

Inhibitory Activities of Gatifloxacin (AM-1155), a Newly Developed Fluoroquinolone, against Bacterial and Mammalian Type II Topoisomerases

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We determined the inhibitory activities of gatifloxacin against *Staphylococcus aureus* topoisomerase IV, *Escherichia coli* DNA gyrase, and HeLa cell topoisomerase II and compared them with those of several quinolones. The inhibitory activities of quinolones against these type II topoisomerases significantly correlated with their antibacterial activities or cytotoxicities (correlation coefficient [r] = 0.926 for *S. aureus*, r = 0.972 for *E. coli*, and r = 0.648 for HeLa cells). Gatifloxacin possessed potent inhibitory activities against bacterial type II topoisomerases (50% inhibitory concentration [IC_{50}] = 13.8 μ g/ml for *S. aureus* topoisomerase IV; IC_{50} = 0.109 μ g/ml for *E. coli* DNA gyrase) but the lowest activity against HeLa cell topoisomerase II (IC_{50} = 265 μ g/ml) among the quinolones tested. There was also a significant correlation between the inhibitory activities of quinolones against *S. aureus* topoisomerase IV and those against *E. coli* DNA gyrase (r = 0.969). However, the inhibitory activity against HeLa cell topoisomerase II did not correlate with that against either bacterial enzyme. The IC_{50} of gatifloxacin for HeLa cell topoisomerase II was 19 and was more than 2,400 times higher than that for *S. aureus* topoisomerase IV and that for *E. coli* DNA gyrase. These ratios were higher than those for other quinolones, indicating that gatifloxacin possesses a higher selectivity for bacterial type II topoisomerases.

Quinolone antibacterial agents have potent activity against gram-positive and -negative bacteria and are currently used as therapy for various bacterial infections. The antibacterial activities of quinolones are related in their inhibitory activities against DNA gyrase and topoisomerase IV (3, 13, 28). Both enzymes are members of the type II topoisomerase family that controls bacterial DNA topology by passing a DNA double helix through another, by using a transient double-strand break (18).

It has recently been reported that the primary target of several quinolones in *Escherichia coli* is DNA gyrase (8, 13, 16, 17) and that in *Staphylococcus aureus* is topoisomerase IV (2, 4, 6, 22, 31). Quinolones also inhibit mammalian type II topoisomerases such as topoisomerase II (11, 12, 14, 21, 25), and their inhibitory potencies for topoisomerase II have been correlated with their cytotoxicity (25). Therefore, it is important to determine the inhibitory activities of quinolones against bacterial and mammalian type II topoisomerases to clarify the selective toxicities.

Gatifloxacin (AM-1155), a newly developed quinolone, has shown potent activity against gram-positive and -negative bacteria, and it has been reported that gatifloxacin inhibits DNA gyrase of *E. coli*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, and *S. aureus*, like other quinolones (10, 30). However, little is known about the inhibitory activities of gatifloxacin against *S. aureus* topoisomerase IV and HeLa cell topoisomerase II. In this study, we determined the inhibitory activity of gatifloxacin against bacterial primary target enzymes and mammalian topoisomerase II, and herein we discuss its selectivity.

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MATERIALS AND METHODS

Quinolones. Gatifloxacin, ciprofloxacin, clinafloxacin, fleroxacin, levofloxacin, lomefloxacin, norfloxacin, ofloxacin, sparfloxacin, tosufloxacin, and pipemidic acid were synthesized by our company. Enoxacin and nalidixic acid were purchased from Sigma Chemical Co. (St. Louis, Mo.).

