ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1989, p. 1980–1988
0066-4804/89/111980-09$02.00/0

Vol. 33, No. 11

In Vitro Activity of AT-4140 against Clinical Bacterial Isolates

TSUYOSHI KOJIMA,[1]* MATSUHISA INOUE,[2] AND SUSUMU MITSUHASHI[1]

Episome Institute, Fujimi, Seta-gun, Gunma 371-01,[1] and Laboratory of Drug Resistance in Bacteria, School of Medicine, Gunma University, Maebashi, Gunma 371,[2] Japan

Received 8 February 1989/Accepted 16 August 1989

The activity of AT-4140, a new fluoroquinolone, was evaluated against a wide range of clinical bacterial isolates and compared with those of existing analogs. AT-4140 had a broad spectrum and a potent activity against gram-positive and -negative bacteria, including *Legionella* spp. and *Bacteroides fragilis*. The activity of AT-4140 against gram-positive and -negative cocci, including *Acinetobacter calcoaceticus*, was higher than those of ciprofloxacin, ofloxacin, and norfloxacin. Its activity against gram-negative rods was generally comparable to that of ciprofloxacin. Some isolates of methicillin-resistant *Staphylococcus aureus* (MIC of methicillin, ≧12.5 μg/ml) were resistant to existing quinolones, but many of them were still susceptible to AT-4140 at concentrations below 0.39 μg/ml. The MICs of AT-4140, ciprofloxacin, ofloxacin, and norfloxacin for 90% of clinical isolates of methicillin-resistant *S. aureus* were 0.2, 12.5, 6.25, and 100 μg/ml, respectively. AT-4140 was bactericidal for each of 20 clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *Pseudomonas aeruginosa* at concentrations near the MICs. AT-4140 inhibited the supercoiling activity of DNA gyrase from *E. coli*.

Over a quarter of a century since the discovery of nalidixic acid by Lesher et al. (14), subsequent efforts have been directed toward finding more potent nalidixic acid derivatives. As a result of these efforts, ciprofloxacin (30), ofloxacin (20), norfloxacin (11), and enoxacin (13), the so-called new quinolones, have become available clinically. Their antibacterial activities are generally broad and potent, although activities against gram-positive cocci are generally not high.

AT-4140 [5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid] (Fig. 1) is a new quinolone antibacterial agent (J. Matsumoto, T. Miyamoto, H. Egawa, and S. Nakamura, Chem. Abstr. **107**:236733v, 1987). Distinct features in the chemical structure of AT-4140 are an amino residue at the 5 position of the quinolone nucleus and *cis*-dimethyl residues at the 3 and 5 positions of the piperazinyl moiety. AT-4140 has a broad antibacterial spectrum covering gram-positive and gram-negative bacteria, *Mycobacterium* spp., *Mycoplasma* spp., and *Chlamydia* spp. (S. Nakamura, A. Minami, T. Kojima, K. Fujimoto, N. Kurobe, S. Kashimoto, T. Ohue, K. Kouno, M. Hashimoto, and M. Shimizu, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1487, 1988).

This report describes the in vitro antibacterial properties of AT-4140 compared with those of the existing quinolones.

MATERIALS AND METHODS

Drugs. AT-4140 was obtained from Dainippon Pharmaceutical Co., Ltd., Osaka, Japan; ciprofloxacin, from Bayer Yakuhin, Ltd., Osaka, Japan; ofloxacin and rifampin, from Daiichi Seiyaku Co., Ltd., Tokyo, Japan; norfloxacin, from Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan; methicillin, from Banyu Pharmaceutical Co., Ltd., Tokyo, Japan; ampicillin, from Meiji Seika Kaisha Co., Ltd., Tokyo, Japan; and erythromycin, from Taisho Pharmaceutical Co., Ltd., Tokyo, Japan.

Organisms. Bacterial strains used in this study were reference strains maintained in our laboratories and recent clinical isolates collected in various hospitals in Japan.

Determination of MIC. MICs were determined by the twofold serial agar dilution method with Sensitivity Disk Agar-N (SDA; Nissui Pharmaceutical, Tokyo, Japan), which was supplemented with 5% defibrinated horse blood for streptococci and 5% Fildes enrichment (Difco Laboratories, Detroit, Mich.) for *Haemophilus influenzae*. GC agar (Difco) supplemented with 1% hemoglobin (Difco) and 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.) was used for *Neisseria gonorrhoeae*. For obligate anaerobes, GAM agar (Nissui) was used. BCYEα agar was used for *Legionella* spp. To prepare BCYEα agar, 1 g of yeast extract (Difco) and 0.2 g of activated charcoal (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were mixed in 90 ml of distilled water for 2 h. The charcoal was removed completely by centrifugation at 9,000 × *g* for 30 min at 4°C, followed by membrane filtration (0.45 μm; Corning Laboratory Science Co., Corning, N.Y.) to avoid any interference with drug activity. After the filtrate was autoclaved with 1.3 g of agar (Eiken Chemical Co., Ltd., Tokyo, Japan), 10% *Legionella* BCYEα growth supplement (Oxoid Ltd., Basingstoke, Hampshire, England) was added aseptically.

An overnight culture or bacterial suspension was diluted with corresponding broth or buffered saline containing 0.01% gelatin to a concentration of approximately 10^6 CFU/ml. A portion (about 5 μl) of the dilution was inoculated with

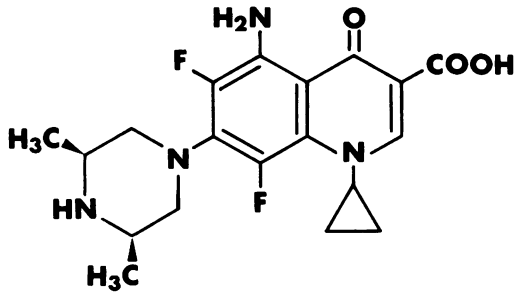

FIG. 1. Chemical structure of AT-4140.

* Corresponding author.

