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Fluoroquinolone Resistance in *Streptococcus pneumoniae*: Area Under the Concentration-Time Curve/MIC Ratio and Resistance Development with Gatifloxacin, Gemifloxacin, Levofloxacin, and Moxifloxacin▽

Kerry L. LaPlante,[1,2,4]† Michael J. Rybak,[1,2,3,4]* Brian Tsuji,[1,2,4]‡
Thomas P. Lodise,[6] and Glenn W. Kaatz[1,3,5]

Anti-Infective Research Laboratory, Eugene Applebaum College of Pharmacy,[1] Department of Pharmacy Practice,[2] and School of Medicine,[3] Wayne State University, Detroit Receiving Hospital and University Health Center,[4] and John D. Dingell Veterans Affairs Medical Center,[5] Detroit, Michigan, and Department of Pharmacy Practice, Albany College of Pharmacy, Albany, New York[6]

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The potential for resistance development in *Streptococcus pneumoniae* secondary to exposure to gatifloxacin, gemifloxacin, levofloxacin, and moxifloxacin at various levels was examined at high inoculum ($10^{8.5}$ to 10^{9} $\log_{10}$ CFU/ml) over 96 h in an in vitro pharmacodynamic (PD) model using two fluoroquinolone-susceptible isolates. The pharmacokinetics of each drug was simulated to provide a range of free areas under the concentration-time curves (*f*AUC) that correlated with various fluoroquinolone doses. Potential first (*parC* and *parE*)- and second-step (*gyrA* and *gyrB*) mutations in isolates with raised MICs were identified by sequence analysis. PD models simulating *f*AUC/MICs of 51 and ≤60, 34 and 37, ≤82 and ≤86, and ≤24 for gatifloxacin, gemifloxacin, levofloxacin, and moxifloxacin, respectively, against each isolate were associated with first-step *parC* (S52G, S79Y, and N91D) and second-step *gyrA* (S81Y and S114G) mutations. For each fluoroquinolone a delay of first- and second-step mutations was observed with increasingly higher *f*AUC/MIC ratios and recovery of topoisomerase mutations in *S. pneumoniae* was related to the *f*AUC/MIC exposure. Clinical doses of gatifloxacin, gemifloxacin, and moxifloxacin exceeded the *f*AUC/MIC resistance breakpoint against wild-type *S. pneumoniae*, whereas those of levofloxacin (500 and 750 mg) were associated with first- and second-step mutations. The exposure breakpoints for levofloxacin were significantly different ($P < 0.001$) from those of the newer fluoroquinolones gatifloxacin, gemifloxacin, and moxifloxacin. Additionally, moxifloxacin breakpoints were significantly lower ($P < 0.002$) than those of gatifloxacin. The order of resistance development determined from *f*AUC/MIC breakpoints was levofloxacin > gatifloxacin > moxifloxacin = gemifloxacin, which may be related to structural differences within the class.

Since their introduction into clinical use the fluoroquinolones have had a major impact on the treatment of moderate-to-severe infections. Their broad spectrum of activity, clinical utility, availability in both oral and parenteral forms, and favorable pharmacokinetic properties have contributed to their extensive worldwide use. However, in recent years bacterial resistance to the fluoroquinolones has become a major concern.

Various reports of fluoroquinolone resistance among previously susceptible organisms have been published, as well as results from a number of longitudinal studies of trends in fluoroquinolone susceptibility (7, 18, 24). Examples include methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae* (29). Determinants of bacterial resistance for this class of antibiotics include patterns of antibiotic prescribing, geographic location, clinical setting, pathogen susceptibility, and overall individual fluoroquinolone characteristics. Low intrinsic activity and poor pharmacodynamic performance against a select group of pathogens have been thought to contribute to the rise in fluoroquinolone resistance.

The rise in gram-positive pathogen resistance in recent years has prompted the pharmaceutical industry to develop fluoroquinolones with greater activity against these rapidly changing pathogens. Structural modifications to the basic fluoroquinolone nucleus have given rise to several new generations of compounds. With each new generation the potency against many gram-positive pathogens, including *S. pneumoniae*, has improved.