Bacterial strains, cell line, and plasmids. *S. aureus* MS5935 is a quinolone-susceptible clinical isolate, as reported previously (9). *E. coli* NIHJ JC-2 and HeLa cells were obtained from stocks in our laboratory. *E. coli* BL21(DE3) and pET11a were purchased from Stratagene (La Jolla, Calif.). pRSET A and kinetoplast DNA were purchased from Invitrogen (San Diego, Calif.) and Topo-

TABLE 1. Inhibitory activities of quinolones against type II topoisomerases

Quinolone	Activity (μ g/ml) against:					
	<i>S. aureus</i> MS5935 topoisomerase IV		<i>E. coli</i> NIHJ JC-2 DNA gyrase		HeLa cell topoisomerase II	
	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	Cytotoxicity
Gatifloxacin	13.8	0.05	0.109	0.0063	265	122
Clinafloxacin	3.23	0.025	0.045	0.0032	29.7	60.1
Ciprofloxacin	11.8	0.39	0.072	0.0032	34.4 ^a	105
Sparfloxacin	22.3	0.05	0.130	0.0125	102	72.5
Ofloxacin	35.7	0.39	0.211	0.0125	132 ^a	301
Tosufloxacin	ND ^b	ND	0.165	0.0125	ND	ND
Levofloxacin	19.9	0.20	0.186	0.0063	ND	ND
Lomefloxacin	35.2	0.39	0.171	0.025	238	216
Norfloxacin	26.0	0.78	0.309	0.025	87.3 ^a	139
Fleroxacin	45.7	0.39	0.378	0.025	193 ^a	349
Enoxacin	55.4	0.78	0.482	0.05	81.8	67.9
Pipemidic acid	350	25	3.18	0.78	ND	ND
Nalidixic acid	435	25	7.25	0.78	169 ^a	277

^a Data from previous study (25).

^b ND, not determined.

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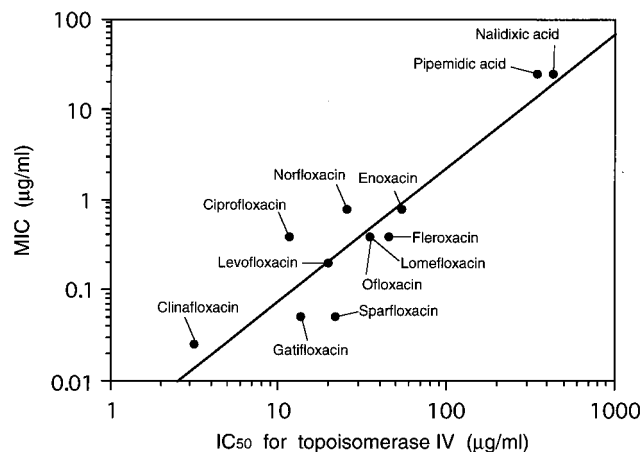


FIG. 1. Correlation between the IC_{50} for topoisomerase IV and the MIC for *S. aureus* ($r = 0.926$; $P < 0.01$).

GEN Inc. (Columbus, Ohio), respectively. Supercoiled pBR322 was prepared by the cesium chloride density gradient method and was relaxed by topoisomerase I (prepared from HeLa cells in our laboratory) (19).

Preparation of type II topoisomerases. The *grlA* and *grlB* genes of *S. aureus* encoding both subunits of topoisomerase IV were amplified by PCR. PCR was performed with genomic DNA from *S. aureus* MS5935 by using eLONGASE enzyme mix (GIBCO BRL, Rockville, Md.) with the oligonucleotides 5'-GGA ATTCATATGAGTGAATAATTCAAGATT-3' and 5'-CGGGATCCATT ATGTGGTGGTATATCTGTGCGC-3' as primers for the first half of *grlA*, 5'-CCTAACTACTAGTGAATGGTTCTACAGG-3' and 5'-CGGGATCCCTA GTTTTAGCTAATATACATGTCTAT-3' for the second half of *grlA*, and 5'-GGCGAAATCATGTCATATGAATAAAACAAAATAATTATTTCAGATGAT TCAATACAGG-3' and 5'-CTTGAATTAGGATCCTCACTAGATTTCCCTC-3' for *grlB*. Based on the sequence published by Ferrero et al. (4), the primers were designed to introduce a *NdeI* site at the initiation codon and a *BamHI* site downstream from the termination codon. DNA was amplified for 30 cycles of 1 min at 95°C for denaturation, 1 min at 55°C for annealing, and 2 min at 70°C for polymerization. The first half and the second half of the *grlA* fragment were digested with *NdeI* and *PstI* and with *BamHI* and *PstI*, respectively. The *grlB* fragment and vector plasmids pET11a and pRSET A were digested with both *NdeI* and *BamHI*. Ligation was performed to construct *grlA*-pET11a (pKY3403) and *grlB*-pRSET A (pKY3405). *GrlA* and *GrlB* were individually induced by the addition of IPTG (isopropyl-1-thio- β -D-galactopyranoside) to cultures of *E. coli* BL21(DE3) transformed by pKY3403 and pKY3405, respectively. Cell extracts of BL21(DE3)/pKY3403 and BL21(DE3)/pKY3405 were prepared by the procedure of Peng and Mariani (26). Fully active topoisomerase IV was reconstituted by preincubating *GrlA* and *GrlB* on ice for at least 30 min.