TABLE 1. Antibacterial activities of AT-4140 and reference compounds against clinical isolates

Organism (no. of isolates)	Drug	MIC (μg/ml)[a]		
		Range	50%	90%
Methicillin-susceptible *S. aureus* (126)	AT-4140	0.0125–50	0.05	0.1
	Ciprofloxacin	0.1–>100	0.39	1.56
	Ofloxacin	0.2–>100	0.39	0.78
	Norfloxacin	0.2–>100	1.56	3.13
	Methicillin	0.78–6.25	1.56	3.13
MRSA[b] (86)	AT-4140	0.025–12.5	0.05	0.2
	Ciprofloxacin	0.2–>100	0.78	12.5
	Ofloxacin	0.2–50	0.39	6.25
	Norfloxacin	0.78–>100	1.56	100
	Methicillin	12.5–>100	>100	>100
Methicillin-susceptible *Staphylococcus epidermidis* (112)	AT-4140	0.025–6.25	0.05	0.1
	Ciprofloxacin	0.1–100	0.2	0.39
	Ofloxacin	0.2–50	0.39	0.39
	Norfloxacin	0.2–>100	0.78	0.78
	Methicillin	0.2–6.25	3.13	6.25
Methicillin-resistant *Staphylococcus epidermidis* (72)	AT-4140	0.05–3.13	0.05	0.1
	Ciprofloxacin	0.2–6.25	0.2	0.39
	Ofloxacin	0.2–6.25	0.39	0.78
	Norfloxacin	0.39–50	0.78	1.56
	Methicillin	12.5–>100	25	>100
Streptococcus pneumoniae (21)	AT-4140	0.05–6.25	0.1	0.2
	Ciprofloxacin	0.2–50	0.78	3.13
	Ofloxacin	0.39–25	1.56	3.13
	Norfloxacin	1.56–>100	6.25	25
Streptococcus pyogenes (100)	AT-4140	0.05–0.39	0.2	0.39
	Ciprofloxacin	0.2–1.56	0.39	0.78
	Ofloxacin	0.39–3.13	1.56	1.56
	Norfloxacin	0.78–50	3.13	3.13
Enterococcus faecalis (102)	AT-4140	0.1–0.39	0.39	0.39
	Ciprofloxacin	0.39–3.13	0.78	1.56
	Ofloxacin	0.78–6.25	1.56	3.13
	Norfloxacin	0.78–25	3.13	6.25
Enterococcus faecium (33)	AT-4140	0.1–0.78	0.2	0.39
	Ciprofloxacin	0.39–3.13	1.56	3.13
	Ofloxacin	0.78–6.25	3.13	6.25
	Norfloxacin	0.78–12.5	3.13	12.5
Escherichia coli (146)	AT-4140	0.0125–1.56	0.025	0.05
	Ciprofloxacin	0.0063–1.56	0.025	0.05
	Ofloxacin	0.025–12.5	0.1	0.1
	Norfloxacin	0.025–3.13	0.1	0.1
Enterobacter cloacae (105)	AT-4140	0.0063–3.13	0.05	0.2
	Ciprofloxacin	0.0125–3.13	0.05	0.2
	Ofloxacin	0.05–12.5	0.1	0.78
	Norfloxacin	0.05–25	0.1	0.78
Enterobacter aerogenes (10)	AT-4140	0.05–0.1	0.05	0.1
	Ciprofloxacin	0.05	0.05	0.05
	Ofloxacin	0.2	0.2	0.2
	Norfloxacin	0.1–0.2	0.2	0.2
Klebsiella pneumoniae (108)	AT-4140	0.025–0.78	0.05	0.1
	Ciprofloxacin	0.0063–1.56	0.05	0.05
	Ofloxacin	0.05–3.13	0.2	0.2
	Norfloxacin	0.05–6.25	0.2	0.2
Klebsiella oxytoca (106)	AT-4140	0.0063–0.1	0.05	0.1
	Ciprofloxacin	0.0125–0.1	0.025	0.05
	Ofloxacin	0.05–0.39	0.1	0.2
	Norfloxacin	0.05–0.39	0.1	0.1

Continued on following page

TABLE 1—*Continued*

Organism (no. of isolates)	Drug	MIC (μg/ml)[a]		
		Range	50%	90%
Serratia marcescens (120)	AT-4140	0.05–100	0.78	12.5
	Ciprofloxacin	0.025–50	0.39	12.5
	Ofloxacin	0.1–>100	1.56	25
	Norfloxacin	0.05–>100	0.78	50
Citrobacter freundii (96)	AT-4140	0.0125–50	0.05	1.56
	Ciprofloxacin	0.0063–12.5	0.05	0.2
	Ofloxacin	0.05–50	0.2	1.56
	Norfloxacin	0.05–50	0.1	0.78
Proteus mirabilis (103)	AT-4140	0.025–25	0.2	0.39
	Ciprofloxacin	0.025–3.13	0.05	0.05
	Ofloxacin	0.1–12.5	0.2	0.2
	Norfloxacin	0.05–25	0.1	0.1
Proteus vulgaris (75)	AT-4140	0.05–3.13	0.1	0.39
	Ciprofloxacin	0.0125–0.2	0.025	0.1
	Ofloxacin	0.05–1.56	0.1	0.39
	Norfloxacin	0.05–0.39	0.05	0.1
Providencia rettgeri (50)	AT-4140	0.0125–3.13	0.2	1.56
	Ciprofloxacin	0.0063–1.56	0.05	0.39
	Ofloxacin	0.05–6.25	0.39	3.13
	Norfloxacin	0.05–3.13	0.2	1.56
Providencia stuartii (75)	AT-4140	0.0125–0.39	0.1	0.2
	Ciprofloxacin	0.025–0.78	0.2	0.39
	Ofloxacin	0.1–1.56	0.39	1.56
	Norfloxacin	0.05–3.13	0.78	1.56
Morganella morganii (108)	AT-4140	0.0063–25	0.1	0.2
	Ciprofloxacin	≦0.0031–3.13	0.025	0.025
	Ofloxacin	0.025–25	0.1	0.1
	Norfloxacin	0.0125–6.25	0.05	0.1
Salmonella spp. (108)	AT-4140	0.0125–0.05	0.025	0.05
	Ciprofloxacin	0.0125–0.05	0.025	0.025
	Ofloxacin	0.1–0.2	0.1	0.1
	Norfloxacin	0.05–0.2	0.1	0.1
Shigella spp. (72)	AT-4140	0.0063–0.2	0.0125	0.025
	Ciprofloxacin	0.0125–0.2	0.0125	0.025
	Ofloxacin	0.05–0.39	0.05	0.1
	Norfloxacin	0.05–0.39	0.05	0.1
Pseudomonas aeruginosa (100)	AT-4140	0.1–100	0.78	3.13
	Ciprofloxacin	0.05–50	0.2	0.78
	Ofloxacin	0.39–>100	1.56	6.25
	Norfloxacin	0.39–>100	0.78	3.13
Pseudomonas cepacia (51)	AT-4140	0.05–12.5	6.25	12.5
	Ciprofloxacin	0.025–25	3.13	12.5
	Ofloxacin	0.78–25	12.5	25
	Norfloxacin	0.78–100	25	50
Xanthomonas maltophilia (50)	AT-4140	0.0125–3.13	0.2	0.78
	Ciprofloxacin	0.39–12.5	3.13	6.25
	Ofloxacin	0.78–12.5	3.13	3.13
	Norfloxacin	3.13–100	12.5	25
Haemophilus influenzae (89)	AT-4140	0.0063–0.1	0.0063	0.0125
	Ciprofloxacin	0.0063–0.1	0.025	0.025
	Ofloxacin	0.025–0.2	0.05	0.05
	Norfloxacin	0.05–0.2	0.05	0.1
	Ampicillin	0.1–>100	0.2	6.25

