasmid	MIC (μg/ml)
parC$^+$	pUC118	1.56
	pUC118-*parC*$^+$	1.56
parC48	pUC118	6.25
	pUC118-*parC*$^+$	0.78
parC15	pUC118	12.5
	pUC118-*parC*$^+$	1.56
parC1	pUC118	50
	pUC118-*parC*$^+$	0.78
parC12	pUC118	50
	pUC118-*parC*$^+$	0.78

[a] Strains used are DNS5101CR (*gyrA parC*::Cm *recA*) carrying pJK282 (mini-F Km^r) plasmid derivatives with the *parC*$^+$ gene or the *parC* mutant genes. MICs were determined under selection with both ampicillin (for pUC118 or pUC118-*parC*$^+$) and kanamycin (for pJK282-*parC*$^+$ or pJK282-*parC*).

TABLE 4. Effect of introduction of the *parC*$^+$ plasmid on resistance to levofloxacin

Strain	Plasmid	MIC (μg/ml)
DNS5101	pJK904Δ (pBR322-*parC*)	50
	pJK904 (pBR322-*parC*$^+$)	1.56
	pJK282 (mini-F)	50
	pJK282-*parC*$^+$	1.56
	pUC118	50
	pUC118-*parC*$^+$	0.78
891145	pJK904Δ	12.5
	pJK904	3.13
900858	pJK904Δ	25
	pJK904	3.13
900859	pJK904Δ	25
	pJK904	3.13

These results show that the *parC* gene on a multicopy plasmid confers a high level of quinolone resistance on the *gyrA* mutants.

Identification of *parC* mutants among clinical isolates. To identify the *parC* mutant in clinical isolates, we made use of the effect of the *parC*$^+$ plasmid on quinolone resistance. Among 204 *E. coli* clinical isolates collected from various hospitals in Japan from 1989 to 1990, 10 levofloxacin-resistant strains (MIC, >0.39 μg/ml) were selected. The *parC*$^+$ Km^r multicopy plasmid (pJK904) or the *parC* Km^r plasmid (pJK904Δ) was introduced into the Km^s clinical strains, which were 8 of the 10 levofloxacin-resistant strains. Three of the eight strains, 891145, 900858, and 900859, became more susceptible to levofloxacin by introduction of the *parC*$^+$ plasmid than by that of the *parC* plasmid (Table 4), suggesting that these strains were the *parC* mutants. This suggestion was confirmed by cloning the *parC* mutant genes of the three strains into a mini-F (Km^r) vector (pJK282) (Table 1). The *parC* gene of a clinical isolate, 900857, which is the Km^r clinical strain of the 10 levofloxacin-resistant strains described above, was also cloned, and it was found to be a *parC* mutant (Table 1). Nucleotide sequences of the regions around the active site (300 bp from the initiation codon) of the four *parC* genes were determined (Table 2). The results confirm that these are the *parC* mutants.

Search for a quinolone that is effective for *gyrA parC* mutants. Susceptibility of the laboratory and clinical *gyrA parC* mutants to the quinolones, ciprofloxacin, sparfloxacin, and nalidixic acid, was investigated. As shown in Table 1, the increase in MIC between *parC*$^+$ and *parC* mutants was eightfold for DU-6859a but from 32- to 128-fold for ciprofloxacin and sparfloxacin. The result indicates that Topo IV inhibition by DU-

TABLE 5. Effect of the *parC* plasmid on susceptibility to levofloxacin[a]

Strain	Plasmid	MIC (μg/ml)
DNS5101CR pJK282-*parC*$^+$	pUC118	1.56
	pUC118-*parC*$^+$	0.78
	pUC118-*parC15*	6.25
	pUC118-*parC48*	6.25
C600*gyrA*	pUC118	0.2
	pUC118-*parC*$^+$	0.2
	pUC118-*parC15*	1.56
	pUC118-*parC48*	1.56

[a] For construction of the pUC118-*parC*$^+$ or pUC118-*parC* plasmids, the *Eco*RI-*Bam*HI fragments containing the *parC* genes of pJK282-*parC*$^+$ or pJK282-*parC* plasmids were subcloned into the *Eco*RI-*Bam*HI sites of pUC118.

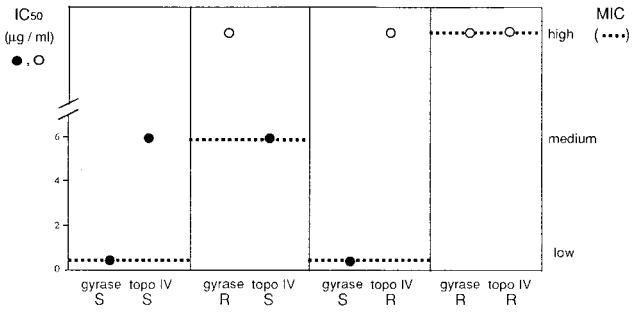

FIG. 2. Model representing relationship between quinolone MIC for *E. coli* and quinolone sensitivity of two topoisomerases. MICs of quinolones for *E. coli* would depend on IC_{50}s for two targets of quinolones, DNA gyrase and Topo IV. In this figure, the case with levofloxacin is shown. IC_{50}s of levofloxacin for wild-type DNA gyrase (0.38 μg/ml) and Topo IV (5.95 μg/ml) are represented by closed circles. Putative IC_{50}s for quinolone-resistant DNA gyrase and Topo IV are shown by open circles. This figure does not show quantitatively the IC_{50}s for the mutant DNA gyrase and mutant Topo IV. The MICs for wild-type and quinolone-resistant Topo IV strains depend on the IC_{50} for DNA gyrase, while the MIC for a quinclone-resistant gyrase mutant depends on the IC_{50} for Topo IV. The MIC for a quinclone-resistant gyrase quinclone-resistant Topo IV double mutant depends on lower IC_{50} for either DNA gyrase or Topo IV.