DNA gyrase from *E. coli* NIHJ JC-2 was prepared by affinity chromatography on novobiocin-Sepharose by the method of Sato et al. (27).

Topoisomerase II from HeLa cells was obtained by the method of Miller et al. (19).

Enzyme assay. The decatenation activity of the reconstituted topoisomerase IV was determined by the method of Peng and Mariani (26) with minor modifications. The reactions were analyzed by electrophoresis, and DNA quantification in agarose gels was carried out after ethidium bromide staining. The brightness of the bands corresponding to decatenated monomers of kinetoplast DNA was determined by densitometric analysis with FMBIO II Multi-View (Hitachi Software Engineering Co., Ltd., Yokohama, Japan).

The supercoiling activity of DNA gyrase was determined by the method of Gellert et al. (7) with minor modifications. Analysis was performed as described for the topoisomerase IV assay.

The relaxation activity of topoisomerase II was determined by the method of Oomori et al. (25).

The inhibitory effect of each quinolone on type II topoisomerase was assayed by determining the concentration required to inhibit 50% of the enzyme reaction (IC_{50}). Selectivity was determined by dividing the IC_{50} for HeLa cell topoisomerase II by the IC_{50} for bacterial type II topoisomerase.

Determination of MICs and cytotoxicities. MICs were measured by an agar dilution method (10) with Mueller-Hinton medium (Difco Laboratories, Detroit, Mich.).

Growth inhibition of HeLa cells was determined by the procedure of Aggarwal et al. (1). Cytotoxicity was expressed as the concentration required to inhibit 50% of HeLa cell growth.

Statistical analysis. Correlation was determined by a linear regression analysis. A P value of <0.05 was considered to be statistically significant.