Continued on following page

TABLE 1—*Continued*

Organism (no. of isolates)	Drug	MIC (μg/ml)[a]		
		Range	50%	90%
Neisseria gonorrhoeae (15)	AT-4140	0.0008–0.0063	0.0031	0.0063
	Ciprofloxacin	0.0063–0.025	0.0063	0.025
	Ofloxacin	0.0125–0.05	0.025	0.05
	Norfloxacin	0.025–0.1	0.05	0.1
	Ampicillin	0.05–50	0.2	25
Branhamella catarrhalis (21)	AT-4140	0.0063–0.025	0.0125	0.0125
	Ciprofloxacin	0.025–0.05	0.025	0.05
	Ofloxacin	0.05–0.1	0.05	0.1
	Norfloxacin	0.2–0.39	0.2	0.39
Acinetobacter calcoaceticus (35)	AT-4140	0.0125–0.1	0.025	0.1
	Ciprofloxacin	0.1–3.13	0.39	0.78
	Ofloxacin	0.2–3.13	0.39	1.56
	Norfloxacin	0.78–50	3.13	12.5
Clostridium perfringens (16)	AT-4140	0.1–0.78	0.39	0.78
	Ciprofloxacin	0.39–1.56	0.78	1.56
	Ofloxacin	0.39–3.13	0.78	1.56
	Norfloxacin	1.56–6.25	3.13	6.25
Clostridium difficile (24)	AT-4140	0.78–6.25	3.13	6.25
	Ciprofloxacin	3.13–12.5	6.25	12.5
	Ofloxacin	3.13–12.5	6.25	12.5
	Norfloxacin	12.5–50	25	50
Bacteroides fragilis (35)	AT-4140	0.39–3.13	0.78	1.56
	Ciprofloxacin	3.13–100	6.25	25
	Ofloxacin	0.78–25	3.13	12.5
	Norfloxacin	25–>100	50	>100

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.
[b] Methicillin MIC, ≧12.5 μg/ml.

an inoculation apparatus (Microplanter; Sakuma Seisakusho, Tokyo, Japan) onto agar plates containing graded concentrations of drug. The final inoculum size was approximately 5×10^3 CFU per spot. The plates were incubated at 37°C for 18 h, except *Legionella* spp., which were cultured for 72 h; *H. influenzae* and *N. gonorrhoeae*, which were cultured in a candle jar for 18 h; and anaerobes, which were incubated in an anaerobic chamber for 24 h. The MIC was defined as the lowest drug concentration at which visible bacterial growth was inhibited.

Determination of bactericidal activity. Bactericidal activity of the drugs was assessed by determination of the MBC and measurement of the reduction in viable cells during incubation with the drugs in sensitivity test broth (Nissui). An overnight culture of test organisms was diluted with fresh sensitivity test broth to about 10^6 CFU/ml, and 0.9 ml of the dilution was added to 0.1 ml of the drug solution in a clear tube, which was incubated without shaking at 37°C for 20 h. The number of inoculated cells was confirmed at $>5 \times 10^5$ CFU/ml. After determination of the MIC, 0.1 ml of growth-negative cultures was mixed with 10 ml of melted SDA and an additional 10 ml of melted SDA was overlaid after solidification of the first layer of SDA. The plates were incubated at 37°C for 48 h for colony formation. The MBC was defined as the lowest drug concentration at which the number of residual viable cells was <0.1% of the number of inoculated cells. The reduction in viable cells during exposure to the drug was measured as follows. An overnight culture of test organisms in sensitivity test broth was diluted to about 10^4 CFU/ml with fresh sensitivity test broth, and 9 ml of the bacterial suspension was incubated at 37°C in an L-shaped tube with shaking. After 2 h of preincubation, 1 ml of drug solution was added to the culture and the incubation was continued. The final drug concentrations were near their MICs. A 0.5-ml portion of each culture was taken at appropriate intervals and serially diluted 10-fold with saline. One milliliter of the dilution or the culture was mixed with 10 ml of melted SDA, and an additional 10 ml of melted SDA was overlaid after solidification of the first layer of SDA. The number of colonies was counted after 48 h of incubation at 37°C. The drug carry-over did not affect colony formation. The MICs of the drugs for the test organisms under these conditions were examined before the killing-curve study.

Determination of frequency of spontaneous mutation. The frequencies of spontaneous mutations resistant to quinolones in *Staphylococcus aureus* Smith, *Escherichia coli* ML4707, and *Pseudomonas aeruginosa* GN11189 were determined by spreading 0.1 ml of overnight cultures of each test organism onto three SDA agar plates containing drugs at concentrations four and eight times the MIC. Cell numbers of the overnight cultures of *S. aureus* Smith, *E. coli* ML4707, and *P. aeruginosa* GN11189 were 1.8×10^9, 4.5×10^9, and 6.8×10^9 CFU/ml, respectively. After incubation at 37°C for 48 h, colonies were counted and the frequency of spontaneous mutation of drug resistance was calculated as the ratio of the number of resistant cells to the number of cells inoculated.