Resistance to the fluoroquinolones among gram-positive bacteria is known to occur by at least two mechanisms, which may be present concomitantly in an individual strain. Chromosomally mediated resistance may occur through alterations in the genes coding for both subunits of DNA gyrase (*gyrA* and *gyrB*) or topoisomerase IV (*parC* and *parE*) (16). Resistance also may occur through the action of efflux pumps such as that encoded by *norA* in *S. aureus* or *pmrA* in *S. pneumoniae* (13, 21). Topoisomerase IV- and DNA gyrase-mediated resistance may occur in combination, but in *S. pneumoniae* mutations in *parC* always precede those in *gyrA* (14). Resistance may develop in a stepwise fashion, with a progressively higher MIC

* Corresponding author. Mailing address: Anti-Infective Research Laboratory, Pharmacy Practice-4148, Eugene Applebaum College of Pharmacy and Health Sciences, Wayne State University, 259 Mack Ave., Detroit, MI 48201. Phone: (313) 577-4376. Fax: (313) 577-8915. E-mail: m.rybak@wayne.edu.

† Present address: University of Rhode Island, Department of Pharmacy Practice, Fogarty Hall, 41 Lower College Road, Kingston, RI 02881.

‡ Present address: University at Buffalo, School of Pharmacy and Pharmaceutical Sciences, Buffalo, NY 14260.

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observed with the accumulation of multiple resistance-conferring mutations. Achieving fluoroquinolone concentrations effective in preventing first-step (*parC*) mutations will decrease the overall emergence of target-based resistance, as mutations in *gyrA* typically do not appear in the absence of a *parC* mutation. Previous research for both animals and humans demonstrates that efficacy, or bacterial eradication, is associated with free area under the concentration-time curve (*f*AUC)/MIC ratios of >33.7 to 52 (2, 4). However, higher *f*AUC/MICs may be needed to prevent the emergence of resistance (1, 8).

To date, no published study has described a head-to-head comparison of resistance development potentials between the four respiratory fluoroquinolones. These fluoroquinolones vary structurally, in their antibacterial activities, and in their pharmacokinetic properties (3). It is therefore hypothesized that the emergence of resistance and the rate of its development also will vary. We thus examined the potential for resistance development in *S. pneumoniae* secondary to various exposures of gatifloxacin, gemifloxacin, levofloxacin, and moxifloxacin at high inoculum ($10^{8.5}$ to 10^{9} $\log_{10}$ CFU/ml) over 96 h using an in vitro pharmacodynamic model.

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MATERIALS AND METHODS

Bacterial strains. Two fluoroquinolone-susceptible strains of *S. pneumoniae* with well-documented phenotypic characteristics were tested. ATCC 49619 (penicillin, erythromycin, and fluoroquinolone susceptible) and BSP2443 (penicillin and fluoroquinolone susceptible, erythromycin resistant), obtained from Darrin Bast (Toronto Center for Antimicrobial Research & Evaluation [ToCARE], Department of Microbiology, Mount Sinai Hospital, Toronto, Canada) were evaluated. These strains were found to possess no mutations in the quinolone resistance-determining regions (QRDRs) of *parC*, *parE*, *gyrA*, and *gyrB* and did not demonstrate efflux, assessed as described below.

Medium. Bacteriologic growth media were obtained from Becton Dickinson (Difco, Sparks, MD). All pharmacodynamic models involving *S. pneumoniae* used Todd-Hewitt broth supplemented with 0.5% yeast extract (THBY). Colony counts for these models were determined using tryptic soy agar (TSA) plates containing 5% sheep red blood cells (TSA/SRBC).

Antimicrobial agents. Analytical-grade powders were obtained from their respective manufacturers as follows: gatifloxacin, Bristol-Myers Squibb Company, Wallingford, CT; gemifloxacin, Oscient Pharmaceutical Corporation, Waltham, MA; levofloxacin, commercially purchased; and moxifloxacin, Bayer Corporation, Pharmaceutical Division, West Haven, CT.

In vitro susceptibility. MICs were determined in THBY using a microdilution technique with an inoculum of 5×10^5 CFU/ml according to established CLSI guidelines (5a). The susceptibility profiles of organisms recovered from the models following fluoroquinolone exposure were determined using E-tests and then confirmed by microdilution. The rationale for selecting the wild-type organisms was based upon typical MIC data published for each fluoroquinolone via antimicrobial susceptibility and surveillance studies (5, 15, 25).

Inoculum preparation. Colonies recovered after an overnight incubation on TSA-SRBC were added to THBY to obtain a suspension of approximately 10^9 CFU/ml. The contents of several plates were required to achieve this organism density. An aliquot of this suspension was added to each model to achieve an organism density of 10^6 CFU/ml, and growth was allowed to proceed until the desired starting inoculum was reached.