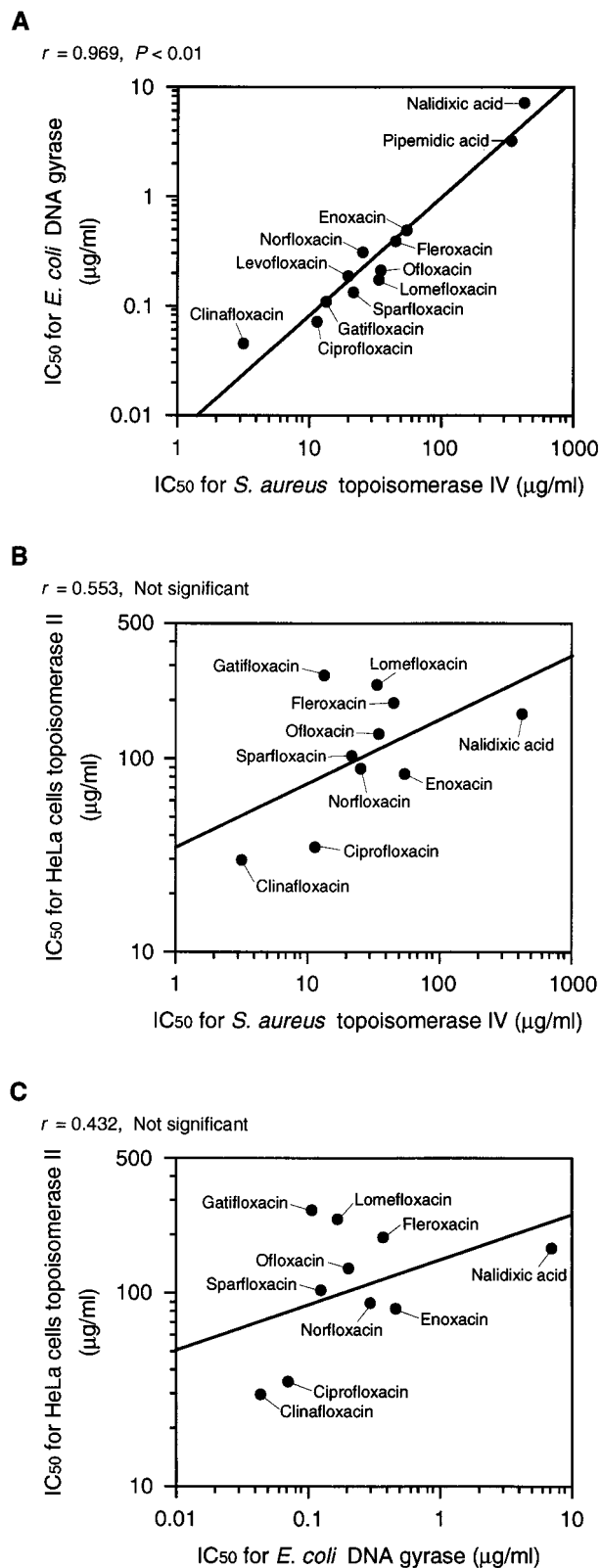


FIG. 2. Correlation between inhibitory activities against type II topoisomerases. (A) *S. aureus* topoisomerase IV vs. *E. coli* DNA gyrase; (B) *S. aureus* topoisomerase IV vs. HeLa cell topoisomerase II; (C) *E. coli* DNA gyrase vs. HeLa cell topoisomerase II. The correlation coefficient and significance are indicated at the top of each panel.

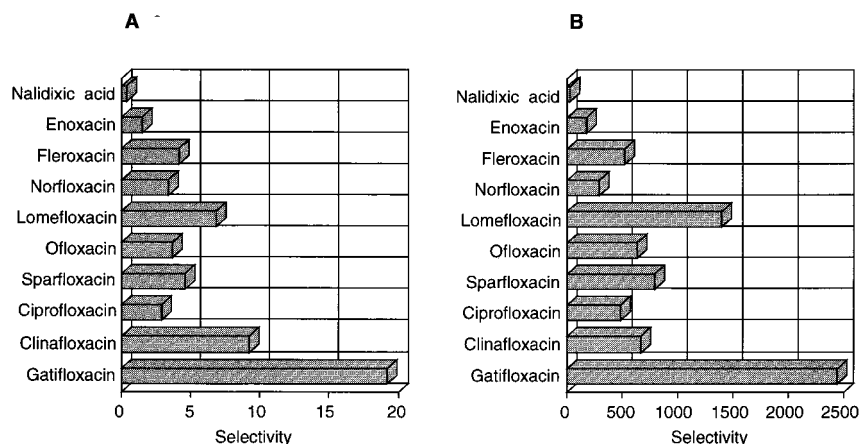


FIG. 3. Selectivity of quinolones against type II topoisomerases. Selectivity was calculated by dividing the IC_{50} for HeLa cell topoisomerase II by the IC_{50} for bacterial type II topoisomerase. (A) *S. aureus* topoisomerase IV vs. HeLa cell topoisomerase II; (B) *E. coli* DNA gyrase vs. HeLa cell topoisomerase II.

RESULTS AND DISCUSSION

Inhibitory activities of quinolones for bacterial type II topoisomerases. Bacterial and mammalian type II topoisomerases are known to be essential enzymes for cell growth (24). Based on genetic analysis, it has been reported that the primary targets of the quinolones tested so far in *E. coli* and *S. aureus* are DNA gyrase and topoisomerase IV, respectively (2, 4–6, 16, 17, 22, 31). In our study, gatifloxacin showed a higher inhibitory activity against *S. aureus* topoisomerase IV ($IC_{50} = 13.8 \mu\text{g/ml}$) and *E. coli* DNA gyrase ($IC_{50} = 0.109 \mu\text{g/ml}$) than did the other quinolones tested, except for clinafloxacin and ciprofloxacin (Table 1).