Assay of DNA gyrase inhibition. DNA gyrase was prepared from *E. coli* KL-16 (7) by a method described previously (19). In brief, *E. coli* cells were treated with lysozyme (Sigma Chemical Co., St. Louis, Mo.) and ammonium sulfate. The crude enzyme fraction was loaded on a novobiocin-Sepha-

TABLE 2. Profile of drug susceptibility of clinical isolates of *S. aureus*

S. aureus strain	MIC (μg/ml)[a]				
	AT-4140	CPFX	OFLX	NFLX	DMPPC
MS16008	0.025	0.2	0.2	0.78	1.56
MS16023	0.05	0.39	0.39	1.56	>100
MS16385	0.1	25	6.25	>100	>100
MS16405	12.5	>100	50	>100	100
MS16412	0.1	3.13	1.56	25	1.56
NMS54	50	>100	>100	>100	1.56

[a] CPFX, Ciprofloxacin; OFLX, ofloxacin; NFLX, norfloxacin; DMPPC, methicillin.

rose column (25), and fractions of DNA gyrase subunits A and B were obtained separately. A reaction mixture containing subunits A and B, a drug solution, and pBR322 plasmid relaxed by topoisomerase I (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) was incubated for 2 h at 37°C. The reaction mixture was analyzed as reported previously (1).

RESULTS

Antibacterial activities. The activities of AT-4140, ciprofloxacin, ofloxacin, norfloxacin, and reference antibiotics against recent clinical isolates of various species of bacteria are shown in Table 1. AT-4140 showed the highest activity of the drugs tested against gram-positive bacteria such as *Staphylococcus*, *Streptococcus*, and *Enterococcus* spp. The MICs of AT-4140 for 90% of the isolates tested ranged from 0.1 to 0.39 μg/ml for gram-positive cocci. In 212 isolates of *S. aureus* tested, about 40% of the isolates were resistant to methicillin (MIC of methicillin, ≧12.5 μg/ml). There was a difference in susceptibility to quinolones between methicillin-susceptible *S. aureus* and methicillin-resistant *S. aureus* (MRSA). Although the MICs of AT-4140, ciprofloxacin, ofloxacin, and norfloxacin for 90% of the methicillin-susceptible *S. aureus* were 0.1, 1.56, 0.78, and 3.13 μg/ml, respectively, those for MRSA were 0.2, 12.5, 6.25, and 100 μg/ml, respectively. These results indicated that >10% of the MRSA tested in this study were also resistant to the existing quinolones, but many of them did not become resistant to AT-4140. The clinical isolates of *S. aureus* were divided into six groups with regard to susceptibility to AT-4140, the existing quinolones, and methicillin. The susceptibility profile of typical strains in each group is shown in Table 2. *S. aureus* MS16008 was quite susceptible to all drugs tested. MS16023 was an MRSA, but was susceptible to all quinolones. MS16385 was resistant to methicillin and the existing quinolones, but was susceptible to AT-4140. MS16405 was resistant to all drugs tested. MS16412 was resistant to at least norfloxacin, but was susceptible to methicillin and AT-4140. NMS54 was resistant to all quinolones, but was methicillin-susceptible. Methicillin resistance was encountered in 40% of the *S. epidermidis* isolates, but they were as susceptible to all quinolones as the methicillin-susceptible *S. epidermidis*. It was noted that *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Enterococcus faecalis*, and *Enterococcus faecium*, which were relatively less susceptible to the existing quinolones, were susceptible to AT-4140 at relatively low concentrations of 0.2 to 0.39 μg/ml.

Against many members of the *Enterobacteriaceae* family, including *Klebsiella pneumoniae* and *Serratia marcescens*, the antibacterial activity of AT-4140 was similar to that of ciprofloxacin. AT-4140 was generally less active than ciprofloxacin and as active as ofloxacin against *Proteus* spp., *Providencia rettgeri*, *Morganella morganii*, and *Citrobacter freundii*. In glucose-nonfermenting gram-negative rods, the antibacterial activity of AT-4140 was greater than that of the existing quinolones against *Xanthomonas maltophilia*, and AT-4140 was less active than ciprofloxacin and more active than ofloxacin against *P. aeruginosa*. All members of the family *Neisseriaceae* tested were most susceptible to AT-4140 of the quinolones. *H. influenzae* was susceptible to AT-4140 as well as to ciprofloxacin. Cross-resistance to AT-4140 and ampicillin was not observed in *N. gonorrhoeae* and *H. influenzae* (data not shown). Against *Bacteroides fragilis*, AT-4140 was the most active of the drugs tested. The comparative activity against *Legionella* spp. is shown in Table 3. Rifampin had a very high activity, followed by quinolones and erythromycin. Of the quinolones, AT-4140 had the highest activity against *Legionella* spp.

TABLE 3. Antibacterial activities of AT-4140 and reference compounds against *Legionella* spp.

Organism	MIC (μg/ml)[a]					
	AT-4140	CPFX	OFLX	NFLX	RFP	EM
L. pneumophila						
ATCC33152	0.0063	0.05	0.05	0.2	0.0008	0.78
ATCC33153	0.0063	0.025	0.05	0.1	0.0016	0.78
ATCC33154	0.0125	0.025	0.025	0.05	0.0016	0.78
ATCC33155	0.0063	0.025	0.05	0.1	0.0016	0.78
ATCC33156	0.0125	0.025	0.025	0.1	0.0031	0.78
ATCC33215	0.0063	0.05	0.05	0.2	0.0008	0.78
ATCC33216	0.025	0.05	0.05	0.1	0.0008	0.78
ATCC33623	0.0125	0.025	0.1	0.1	0.0008	3.13
GIFU9799	0.0031	0.0125	0.05	0.05	0.0008	1.56
L. bozemanii ATCC33217	0.025	0.05	0.1	0.39	0.0063	0.78
L. micdadei ATCC33218	0.025	0.05	0.1	0.1	0.0125	0.78
L. gormanii ATCC33297	0.0125	0.025	0.025	0.1	0.0031	0.78
L. dumoffii						
ATCC33279	0.025	0.025	0.05	0.1	0.0031	0.78
ATCC33343	0.025	0.05	0.1	0.2	0.0031	1.56
L. longbeachae ATCC33462	0.0063	0.05	0.05	0.2	0.0016	0.39

[a] CPFX, Ciprofloxacin; OFLX, ofloxacin; NFLX, norfloxacin; RFP, rifampin; EM, erythromycin.