Fluoroquinolone regimen simulations. A range of *f*AUC/MIC exposures were simulated starting with the reported *f*AUC/MICs achieved via the manufacturers' recommended doses and intervals for patients with normal renal function. As protein binding differs for each agent, free concentrations were simulated using the reported protein binding of 20% for gatifloxacin (Tequin product information; Bristol-Myers Squibb), 60% for gemifloxacin (Factive product information; LG Life Sciences), 30% for levofloxacin (Levaquin product information; Ortho-McNeil), and 40% for moxifloxacin (Avelox product information; Bayer Corporation). Initial regimen simulations were as follows: gatifloxacin administered to simulate 400 mg (free peak, 3.36 μg/ml) dosed every 24 h with pump rate set to achieve a half-life of 8 h; gemifloxacin administered to simulate 320 mg (free peak, 0.64 μg/ml) every 24 h with the pump rate set to achieve a half-life of 7 h; levofloxacin administered to simulate 500 mg (free peak, 4.13 μg/ml) every 24 h with a pump rate was set to achieve a half-life of 7 h; moxifloxacin administered to simulate 400 mg (free peak, 2.25 μg/ml) every 24 h with a pump rate was set to achieve a half-life of 12 h. Each subsequent *f*AUC/MIC increment was then generated based upon whether resistance developed in the model. In models where resistance developed, the *f*AUC/MIC exposure was increased by 50% until no resistance was detected. If no resistance developed, then the *f*AUC/MIC was decreased in increments of 50% until resistance did occur. The breakpoint for resistance determined for *S. pneumoniae* ATCC 49169 was then used as the starting point for verification of the resistance breakpoint for strain BSP2443.

In vitro pharmacodynamic model. An in vitro infection model consisting of a 250-ml one-compartment glass chamber with multiple ports for the delivery and removal of medium, delivery of antibiotics, and collection of bacterial and antimicrobial samples was utilized (1). All model experiments were performed in triplicate to ensure reproducibility. The model was prefilled with medium, and antibiotics were administered as boluses into the central compartment via an injection port. The model also was placed in a 37°C water bath for the duration of the experiment, with magnetic stir bars to allow for continuous mixing. A peristaltic pump (Masterflex; Cole-Parmer Instrument Company, Chicago, IL) was used to replace antibiotic-containing medium continuously with fresh medium at a rate to simulate the half-life of each tested antibiotic. Samples were removed at various times over a 96-hour period to determine organism density, pharmacokinetics, and organism susceptibility. Each fluoroquinolone was run at the specific simulated *f*AUC/MIC exposure as indicated above. In addition, models containing no antibiotic were run to assure adequate growth of test organisms in this system.

Pharmacokinetic analysis. Antibiotic concentrations were determined from samples drawn in duplicate from each of the three models (A, B, and C) at 0, 0.5, 2, 4, 8, 24, 32, 48, 56, 72, 80, and 96 h. Samples were stored at −70°C until analysis. Peak and trough concentrations and half-lives were calculated using concentration-time plots of the model samples. The *f*AUC from 0 to 24 h was calculated using the linear trapezoid method and the PKANALYST program (version 1.10; MicroMath Scientific Software, Salt Lake City, UT).

Pharmacodynamic analysis. Quadruplicate samples were removed from each model at each time point indicated above. Bacterial counts were determined by serial dilution and plating techniques using TSA/SRBC. Plates were incubated at 37°C for 24 h, and colony counts ($\log_{10}$ CFU/ml) were determined using a laser colony counter (ProtoCOL, version 2.05.02; Synbiosis, Cambridge, United Kingdom). Model time-kill curves were determined by plotting mean colony counts ($\log_{10}$ CFU/ml) and resistance from each model versus time. Reductions in colony counts were determined over the 96-hour period and compared between regimens. To prevent antibiotic carryover, samples removed from the peripheral compartment were treated with 0.2 g of nonionic polymeric adsorbent beads (Amberlite XAD-4; Sigma Chemical Co., St. Louis, MO) for 15 min (31). Reductions in colony counts were determined over a 96-h period and were compared between regimens. The resultant *f*AUC/MICs were determined for all regimens.