The inhibitory activities of quinolones against *E. coli* DNA gyrase have been shown to correlate with their antimicrobial activities (12). However, little is known about the relationship between the antimicrobial activities of quinolones and their inhibitory activity against *S. aureus* topoisomerase IV (29). In this study, we statistically determined the correlation between the inhibitory activity of many quinolones against *S. aureus* topoisomerase IV and their antibacterial activity and observed a significant correlation between not only the IC_{50} s of the quinolones for *E. coli* DNA gyrase and their MICs (correlation coefficient [r] = 0.972, $P < 0.01$) but also the IC_{50} s of the quinolones for *S. aureus* topoisomerase IV and their MICs ($r = 0.926$, $P < 0.01$) (Fig. 1). These results suggest that the antibacterial activities of the quinolones tested against *E. coli* and *S. aureus* are basically determined by the inhibition of DNA gyrase and topoisomerase IV, respectively.

The IC_{50} of gatifloxacin for *S. aureus* topoisomerase IV was almost equal to that of ciprofloxacin. However, the antibacterial activity of gatifloxacin against *S. aureus* was eight times higher than that of ciprofloxacin. A similar discrepancy was also observed between sparfloxacin and norfloxacin. These phenomena suggest that some factors besides the inhibition of topoisomerase IV influence the antibacterial activity of quinolones against *S. aureus*. These factors might include inhibition of *S. aureus* DNA gyrase and excretion mechanisms of quinolones in *S. aureus*, such as the quinolone efflux protein NorA (6, 15, 23).

Inhibitory activities of quinolones for mammalian topoisomerase II. All the quinolones tested inhibited the relaxation activity of topoisomerase II from HeLa cells (Table 1). However, the IC_{50} s of quinolones, except for nalidixic acid, for HeLa cell topoisomerase II were higher than those for both

bacterial enzymes. The IC_{50} s for topoisomerase II roughly correlated with the cytotoxicity ($r = 0.648$, $P < 0.05$). Therefore, it was suggested that the inhibitory activity of quinolones against topoisomerase II is one of the causes of the inhibition of HeLa cell growth. Gatifloxacin, with an IC_{50} of $265 \mu\text{g/ml}$, showed the lowest inhibitory activity for topoisomerase II. In contrast, clinafloxacin and ciprofloxacin showed the highest inhibitory activities among the quinolones tested.

Selectivity of quinolones against bacterial and mammalian type II topoisomerases. The correlations between the inhibitory activities for each type II topoisomerase are shown in Fig. 2.

Ferrero et al. have reported that the *S. aureus* topoisomerase IV subunit GrIA and GrIB proteins have 32 and 49% identities with the *E. coli* DNA gyrase subunit GyrA and GyrB proteins, respectively (4). We showed that the inhibitory activities of quinolones against *S. aureus* topoisomerase IV closely correlate with those against *E. coli* DNA gyrase ($r = 0.969$, $P < 0.01$) (Fig. 2A). These data suggest that quinolones inhibit both enzymes in a similar manner. On the other hand, Hoshino et al. have reported that the inhibitory activities of various quinolones against *E. coli* DNA gyrase do not correlate with those against fetal calf thymus topoisomerase II (12). In this study, all the quinolones except nalidixic acid required concentrations (IC_{50} s) to inhibit the topoisomerase II of HeLa cells that were higher than those needed to inhibit bacterial type II topoisomerases, and no significant correlation was observed between the inhibition of HeLa cell topoisomerase II and that of *S. aureus* topoisomerase IV as well as *E. coli* DNA gyrase (Fig. 2B and C). Morais Cabral et al. have reported differences in the rotation of the quaternary organization of the breakage-reunion domain, which includes the quinolone resistance-determining region, between prokaryotic (*E. coli*) DNA gyrase and eukaryotic (yeast) topoisomerase II (20). These differences might contribute to the only slight correlation between the inhibition of HeLa cell topoisomerase II and the inhibition of bacterial enzymes. Thus, there might be a large difference in the selectivities of the quinolones for bacterial and mammalian enzymes.