TABLE 4. Bactericidal activities of AT-4140 and reference compounds against recent clinical isolates

Organism	Drug	MIC (μg/ml)[a]			MBC (μg/ml)[b]		
		Range	50%	90%	Range	50%	90%
Staphylococcus aureus	AT-4140	0.05–0.1	0.1	0.1	0.1–0.2	0.2	0.2
	Ciprofloxacin	0.39–3.13	0.78	3.13	0.39–12.5	0.78	3.13
	Ofloxacin	0.39–1.56	0.78	1.56	0.39–3.13	0.78	1.56
Escherichia coli	AT-4140	0.025–1.56	0.05	0.39	0.025–3.13	0.05	0.39
	Ciprofloxacin	0.025–1.56	0.05	0.2	0.025–3.13	0.05	0.2
	Ofloxacin	0.1–12.5	0.2	0.78	0.1–25	0.2	1.56
Klebsiella pneumoniae	AT-4140	0.05–1.56	0.2	0.78	0.05–1.56	0.2	0.78
	Ciprofloxacin	0.1–3.13	0.39	0.78	0.1–6.25	0.39	0.78
	Ofloxacin	0.39–6.25	0.39	1.56	0.39–6.25	0.78	1.56
Serratia marcescens	AT-4140	0.39–12.5	1.56	6.25	0.39–12.5	1.56	12.5
	Ciprofloxacin	0.2–12.5	1.56	6.25	0.2–12.5	1.56	6.25
	Ofloxacin	0.39–12.5	3.13	12.5	0.39–25	3.13	12.5
Pseudomonas aeruginosa	AT-4140	0.39–6.25	1.56	3.13	0.39–12.5	1.56	3.13
	Ciprofloxacin	0.2–3.13	0.78	0.78	0.39–3.13	0.78	1.56
	Ofloxacin	1.56–12.5	1.56	6.25	1.56–12.5	3.13	6.25

[a] 50% and 90%, MICs for 50 and 90% of 20 recent clinical isolates of each organism tested, respectively.
[b] 50% and 90%, MBCs for 50 and 90% of 20 recent clinical isolates of each organism tested, respectively.

Bactericidal activity. The bactericidal activity of AT-4140 against 20 recent clinical isolates each of *S. aureus*, *E. coli*, *K. pneumoniae*, *Serratia marcescens*, and *P. aeruginosa* was compared with the activities of ciprofloxacin and ofloxacin (Table 4). The MBCs of AT-4140 were equal to or twofold higher than the MICs, as were those of ciprofloxacin and ofloxacin. The rapid decrease in number of viable cells was observed at concentrations equal to or twice the MICs of AT-4140 in the killing-curve study with *S. aureus* Smith, *E. coli* ML4707, and *P. aeruginosa* GN11189 (Fig. 2). The regrowth of *S. aureus* Smith and *P. aeruginosa* GN11189 was observed at concentrations equal to or twice the MICs after 24 h of incubation with all quinolones tested. The MICs of AT-4140 by the agar dilution method were 0.1 μg/ml for the regrowing cells of *S. aureus* Smith incubated with AT-4140 at a concentration of 0.05 μg/ml for 24 h and 3.13 μg/ml for the regrowing cells of *P. aeruginosa* GN11189 incubated with AT-4140 at a concentration of 1.56 or 3.13 μg/ml for 24 h. No regrowth of *E. coli* ML4707 was observed throughout the 24 h of incubation. AT-4140 was similar to ciprofloxacin and ofloxacin in bactericidal activity.

Frequency of spontaneous quinolone-resistant mutation. The frequencies of appearance of spontaneous mutants resistant to AT-4140, ciprofloxacin, and ofloxacin are shown in Table 5. No mutants of *S. aureus* Smith resistant to each drug were detected at a concentration four times the MIC. In *E. coli* ML4707 and *P. aeruginosa* GN11189, some spontaneous mutants selected by quinolones were observed at concentrations four times the MICs, but none were observed at concentrations eight times the MICs.

Inhibition of DNA gyrase activity. The supercoiling activity of DNA gyrase from *E. coli* KL-16, using plasmid pBR322, was inhibited by quinolones (Table 6). The 50% inhibitory concentrations of AT-4140, ciprofloxacin, and ofloxacin were 0.14, 0.086, and 0.56 μg/ml, respectively, roughly parallel to the MICs. AT-4140 was a potent DNA gyrase inhibitor, as were ciprofloxacin and ofloxacin.

DISCUSSION

A susceptibility study with a large number of recent clinical isolates revealed that a variety of organisms were highly susceptible to AT-4140 in vitro. The antibacterial activity of the existing quinolones, such as ciprofloxacin, ofloxacin, and norfloxacin, against gram-positive cocci is relatively low compared with that against gram-negative rods. The activity of AT-4140 against gram-positive and -negative cocci, including *Acinetobacter calcoaceticus*, was higher than those of other quinolones, and the activity of AT-4140 against gram-negative rods was generally comparable to that of ciprofloxacin and higher than those of ofloxacin and norfloxacin. AT-4140 appears to have an increased potency against gram-positive and -negative cocci and has an expanded antibacterial spectrum, including *Legionella* spp. and *B. fragilis*, compared with the existing quinolones.