Antibiotic assays. Gatifloxacin, levofloxacin, and moxifloxacin concentrations were determined by bioassay using antibiotic medium 1 (AM-1; Becton Dickinson [Difco, Sparks, MD]) and *Klebsiella pneumoniae* ATCC 33495 as the indicator organism (1). Blank 1/4-inch sterile disks were spotted with 20 μl of a standard antibiotic concentration or of model samples. Each standard was tested in triplicate by placing disks on AM-1 agar plates which were preswabbed with a 0.5 McFarland suspension of the test organism. Plates were incubated for 18 to 24 h at 37°C, at which time growth inhibition zone sizes were measured. Concentrations of 10, 5, 1.25, and 0.3125 μg/ml were used as standards. Antibiotic concentrations were determined by comparing zone sizes with those produced by the standards. Coefficients of variation for the gatifloxacin, levofloxacin, and moxifloxacin assays were less than 10%. Gemifloxacin concentrations were determined using a validated high-performance liquid chromatography assay at ToCARE, Department of Microbiology, Mount Sinai Hospital, Toronto, Ontario, Canada. The standard curves ranged from 0.084 to 3.016 μg/ml, with a between-day sample coefficient of variation of 6%.

Detection of resistance. Samples (100 μl) taken at each time point were plated onto TSA supplemented with 0.5% lysed horse blood containing an antibiotic concentration of four to eight times the MIC for each organism and were incubated for 24 and 48 h at 37°C to monitor the development of resistance. Plates were visually inspected for growth of resistant subpopulations after 24, 32, and 48 h. The MIC for resistant organisms was then determined using E-test methods (AB Biodisk, Solna, Sweden) in order to detect all possible MIC

elevations. The possible contribution of efflux to MIC increases in resistant isolates was assessed by determining microdilution MICs of the common efflux pump substrates acriflavine, benzalkonium chloride, ethidium bromide, and tetraphenylphosphonium (as well as the fluoroquinolone by which resistance was selected) in the presence and absence of the efflux pump inhibitor reserpine (final concentration, 20 μg/ml). MIC reductions of at least fourfold in the presence of reserpine were considered indicative of efflux. All resistant organisms underwent sequence analysis in order to identify QRDR mutations occurring concomitantly with the development of resistance (16).

PCR procedures. Codons 46 to 172, 371 to 512, 35 to 157, and 398 to 483 of *gyrA*, *gyrB*, *parC*, and *parE*, encompassing the QRDR of each gene, were amplified from genomic DNA using primers and PCR parameters previously described (9, 11, 20, 22, 23, 26, 28). For all gene amplifications, PCR parameters were 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 3 min.

DNA sequence determination. QRDR sequence determinations of parent and putative mutant strains were performed using an automated dideoxy chain termination method by the Applied Genomics Technology Center, Wayne State University, Detroit, MI. Sequences of two independently generated PCR products were determined to control for the possibility of polymerase-induced errors. Sequence analyses were performed using DS Gene 1.5 (Accelrys, San Diego, CA). The *parC* and *gyrA* QRDRs of both parent strains were wild type (data not shown).

Statistical analysis. All statistical analyses were performed using SPSS version 11.0 (SPSS, Inc., Chicago, IL), and a P value of ≤ 0.05 was considered indicative of statistical significance. Mean differences in resistance breakpoints between fluoroquinolones were evaluated by analysis of variance, with Tukey's post hoc test for multiple comparisons. The relationship between *f*AUC/MIC and the emergence of resistance was examined using logistic regression.

RESULTS

Susceptibility testing. The isolates used in this study were susceptible to all fluoroquinolones tested, with MICs typical for each drug against *S. pneumoniae* (5, 15, 25). MICs for ATCC 49619 and BSP2443 were 0.025 and 0.025 mg/liter for gemifloxacin, 0.125 and 0.25 mg/liter for moxifloxacin, 0.19 and 0.25 mg/liter for gatifloxacin, and 0.75 and 0.75 mg/liter for levofloxacin, respectively.

Pharmacokinetics. Observed pharmacokinetic parameters for all tested therapeutic regimens are shown in Table 1. Pharmacokinetic parameters for all regimens were within 10% of expected values.