The selectivities of the quinolones are expressed in Fig. 3 as ratios of the IC_{50} for HeLa cell topoisomerase II to that for the bacterial enzyme. The IC_{50} of gatifloxacin for HeLa cell topoisomerase II was 19 and was more than 2,400 times higher than that for *S. aureus* topoisomerase IV and *E. coli* DNA gyrase. These ratios were higher than those for the other quinolones

tested, indicating that gatifloxacin possesses a higher selectivity for bacterial type II topoisomerases.

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REFERENCES

- Aggarwal, B. B., B. Moffat, and R. N. Harkins. 1984. Human lymphotoxin. *J. Biol. Chem.* **259**:686–691.
- Blanche, F., B. Cameron, F.-X. Bernard, L. Maton, B. Manse, L. Ferrero, N. Ratet, C. Lecoq, A. Goniot, D. Bisch, and J. Crouzet. 1996. Differential behaviors of *Staphylococcus aureus* and *Escherichia coli* type II DNA topoisomerases. *Antimicrob. Agents Chemother.* **40**:2714–2720.
- Drlica, K., and X. Zhao. 1997. DNA gyrase, topoisomerase IV, and 4-quinolones. *Microbiol. Mol. Biol. Rev.* **61**:377–392.
- Ferrero, L., B. Cameron, B. Manse, D. Lagneau, J. Crouzet, A. Famechon, and F. Blanche. 1994. Cloning and primary structure of *Staphylococcus aureus* DNA topoisomerase IV: a primary target of fluoroquinolones. *Mol. Microbiol.* **13**:641–653.
- Ferrero, L., B. Cameron, and J. Crouzet. 1995. Analysis of *gyrA* and *glaA* mutations in stepwise-selected ciprofloxacin-resistant mutants of *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **39**:1554–1558.
- Fukuda, H., S. Hori, and K. Hiramatsu. 1998. Antibacterial activity of gatifloxacin (AM-1155, CG5501, BMS-206584), a newly developed fluoroquinolone, against sequentially acquired quinolone-resistant mutants and the *norA* transformant of *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **42**:1917–1922.
- Gellert, M., K. Mizauchi, M. H. O'Dea, and H. A. Nash. 1976. DNA gyrase: an enzyme that introduces superhelical turns into DNA. *Proc. Natl. Acad. Sci. USA* **73**:3872–3876.
- Hooper, D. C., and J. S. Wolfson. 1993. Mechanisms of quinolone action and bacterial killing, p. 53–57. In D. C. Hooper and J. S. Wolfson (ed.), *Quinolone antibacterial agents*. American Society for Microbiology, Washington, D.C.
- Hori, S., Y. Ohshita, Y. Utsui, and K. Hiramatsu. 1993. Sequential acquisition of norfloxacin and ofloxacin resistance by methicillin-resistant and -susceptible *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **37**:2278–2284.
- Hosaka, M., T. Yasue, H. Fukuda, H. Tomizawa, H. Aoyama, and K. Hirai. 1992. In vitro and in vivo antibacterial activities of AM-1155, a new 6-fluoro-8-methoxy quinolone. *Antimicrob. Agents Chemother.* **36**:2108–2117.
- Hoshino, K., K. Sato, T. Une, and Y. Osada. 1989. Inhibitory effects of quinolones on DNA gyrase of *Escherichia coli* and topoisomerase II of fetal calf thymus. *Antimicrob. Agents Chemother.* **33**:1816–1818.
- Hoshino, K., K. Sato, K. Akahane, A. Yoshida, I. Hayakawa, M. Sato, T. Une, and Y. Osada. 1991. Significance of the methyl group on the oxazine ring of ofloxacin derivatives in the inhibition of bacterial and mammalian type II topoisomerases. *Antimicrob. Agents Chemother.* **35**:309–312.
- Hoshino, K., A. Kitamura, I. Morrissey, K. Sato, J. Kato, and H. Ikeda. 1994. Comparison of inhibition of *Escherichia coli* topoisomerase IV by quinolones with DNA gyrase inhibition. *Antimicrob. Agents Chemother.* **38**:2623–2627.