6859a is significantly less affected by the levofloxacin-resistance mutations than is the inhibition by levofloxacin, ciprofloxacin, and sparfloxacin. Susceptibility of the *gyrA*$^+$ *parC* strain to the quinolones tested in Table 1 was the same level as that of the *gyrA*$^+$ *parC*$^+$ isogenic strain (data not shown). The result suggests that Topo IV is not a primary target of these quinolones in a wild-type strain.

DISCUSSION

We have succeeded in isolating quinolone-resistant mutants of Topo IV and have indicated that Topo IV is a primary target of quinolones in *E. coli* with the *gyrA* background. Previous studies showed that Topo IV is not sensitive to quinolones as much as is DNA gyrase (9, 14, 18). On the other hand, the *parC* mutants with the *gyrA*$^+$ background are as susceptible to quinolones as is the isogenic *parC*$^+$ strain. Therefore, DNA gyrase is thought to be a primary target of quinolones in a wild-type strain. These results may be interpreted by a model showing the relationship between MICs of quinolones for *E. coli* and quinolone sensitivity of DNA gyrase and Topo IV (Fig. 2). In this model, the MIC would depend on the more sensitive target. Purification and measurement of 50% inhibitory concentration (IC_{50}) for the mutant Topo IV will confirm the model.

Analysis of the *parC* mutations showed that the *parC1*, *parC15*, and *parC48* mutant genes contain multiple missense mutations. The other *parC* mutations have not been known to be single mutations, because only the regions around the active site were sequenced. The mutations responsible for quinolone resistance are also not known, but some mutations are suggested to be quinolone resistance mutations. Four of the eight mutants have missense mutations at Ser-80. The mutations at Ser-80 were found in both the laboratory *parC* strains and the clinical *parC* strains. There are the same missense mutations at Gly-78 and Glu-84 in two independently isolated mutants (*parC1* and *parC101* for Gly-78 and *parC858* and *parC859* for Glu-84). The Ser-80, Gly-78, and Glu-84 of ParC correspond to Ser-83, Gly-81, and Asp-87 of GyrA, respectively. The mutations at Ser-83, Gly-81, and Asp-87 of DNA gyrase are well known to confer quinolone resistance (7, 17, 22, 25). The result suggests that the conformation of Topo IV and physical interaction of Topo IV with quinolones might be homologous to those of DNA gyrase.

The *parC* mutants were shown to become susceptible to quinolones by introduction of the *parC*$^+$ plasmids. A similar phenomenon has been reported with *gyrA* mutants (26). ParC$^+$ proteins derived from the *parC*$^+$ gene on the introduced plasmid may predominantly form a complex with ParE$^+$ proteins, leading to a decrease of the portion of mutant Topo IV composed of ParC-ParE$^+$. It was also shown that a *parC*$^+$ strain carrying the *parC* multicopy plasmids became more resistant to quinolones than did the cells carrying the *parC*$^+$ plasmids. In this case, ParE$^+$ proteins may be titrated by ParC proteins, which are derived from the *parC* multicopy plasmid, leading to a decrease of wild-type Topo IV composed of ParC$^+$-ParE$^+$.

Quinolones may become a powerful tool for studying chromosome segregation in *gyrA* cells, since the C600*gyrA* mutant (MIC of levofloxacin, 0.2 μg/ml) formed large aggregated chromosomes in the presence of 0.5 μg of levofloxacin per ml, suggesting that chromosome segregation was blocked (data not shown). The results obtained in this work suggest that the blocking of chromosome segregation is ascribed to inhibition of Topo IV functions by levofloxacin.

Although the *parC* mutations are shown to confer resistance to quinolones, mutant Topo IV may be no more than one of the causes for a high level of resistance and there may be other mechanisms of resistance, for example, alteration of the permeability of quinolones across the cell membrane. However, since at least a part of the high level of resistance of some clinical isolates is caused by additional mutations in Topo IV, the quinolones, which have inhibitory potency against mutant Topo IV, would be very useful for killing of the quinolone-resistant pathogenic bacteria. In this work, DU-6859a is found to be such a type of quinolone. DU-6859a is also known to be effective against quinolone-resistant DNA gyrase mutants (10, 15). On the other hand, DNS5101CR carrying the *parC858* or *parC859* plasmid was resistant to levofloxacin but was more susceptible to nalidixic acid than was the control strain, DNS5101CR carrying the *parC*$^+$ plasmid (Table 1). Analysis of the conformation of Topo IV and the physical interaction of Topo IV with these quinolones would be necessary for understanding the features of these mutants and quinolones.

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