Pharmacodynamics and resistance development. Results of 96-h pharmacodynamic models for the tested isolates, mean standard deviations, *f*AUC/MIC breakpoint exposures, and point mutations are shown in Table 2. The time line of mutant development at four times the respective MIC for each drug is demonstrated in Fig. 1, in which the range represents different isolates. Simulated free gatifloxacin exposure at *f*AUC/MICs of 51 and ≤60 led to first-step *parC* (S79Y, S52G, and N91D) and second-step *gyrA* (S81Y and S114G) mutations for the BSP2443 and ATCC 49619 strains, respectively (Fig. 1a). Gemifloxacin killing is demonstrated in Fig. 1b. Gemifloxacin exposure at *f*AUC/MICs of 34 and 37 led to first-step *parC* (S79Y, S52G, and N91D) and second-step *gyrA* (S114G) mutations in the BSP2443 and ATCC 49619 strains, respectively. Levofloxacin killing activity and resistance development are shown in Fig. 1c. For both test strains, levofloxacin exposure at *f*AUC/MICs of ≤86 and ≤82 led to isolation of first-step *parC* (S52G, S79Y, and N91D) and second-step *gyrA* (S81Y) mutations in the BSP2443 and ATCC 49619 strains, respectively. Moxifloxacin exposure led to first-step *parC* (S52G and N91D) and second-step *gyrA* (S81Y or S114G) mutations only in the BSP2443 strain at *f*AUC/MIC of ≤24. The *f*AUC/MICs for emergence of resistance breakpoints were significantly different among the various fluoroquinolones tested. In the post hoc analysis, the significant breakpoints differed in comparisons between levofloxacin and gatifloxacin ($P = 0.001$; 95% confidence interval [CI], 13 to 50), gemifloxacin ($P = 0.001$; 95% CI, 29 to 68), and moxifloxacin ($P = 0.0001$; 95% CI, 44 to 81) and between moxifloxacin and gatifloxacin ($P = 0.002$; 95% CI, 12 to 48). However, there were no significant differences between the breakpoints of moxifloxacin and gemifloxacin or gemifloxacin and gatifloxacin. The *f*AUC/MIC ratios of 62 and ≥66 (gatifloxacin), ≥50.5 and ≥51.3 (gemifloxacin), ≥89 and ≥94 (levofloxacin), and 31 (moxifloxacin) for the BSP2443 and ATCC 49619 strains, respectively, prevented the development of first-step *parC* and second-step *gyrA* mutations.

TABLE 1. Pharmacokinetic parameters obtained with in vitro models

Drug	Regimen (mg/24 h)	Peak free concn (μg/ml) ± SD		Obtained half-life (h) ± SD
		Expected	Obtained	
Gatifloxacin	100	0.84	0.71 ± 0.02	7.65 ± 0.3
	150	1.26	1.13 ± 0.01	8.16 ± 0.95
	175	1.47	1.22 ± 0.03	7.57 ± 0.62
	200	1.68	1.38 ± 0.01	8.65 ± 0.05
	400	3.36	2.79 ± 0.01	8.01 ± 0.18
Gemifloxacin	50	0.10	0.12 ± 0.03	7.45 ± 0.99
	75	0.15	0.17 ± 0.01	7.19 ± 0.22
	100	0.20	0.23 ± 0.01	8.22 ± 0.00
	150	0.30	0.31 ± 0.01	7.50 ± 0.01
	250	0.50	0.51 ± 0.02	7.22 ± 0.76
	320	0.64	0.62 ± 0.16	7.58 ± 0.88
Levofloxacin	500	4.13	4.10 ± 0.50	8.01 ± 0.69
	750	6.20	6.13 ± 0.10	7.52 ± 0.66
	812.5	6.71	6.78 ± 0.87	7.06 ± 0.26
	843.5	6.97	7.09 ± 0.55	6.98 ± 0.68
	875	7.23	7.19 ± 0.10	8.02 ± 0.02
	1000	8.26	8.75 ± 0.18	7.85 ± 0.90
Moxifloxacin	50	0.28	0.30 ± 0.01	12.33 ± 0.42
	75	0.42	0.45 ± 0.01	12.42 ± 0.46
	100	0.56	0.60 ± 0.00	12.02 ± 0.11
	200	1.13	1.10 ± 0.03	11.90 ± 0.42
	400	2.25	2.48 ± 0.15	11.66 ± 0.33
	800	4.50	4.46 ± 0.04	12.40 ± 0.96

For each compound evaluated, a delay in the appearance of first- and second-step mutations was observed with increasing *f*AUC/MIC ratios. Logistic-regression analyses revealed a significant association ($P = <0.05$) between *f*AUC/MIC and emergence of resistance for all fluoroquinolones studied.