- Hussy, P., G. Maass, B. Tümmler, F. Grosse, and U. Schomburg. 1986. Effect of 4-quinolones and novobiocin on calf thymus DNA polymerase α primase complex, topoisomerase I and II, and growth of mammalian lymphoblasts. *Antimicrob. Agents Chemother.* **29**:1073–1078.
- Kaatz, G. W., S. M. Seo, and C. A. Ruble. 1993. Efflux-mediated fluoroquinolone resistance in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **37**:1086–1094.
- Khodursky, A. B., E. L. Zechiedrich, and N. R. Cozzarelli. 1995. Topoisomerase IV is a target of quinolones in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **92**:11801–11805.
- Kumagai, Y., J.-I. Kato, K. Hoshino, T. Akasaka, K. Sato, and H. Ikeda. 1996. Quinolone-resistant mutants of *Escherichia coli* DNA topoisomerase IV *parC* gene. *Antimicrob. Agents Chemother.* **40**:710–714.
- Luttinger, A. 1995. The twisted life of DNA in the cell: bacterial topoisomerases. *Mol. Microbiol.* **15**:601–606.
- Miller, K. G., L. F. Liu, and P. T. Englund. 1981. A homogeneous type II DNA topoisomerase from HeLa cell nuclei. *J. Biol. Chem.* **256**:9334–9339.
- Morais Cabral, J. H., A. P. Jackson, C. V. Smith, N. Shikotra, A. Maxwell, and R. C. Liddington. 1997. Crystal structure of the breakage-reunion domain of DNA gyrase. *Nature* **388**:903–906.
- Moreau, N. J., H. Robaux, L. Baron, and X. Tabary. 1990. Inhibitory effects of quinolones on pro- and eucaryotic DNA topoisomerases I and II. *Antimicrob. Agents Chemother.* **34**:1955–1960.
- Ng, E. Y., M. Trucksis, and D. C. Hooper. 1996. Quinolone resistance mutations in topoisomerase IV: relationship to the *flqA* locus and genetic evidence that topoisomerase IV is the primary target and DNA gyrase is the secondary target of fluoroquinolones in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **40**:1881–1888.
- Ng, E. Y. W., M. Trucksis, and D. C. Hooper. 1994. Quinolone resistance mediated by *norA*: physiologic characterization and relationship to *flqB*, a quinolone resistance locus on the *Staphylococcus aureus* chromosome. *Antimicrob. Agents Chemother.* **38**:1345–1355.
- Nitiss, J. L. 1994. Roles of DNA topoisomerases in chromosomal replication and segregation, p. 103–134. In L. F. Liu (ed.), *DNA topoisomerases: biochemistry and molecular biology*. Academic Press, Inc., San Diego, Calif.
- Oomori, Y., T. Yasue, H. Aoyama, K. Hirai, S. Suzue, and T. Yokota. 1988. Effects of feroxacin on HeLa cell functions and topoisomerase II. *J. Antimicrob. Chemother.* **22**(Suppl. D):91–97.
- Peng, H., and K. J. Mariani. 1993. *Escherichia coli* topoisomerase IV. Purification, characterization, subunit structure, and subunit interaction. *J. Biol. Chem.* **268**:24481–24490.
- Sato, K., Y. Inoue, T. Fujii, H. Aoyama, M. Inoue, and S. Mitsuhashi. 1986. Purification and properties of DNA gyrase from a fluoroquinolone-resistant strain of *Escherichia coli*. *Antimicrob. Agents Chemother.* **30**:777–780.
- Shen, L. L., and D. T. W. Chu. 1996. Type II DNA topoisomerases as antibacterial targets. *Curr. Pharm. Design* **2**:195–208.
- Tanaka, M., Y. Onodera, Y. Uchida, K. Sato, and I. Hayakawa. 1997. Inhibitory activities of quinolones against DNA gyrase and topoisomerase IV purified from *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **41**:2362–2366.
- Wakabayashi, E., and S. Mitsuhashi. 1994. In vitro antibacterial activity of AM-1155, a novel 6-fluoro-8-methoxy quinolone. *Antimicrob. Agents Chemother.* **38**:594–601.
- Yamagishi, J., T. Kojima, Y. Oyamada, K. Fujimoto, H. Hattori, S. Nakamura, and M. Inoue. 1996. Alterations in the DNA topoisomerase IV *glaA* gene responsible for quinolone resistance in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **40**:1157–1163.