

Pharmacodynamics of Moxifloxacin and Levofloxacin at Simulated Epithelial Lining Fluid Drug Concentrations against *Streptococcus pneumoniae*

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Recent clinical failures associated with levofloxacin treatment for *Streptococcus pneumoniae* infections and growing evidence of frequent mutations in the isolate population have led to increased concerns regarding fluoroquinolone resistance. Our objective was to characterize the efficacies of levofloxacin and moxifloxacin against various genotypes of *S. pneumoniae* after simulated bronchopulmonary exposures. An in vitro model was used to simulate a levofloxacin concentration of 500 mg and a moxifloxacin concentration of 400 mg, which were previously determined to be the concentrations in the epithelial lining fluid of older adults receiving once-daily dosing. The effects of the drugs were tested against six *S. pneumoniae* containing various mutations. Bacterial density and resistance were quantitatively assessed over 48 h. The *S. pneumoniae* isolate with no mutation displayed a 4-log reduction in CFU after treatment with both agents and did not develop resistance. Isolates containing the *parC* or *parE* mutation or both mutations regrew and developed resistance when they were exposed to levofloxacin, despite an unbound area under the concentration-time curve (AUC):MIC ratio of ~100. When the isolate containing the *parC* and *gyrA* mutations was exposed to levofloxacin, there was a half-log reduction in the number of CFU compared to that for the control, but the isolate subsequently regrew. Likewise, levofloxacin did not kill the isolate containing the *parC*, *gyrA*, and *parE* mutations. Moxifloxacin sustained the killing of all bacterial isolates tested without the development of resistance. Levofloxacin did not sustain bacterial killing and did not prevent the emergence of further resistance in mutants with the *parC* or *parE* mutation or both mutations, even though an unbound AUC:MIC ratio for exposure well above the breakpoint of 30 to 40 established in the literature for *S. pneumoniae* was maintained. Moxifloxacin was effective against all isolates tested, despite the presence of isolates with two- and three-step mutations, for which the MICs were increased.

Streptococcus pneumoniae is the most common organism associated with lower respiratory tract infections and accounts for approximately 50% of all cases of community-acquired pneumonia, 35% of cases of acute sinusitis and acute otitis media, and 20% of chronic bronchitis infections (15, 28). Of growing concern with *S. pneumoniae* is the increasing levels of resistance to commonly used antimicrobials, namely, the penicillins and other β -lactams, as well as the macrolides. The frequencies of resistance to penicillin and the macrolides among *S. pneumoniae* strains are estimated to be 40 and 30%, respectively (10). As such, use of one of the antipneumococcal fluoroquinolones (levofloxacin, moxifloxacin, gatifloxacin) as monotherapy for lower respiratory tract infections is fast becoming common practice in both the inpatient and the outpatient settings.

With the increasing use of the antipneumococcal fluoroquinolones, issues surrounding the development of resistance to these agents must be studied. The present rates of fluoroquinolone resistance among *S. pneumoniae* isolates in the United States and Canada are relatively low. In the United States, the overall rates of fluoroquinolone resistance among

4,650 *S. pneumoniae* isolates were 1.4% for ciprofloxacin, 0.5% for levofloxacin, 0.3% for gatifloxacin, and 0.3% for moxifloxacin (3). Similar rates of resistance to ciprofloxacin (1.4%), levofloxacin (0.9%), and gatifloxacin or moxifloxacin (<1%) were found in Canada (17). Of perhaps greater concern are the recent clinical failures of levofloxacin treatment of *S. pneumoniae* infections and growing evidence of frequent mutations in the isolate population. Since 1999, there have been 21 case reports of levofloxacin treatment failures in the United States and Canada (6, 8, 12, 13, 26, 27, 29; N. O. Fishman, B. Suh, L. M. Weigel, B. Lorber, S. Gelone, A. L. Truant, T. D. Gootz, J. D. Christie, and P. H. Edelstein, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C1-825, 1999; J. Piper, K. Couch, D. Tuttle, and L. Steele-Moore, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. L-902, 2001). Furthermore, a recent report (7) of a study with a subset of the levofloxacin-susceptible pneumococcal isolates from the 1999–2000 respiratory season evaluated as part of the TRUST surveillance program suggests that 6.6 and 71% of the *S. pneumoniae* isolates for which the levofloxacin MICs are 1.0 and 2.0 $\mu\text{g/ml}$, respectively, contain a first-step *parC* mutation. Of perhaps more clinical significance was a recent report showing that of 164 unique patient isolates of *S. pneumoniae*, 29.9% harbored a mutation in either the *parC* or the *gyrA* gene, with