The emergence and epidemic of MRSA have become an increasing problem in antibacterial chemotherapy (29). MRSA is one of the major pathogens in patients who have nosocomial infections (2, 18) and is usually resistant to β-lactam antibiotics (26, 27). Although vancomycin has proven useful for treating staphylococcal infections, this therapy is relatively limited because of adverse effects, poor oral absorption, and relatively low cost-effectiveness. Cip-

TABLE 5. Frequencies of spontaneous mutations resistant to AT-4140 and reference compounds

Organism	Drug	Selected concn (μg/ml)	Mutation frequency
S. aureus Smith	AT-4140	4 × MIC (0.2)	$<5.6 \times 10^{-9}$
	Ciprofloxacin	4 × MIC (0.78)	$<5.6 \times 10^{-9}$
	Ofloxacin	4 × MIC (0.78)	$<5.6 \times 10^{-9}$
E. coli ML4707	AT-4140	4 × MIC (0.05)	2.2×10^{-9}
		8 × MIC (0.1)	$<2.2 \times 10^{-9}$
	Ciprofloxacin	4 × MIC (0.05)	5.2×10^{-9}
		8 × MIC (0.1)	$<2.2 \times 10^{-9}$
	Ofloxacin	4 × MIC (0.2)	$<2.2 \times 10^{-9}$
P. aeruginosa GN11189	AT-4140	4 × MIC (3.13)	1.5×10^{-9}
		8 × MIC (6.25)	$<1.5 \times 10^{-9}$
	Ciprofloxacin	4 × MIC (0.78)	$<1.5 \times 10^{-9}$
	Ofloxacin	4 × MIC (6.25)	1.5×10^{-8}
		8 × MIC (12.5)	$<1.5 \times 10^{-9}$

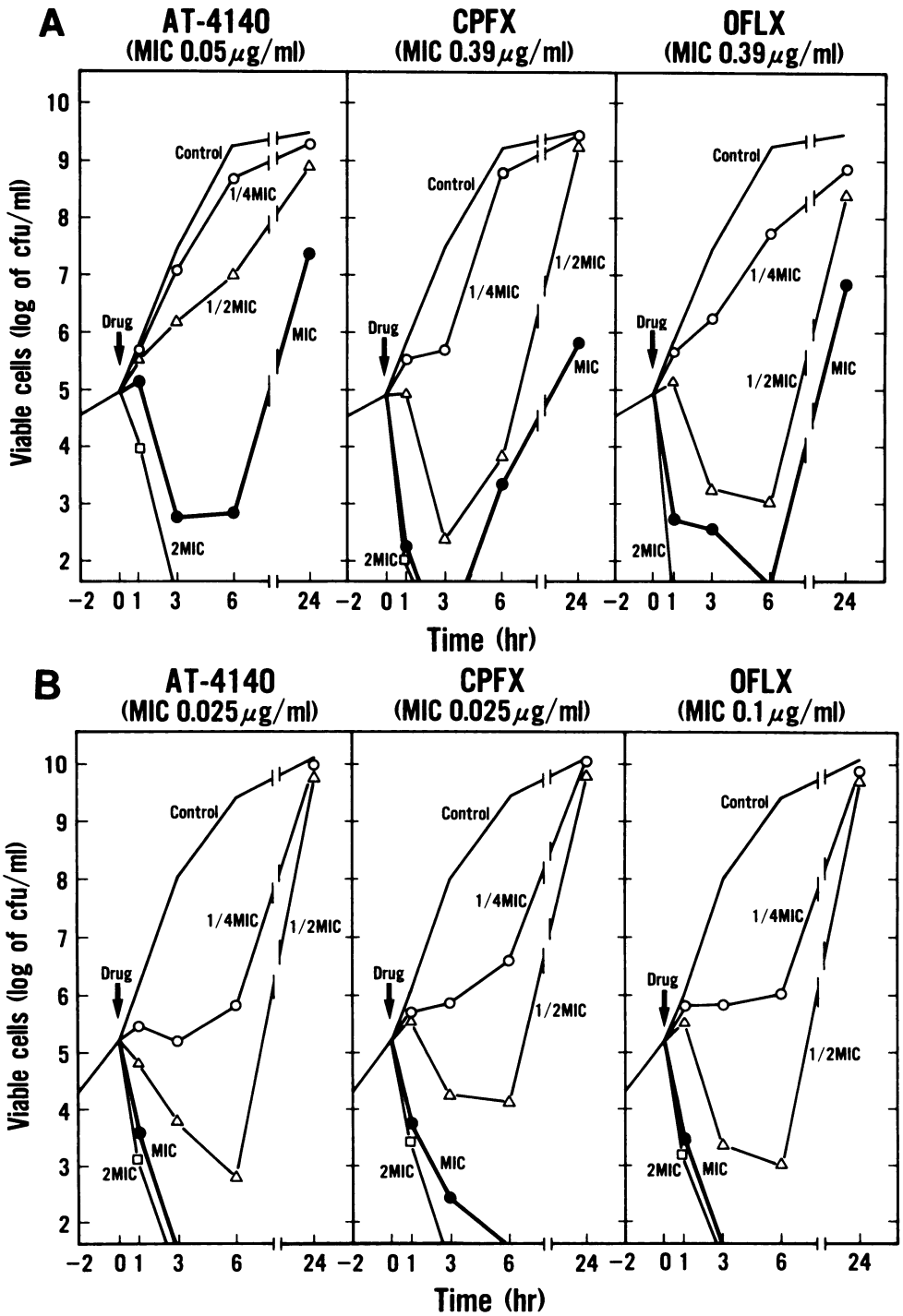

FIG. 2. Bactericidal activity of AT-4140, ciprofloxacin (CPFX), and ofloxacin (OFLX) against *S. aureus* Smith (A), *E. coli* ML4707 (B), and *P. aeruginosa* GN11189 (C).

rofloxacin is a candidate for use in the therapy of MRSA infections (22–24) and has been increasingly administered to patients infected by MRSA. However, the emergence of ciprofloxacin-resistant MRSA has diminished the clinical efficacy of ciprofloxacin therapy (10, 16, 17). Selective pressure of ciprofloxacin on the MRSA may have promoted the resistance; a spontaneous single-step mutation causing resistance to quinolones was very rare in *S. aureus* Smith, a laboratory strain (Table 5), although the mutational frequency in clinical strains of MRSA may be different from that in a laboratory strain. Recent clinical isolates of MRSA tend to have multiple resistances to the various antibacterial agents in Japan (12).