Efflux. As shown by the occurrence of at least fourfold decreases in MICs for common efflux pump substrates in the presence of reserpine, efflux contributed to MIC increases in the ATCC 49619 mutant exposed to moxifloxacin dosed with a simulated *f*AUC/MIC of 32. No efflux-mediated resistance was observed with exposure to gatifloxacin, gemifloxacin, or levofloxacin. For the moxifloxacin-exposed mutant reserpine-mediated reductions in MICs for acriflavine, benzalkonium chloride, ethidium bromide, and tetraphenylphosphonium were 8, 0, 8, and 2, respectively. In addition, the moxifloxacin MIC for this isolate was reduced more than fourfold in the presence of

TABLE 2. Gatifloxacin, Gemifloxacin, Levofloxacin, and Moxifloxacin MICs at various time points throughout 96 hours

Drug and isolate	Dose (mg)	Achieved *f*AUC/MIC	MIC (mg/liter); mutation(s) at:				
			0 h	24 h	48 h	72 h	96 h
Gatifloxacin							
ATCC 49619	100	35 ± 2.3	0.19	0.38; *parC* (S79Y)	6; *parC* (S79Y) and *gyrA* (S81F)	6; *parC* (S79Y) and *gyrA* (S81F)	8; *parC* (S79Y) and *gyrA* (S81F)
	150	60 ± 4.6	0.19	0.25	0.25	0.75; *parC* (S79Y)	4; *parC* (S79Y) and *gyrA* (S81Y)
	175	66 ± 10	0.19	wt[a]	wt	wt	wt
	200	67 ± 15	0.19	wt	wt	wt	wt
	400	144 ± 4.0	0.19	wt	wt	wt	wt
BSP2443	198	51 ± 11.4	0.25	wt	wt	0.38; *parC* (S52G and N91D)	3; *parC* (S52G and N91D) and *gyrA* (S114G)
	235	62 ± 13.6	0.25	wt	wt	wt	wt
Gemifloxacin							
ATCC 49619	50	37 ± 7.4	0.03	wt	wt	0.25; *parC* (S79Y)	0.25; *parC* (S79Y)
	75	50.5 ± 2	0.03	wt	wt	wt	wt
	100	65.3 ± 11	0.03	wt	wt	wt	wt
	150	78 ± 2.5	0.03	wt	wt	wt	wt
	250	119 ± 2.8	0.03	wt	wt	wt	wt
	320	146 ± 4	0.03	wt	wt	wt	wt
BSP2443	50	34 ± 4.1	0.03	wt	wt	0.125; *parC* (S79Y, S52G, and N91D) and *gyrA* (S114G)	0.125; *parC* (S79Y, S52G, and N91D) and *gyrA* (S114G)
	75	51.3 ± 1.3	0.03	wt	wt	wt	wt
	100	69.2 ± 7.1	0.03	wt	wt	wt	wt
Levofloxacin							
ATCC 49619	500	55 ± 3.8	0.75	wt	>32; *parC* (S79Y) and *gyrA* (S81Y)	>32; *parC* (S79Y) and *gyrA* (S81Y)	>32; *parC* (S79Y) and *gyrA* (S81Y)
	750	79 ± 3.8	0.75	1.5; *parC* (S79Y)	>32; *parC* (S79F) and *gyrA* (S81Y)	>32; *parC* (S79F) and *gyrA* (S81Y)	>32; *parC* (S79F) and *gyrA* (S81Y)
	812.5	82 ± 6.1	0.75	1.5; *parC* (S79Y)	>32; *parC* (S79F) and *gyrA* (E85G)	>32; *parC* (S79F) and *gyrA* (E85G)	>32; *parC* (S79F) and *gyrA* (E85G)
	843.5	89 ± 1.3	0.75	wt	wt		wt
	875	97 ± 0.1	0.75	wt	wt		wt
	1,000	114 ± 5.1	0.75	wt	wt		wt
BSP2443	750	75 ± 3.7	0.75	>32; *parC* (S52G and N91D) and *gyrA* (S81Y)	>32; *parC* (S52G and N91D) and *gyrA* (S81Y)	>32; *parC* (S52G and N91D) and *gyrA* (S81Y)	>32; *parC* (S52G and N91D) and *gyrA* (S81Y)
	812.5	83 ± 8.5	0.75	wt	wt	wt	2; *parC* (S52G and N91D)
	843.5	86 ± 0.4	0.75	wt	wt	wt	>32; *parC* (S52G and N91D) *gyrA* (S81Y)
	875	94 ± 1.3	0.75	wt	wt	wt	wt
	937.5	104 ± 0.8	0.75	wt	wt	wt	wt
	1,000	117 ± 6.6	0.75	wt	wt	wt	wt
Moxifloxacin							
ATCC 49619	50	32 ± 0.3	0.125	wt	wt	0.19	0.75
	100	63 ± 0.1	0.125	wt	wt	wt	wt
	200	114 ± 5.0	0.125	wt	wt	wt	wt
	400	236 ± 10.7	0.125	wt	wt	wt	wt
	800	449 ± 16	0.125	wt	wt	wt	wt
BSP2443	50	16 ± 0.2	0.25	wt	wt	4; *parC* (S52G and N91D) and *gyrA* (S81Y)	4–6; *parC* (S52G and N91D) and *gyrA* (S81Y)
	75	24 ± 0.1	0.25	wt	wt	0.38; *parC* (S52G and N91D) and *gyrA* (S114G)	1.5; *parC* (S52G and N91D) and *gyrA* (S114G)
	100	31 ± 0.1	0.25	wt	wt	wt	wt