In this study, 19 of 86 isolates (22%) of MRSA were resistant to ciprofloxacin (MIC of ciprofloxacin, ≧3.13 μg/ml), and 11 of 19 isolates (58%) of ciprofloxacin-resistant

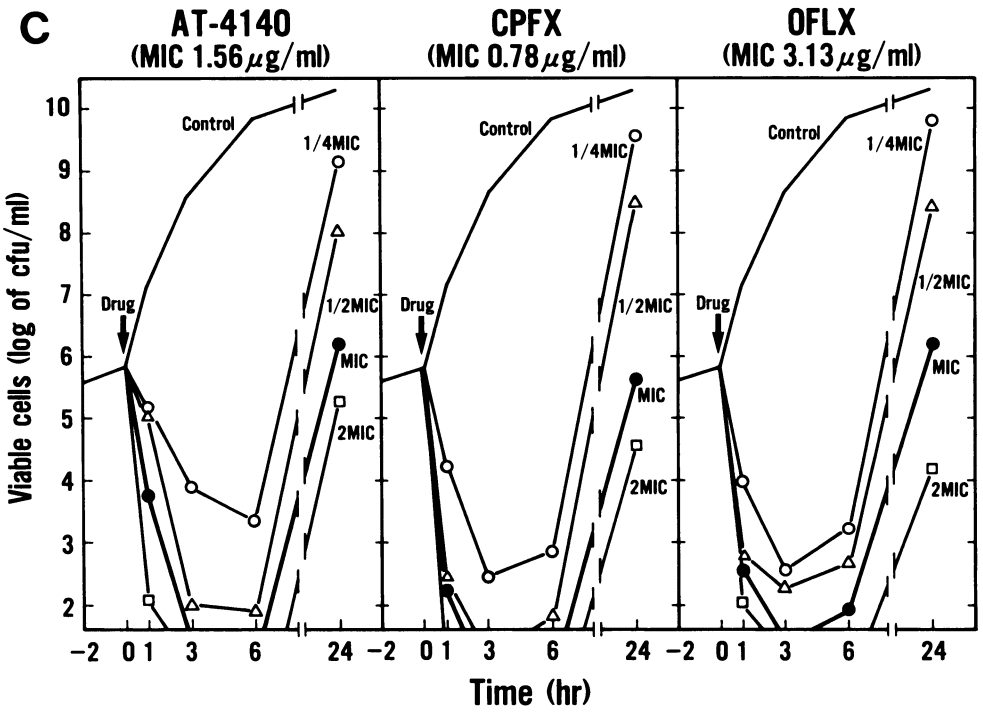

FIG. 2—*Continued*

MRSA were susceptible to AT-4140 at concentrations below 0.39 μg/ml. The typical strain of these isolates is *S. aureus* MS16385 (Table 2). MS16385 does not appear to be a DNA gyrase mutant. If it were, it should be resistant to all quinolones, because the quinolone-resistant mutations affecting DNA gyrase in *E. coli* confer resistance to all quinolones with few exceptions (32, 33). MS16385 did not develop resistance to AT-4140. MS16385 appears to be a mutation which affects drug permeability as the mechanism of quinolone resistance, and AT-4140 is less affected by this mutation, probably due to the moderate lipophilicity of the AT-4140 molecule. *S. aureus* MS16405 and NMS54 (Table 2) may be DNA gyrase mutants with quinolone resistance. Mutations affecting drug permeability are likely to occur in MRSA, and this may be one of the reasons why MRSA can rapidly develop multiple resistances to various antibacterial agents.

AT-4140 was bactericidal to clinical isolates at levels near its MICs and rapidly killed cells of standard strains in a killing-curve study. It is generally accepted that the mode of antibacterial action of quinolones is bactericidal through its inhibitory activity on DNA gyrase (3, 31), which is an essential enzyme for bacterial existence (5, 28). AT-4140 actually inhibited the supercoiling activity of DNA gyrase from *E. coli*. However, there remains to be investigated the molecular mechanism of DNA gyrase inhibition by quinolones (8, 32, 33), whether DNA gyrase is the sole target of quinolones (34), and an explanation of the selective toxicity of quinolones (4, 6, 9, 15, 21).

TABLE 6. Inhibition of supercoiling activity of *E. coli* DNA gyrase by AT-4140

Drug	MIC (μg/ml)	IC_{50} (μg/ml)[a]
AT-4140	0.0125	0.14
Ciprofloxacin	0.0125	0.086
Ofloxacin	0.05	0.56

[a] IC_{50}, 50% Inhibitory concentration.

AT-4140 was well absorbed with oral administration and was well distributed to almost all tissues except brain, spinal fluid, and testis (Y. Sekine, Y. Matsunaga, H. Miyazaki, T. Yamaguchi, Y. Mizuki, T. Itoh, N. Kurobe, S. Nakamura, M. Hashimoto, and M. Shimizu, 28th ICAAC, abstr. no. 1489, 1988). The excellent antibacterial potency and pharmacokinetic properties of AT-4140 suggest that it may be a quinolone worth further study as an antibacterial agent with potential broad clinical application.

LITERATURE CITED

1. **Aoyama, H., K. Sato, T. Fujii, K. Fujimaki, M. Inoue, and S. Mitsuhashi.** 1988. Purification of *Citrobacter freundii* DNA gyrase and inhibition by quinolones. Antimicrob. Agents Chemother. **32**:104–109.
2. **Boyce, J. M., O. A. Landry, T. R. Deetz, and H. L. Dupont.** 1981. Epidemiologic studies of an outbreak of nosocomial methicillin-resistant *Staphylococcus aureus* infections. Infect. Control **2**:110–116.
3. **Chow, R. T., T. J. Dougherty, H. S. Fraimow, E. Y. Bellin, and M. H. Miller.** 1988. Association between early inhibition of DNA synthesis and the MICs and MBCs of carboxyquinolone antimicrobial agents for wild-type and mutant [*gyrA nfxB* (*ompF*) *acrA*] *Escherichia coli* K-12. Antimicrob. Agents Chemother. **32**:1113–1118.
4. **Forsgren, A., A.-K. Bergh, M. Brandt, and G. Hansson.** 1986. Quinolones affect thymidine incorporation into the DNA of human lymphocytes. Antimicrob. Agents Chemother. **29**:506–508.
5. **Gellert, M.** 1981. DNA topoisomerase. Annu. Rev. Biochem. **50**:879–910.
6. **Gollapudi, S. V., B. Vayuvegula, S. Gupta, M. Fok, and H. Thadepalli.** 1986. Aryl-fluoroquinolone derivatives A-56619 (difloxacin) and A-56620 inhibit mitogen-induced human mononuclear cell proliferation. Antimicrob. Agents Chemother. **30**: 390–394.

7. **Hane, M. W., and T. H. Wood.** 1969. *Escherichia coli* K-12 mutants resistant to nalidixic acid: genetic mapping and dominance studies. J. Bacteriol. **99**:238–241.
8. **Horowitz, D. S., and J. C. Wang.** 1987. Mapping the active site tyrosine of *Escherichia coli* DNA gyrase. J. Biol. Chem. **262**: 5339–5344.
9. **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, topoisomerases I and II, and growth of mammalian lymphoblasts. Antimicrob. Agents Chemother. **29**:1073–1078.
10. **Isaacs, R. D., P. J. Kunke, R. L. Cohen, and J. W. Smith.** 1988. Ciprofloxacin resistance in epidemic methicillin-resistant *Staphylococcus aureus*. Lancet **ii**:843.
11. **Ito, A., K. Hirai, M. Inoue, H. Koga, S. Suzue, T. Irikura, and S. Mitsuhashi.** 1980. In vitro antibacterial activity of AM-715, a new nalidixic acid analog. Antimicrob. Agents Chemother. **17**:103–108.
12. **Kanda, K., and T. Yokota.** 1988. Susceptibility of recently isolated highly methicillin-resistant *Staphylococcus aureus* to 13 antimicrobial agents. Chemotherapy (Tokyo) **36**:289–293.
13. **Kouno, K., M. Inoue, and S. Mitsuhashi.** 1983. In vitro and in vivo antibacterial activity of AT-2266. Antimicrob. Agents Chemother. **24**:78–84.
14. **Lesher, G. Y., E. J. Froelich, M. D. Gruett, J. H. Bailey, and R. P. Brundage.** 1962. 1,8-Naphthyridine derivatives. A new class of chemotherapeutic agents. J. Med. Pharm. Chem. **5**: 1063–1065.
15. **Mitelman, F., A.-M. Kolnig, B. Strömbeck, R. Norrby, B. Kromann-Andersen, P. Sommer, and J. Wadstein.** 1988. No cytogenetic effects of quinolone treatment in humans. Antimicrob. Agents Chemother. **32**:936–937.
16. **Mulligan, M. E., P. J. Ruane, L. Johnston, P. Wong, J. P. Wheelock, K. MacDonald, J. F. Reinhardt, C. C. Johnson, B. Statner, I. Blomquist, J. McCarthy, J. O'Brien, S. Gardner, L. Hammer, and D. M. Citron.** 1987. Ciprofloxacin for eradication of methicillin-resistant *Staphylococcus aureus* colonization. Am. J. Med. **82**(Suppl. 4A):215–219.
17. **Piercy, E. A., D. Barbaro, J. P. Luby, and P. A. Mackowiak.** 1989. Ciprofloxacin for methicillin-resistant *Staphylococcus aureus* infections. Antimicrob. Agents Chemother. **33**:128–130.
18. **Saravolatz, D. L., D. J. Pohlod, and L. M. Arking.** 1982. Community-acquired methicillin-resistant *Staphylococcus aureus* infections: a new source for nosocomial outbreaks. Ann. Intern. Med. **97**:325–329.
19. **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.
20. **Sato, K., Y. Matsuura, M. Inoue, T. Une, Y. Osada, H. Ogawa, and S. Mitsuhashi.** 1982. In vitro and in vivo activity of DL-8280, a new oxazine derivative. Antimicrob. Agents Chemother. **22**:548–553.
21. **Shen, L. L., and A. G. Pernet.** 1985. Mechanism of inhibition of DNA gyrase by analogues of nalidixic acid: the target of the drugs is DNA. Proc. Natl. Acad. Sci. USA **82**:307–311.
22. **Simberkoff, M. S., and J. J. Rahal, Jr.** 1986. Bactericidal activity of ciprofloxacin against amikacin- and cefotaxime-resistant gram-negative bacilli and methicillin-resistant staphylococci. Antimicrob. Agents Chemother. **29**:1098–1100.
23. **Smith, S. M.** 1986. In vitro comparison of A-56619, A-56620, amifloxacin, ciprofloxacin, enoxacin, norfloxacin, and ofloxacin against methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **29**:325–326.
24. **Smith, S. M., and R. H. K. Eng.** 1985. Activity of ciprofloxacin against methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **27**:688–691.
25. **Staudenbauer, W. L., and E. Orr.** 1981. DNA gyrase: affinity chromatography on novobiocin-Sepharose and catalytic properties. Nucleic Acids Res. **9**:3589–3603.
26. **Ubukata, K., N. Yamashita, and M. Konno.** 1985. Occurrence of a β-lactam-inducible penicillin-binding protein in methicillin-resistant staphylococci. Antimicrob. Agents Chemother. **27**: 851–857.
27. **Utsui, Y., and T. Yokota.** 1985. Role of an altered penicillin-binding protein in methicillin- and cephem-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **28**:397–403.
28. **Wang, J. C.** 1987. Recent studies of DNA topoisomerases. Biochim. Biophys. Acta **909**:1–9.
29. **Wenzel, R. P.** 1982. The emergence of methicillin-resistant *Staphylococcus aureus*. Ann. Intern. Med. **97**:440–442.
30. **Wise, R., J. M. Andrews, and L. J. Edwards.** 1983. In vitro activity of Bay 09867, a new quinoline derivative, compared with those of other antimicrobial agents. Antimicrob. Agents Chemother. **23**:559–564.
31. **Wolfson, J. S., and D. C. Hooper.** 1985. The fluoroquinolones: structures, mechanisms of action and resistance, and spectra of activity in vitro. Antimicrob. Agents Chemother. **28**:581–586.
32. **Yamagishi, J., H. Yoshida, M. Yamayoshi, and S. Nakamura.** 1986. Nalidixic acid-resistant mutations of the *gyrB* gene of *Escherichia coli*. Mol. Gen. Genet. **204**:367–373.
33. **Yoshida, H., T. Kojima, J. Yamagishi, and S. Nakamura.** 1988. Quinolone-resistant mutations of the *gyrA* gene of *Escherichia coli*. Mol. Gen. Genet. **211**:1–7.
34. **Zweerink, M. M., and A. Edison.** 1986. Inhibition of *Micrococcus luteus* DNA gyrase by norfloxacin and 10 other quinolone carboxylic acids. Antimicrob. Agents Chemother. **29**:598–601.