[a] wt, wild-type MIC.

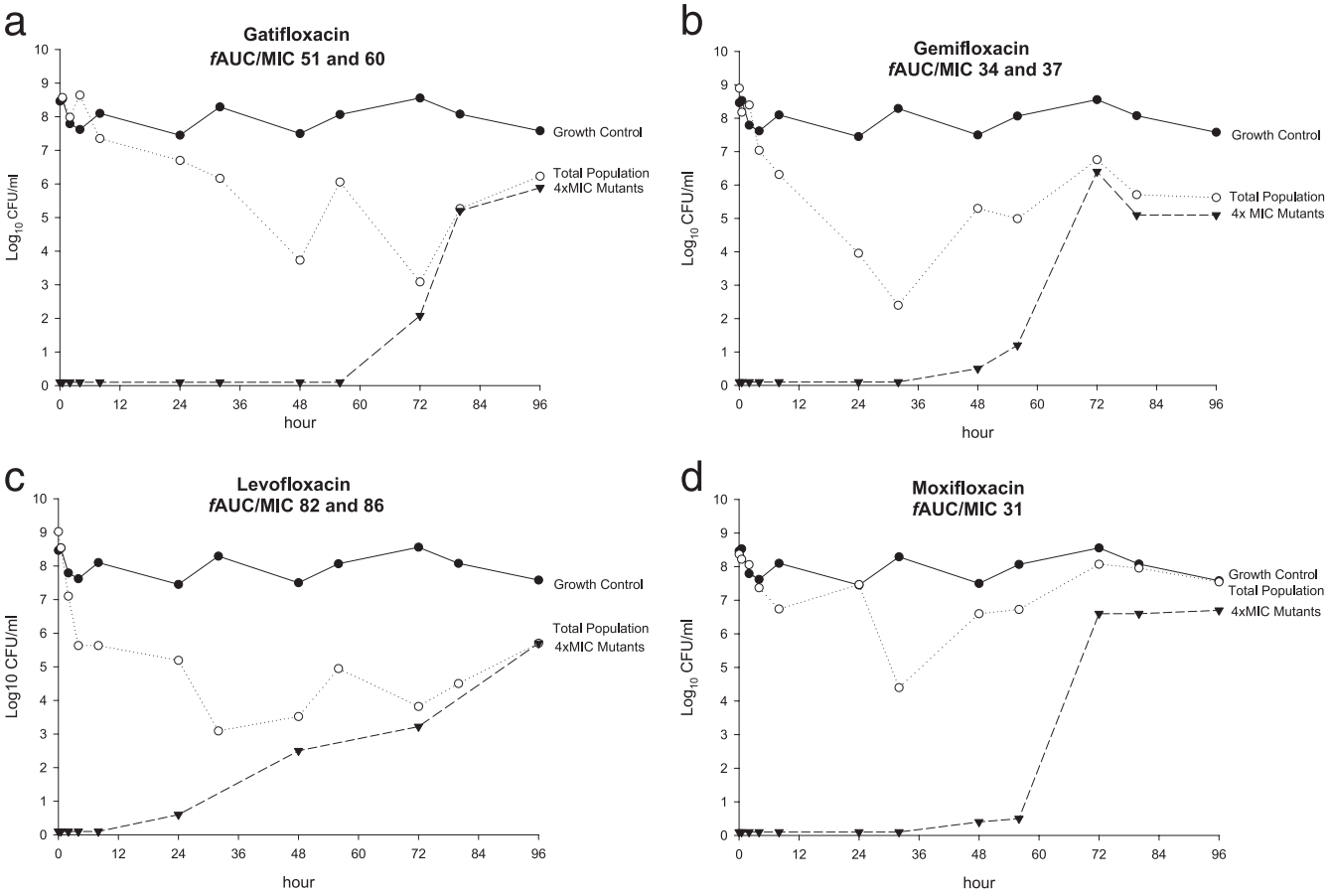

FIG. 1. Time-kill assessment and resistance development at *f*AUC/MIC of gatifloxacin, gemifloxacin, levofloxacin, and moxifloxacin versus wild-type *Streptococcus pneumoniae (*BSP2443 and ATCC 49619). Each graph represents in vitro model results at the highest simulated *f*AUC/MIC for each organism where resistance development occurred.

reserpine. Thus, it is likely that efflux contributes to the raised moxifloxacin MIC observed for this strain.

DISCUSSION

Inappropriate use of any antibiotic can contribute to the emergence of resistance to that and related agents. Canadian, TRUST, and PROTEKT US surveillance data reveal resistance rates among fluoroquinolones to be increasing (0.8% to 1.8%) (17, 18). In addition, it has been suggested that ciprofloxacin-resistant, levofloxacin-susceptible *S. pneumoniae* may already possess first-step mutations (19, 27). A Canadian longitudinal study that evaluated resistance in *S. pneumoniae* between 1988 and 1998 identified an increase in resistance from 0 in 1993 to 1.7% in 1997 (17). A concomitant increase in the number of fluoroquinolone prescriptions (0.8 to 5.5 per 100 persons per year) was also noted. Additionally, previous studies show that in geographical areas where fluoroquinolone use increased there was an associated decrease in pneumococcal susceptibility (19). If this valuable antimicrobial class is to be preserved, it is essential to control inappropriate prescribing and to minimize durations of therapy.

We have shown previously in an in vitro model that a fluoroquinolone AUC/MIC ratio of ≥80 is required for bactericidal activity against *S. pneumoniae* (6). This minimum breakpoint was also important to reduce the potential for the development of resistance to several different fluoroquinolones. These experiments were carried out at an organism inoculum ranging from 10^6 to 10^7 CFU/ml. Resistance associated with an AUC/MIC of ≤80 tended to be related to efflux mechanisms, which seem more likely to occur at a lower organism inoculum (6).

The results of this study were similar to other studies that have ranked the activity of the various fluoroquinolones against *S. pneumoniae*, with optimal activity (from highest to lowest) being gemifloxacin = moxifloxacin > gatifloxacin > levofloxacin > ciprofloxacin against both wild-type and quinolone-resistant *S. pneumoniae* (1, 12). This may be due to greater potency of newer-generation fluoroquinolones and enhanced stability of the ternary gyrase-topoisomerase IV–DNA complex (6, 30). Although previous pharmacodynamic models have documented differences between levofloxacin and moxifloxacin for the development of resistance, there has been little work evaluating differences that may exist between gatifloxacin, gemifloxacin, levofloxacin, and moxifloxacin (1, 10). We found that for each compound evaluated a delay of first- and second-step mutations was observed with increasing *f*AUC/MIC ratios. There also appear to be differences among the

various fluoroquinolones with respect to their *f*AUC/MIC breakpoints that will prevent QRDR mutations from occurring. Our data suggest that the recovery of topoisomerase mutations in *S. pneumoniae* is related to the *f*AUC/MIC exposure.

We conclude that clinical doses of gatifloxacin, gemifloxacin, and moxifloxacin exceed the *f*AUC/MIC resistance breakpoint against wild-type *S. pneumoniae* and that the exposure breakpoints differ between levofloxacin, gatifloxacin, gemifloxacin, and moxifloxacin. Additionally, moxifloxacin breakpoints are significantly lower than those for gatifloxacin. With regard to the prevention of resistance, moxifloxacin = gemifloxacin > gemifloxacin > levofloxacin. These differences may be related to structural variations within the class. Using a fluoroquinolone regimen that exceeds the pharmacodynamic breakpoint for resistance development may decrease the emergence of resistance in patients with *S. pneumoniae* respiratory infections.

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