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TABLE 1. Preexperimental phenotypic and genotypic profiles of the *S. pneumoniae* isolates

<i>S. pneumoniae</i> isolate	Mutation locus or loci for levofloxacin	Levofloxacin MIC ($\mu\text{g/ml}$)	Mutation locus or loci for moxifloxacin	Moxifloxacin MIC ($\mu\text{g/ml}$)
53	None	1.0	None	0.125
1911	<i>parC</i> + <i>gyrA</i>	16	<i>parC</i> + <i>gyrA</i>	2
93	<i>parC</i> + <i>gyrA</i> + <i>parE</i>	128	<i>parC</i> + <i>gyrA</i> + <i>parE</i>	8
1312	<i>parC</i> + <i>parE</i>	2	<i>parC</i> + <i>parE</i>	0.25
1386	<i>parC</i> + <i>parE</i>	2 (32) ^a	NA ^b	(4)
1610	<i>parC</i>	2 (32)	NA	(4)

^a The values in parentheses are the postexperimental MICs (see Table 4).

^b NA, not applicable, as in vitro experimentation was not performed with these isolates and moxifloxacin.

the majority of isolates (67.3%) having a mutation in the *parC* locus only. Mutant isolates were found only when the ciprofloxacin MIC was ≥ 2 $\mu\text{g/ml}$ (3).

The first issue surrounding the prevention of the development of resistance in *S. pneumoniae* involves maintenance of adequate levels of exposure to the fluoroquinolone. For the fluoroquinolones, the pharmacodynamic parameter that best predicts exposure and that best correlates with the outcome is the area under the concentration-time curve (AUC):MIC ratio. In vitro, in vivo, and clinical trials have determined that maintenance of an unbound AUC:MIC ratio of 30 to 40 maximizes the efficacies of fluoroquinolones against *S. pneumoniae* (1, 14, 16, 21). In addition to exposure of the organism to the antimicrobial agent, the target binding site and the genotypic profile of the organism are becoming increasingly important. *S. pneumoniae* isolates with chromosomal mutations in either the *parC* or the *parE* region of topoisomerase IV or the *gyrA* or *gyrB* region of DNA gyrase display low-level resistance, whereas isolates with second-step mutations involving alterations in both the *parC* (*parE*) and *gyrA* (*gyrB*) regions have high-level fluoroquinolone resistance. These mechanisms of resistance are more likely to affect the older antipneumococcal fluoroquinolone levofloxacin, since it binds primarily to DNA gyrase or DNA topoisomerase IV, while the new fluoroquinolones, moxifloxacin and gatifloxacin, bind strongly to both DNA gyrase and topoisomerase IV (18, 24, 25). This could perhaps begin to explain the clinical failures associated with levofloxacin.

To study this relationship, we used an in vitro model which simulated the steady-state bronchopulmonary pharmacokinetic profiles of levofloxacin and moxifloxacin in patients receiving conventional once-daily dosing regimens to examine the rate of killing and the development of resistance among *S. pneumoniae* isolates with various genotypic mutations.

MATERIALS AND METHODS

Bacterial strains and susceptibility testing. Six *S. pneumoniae* isolates with various genotypic profiles (kindly provided by G. V. Doern, University of Iowa, Iowa City) were selected for inclusion (Table 1). Genotypic confirmation was performed by the DNA amplification and sequencing procedures previously described by Doern and colleagues (3). Isolates containing *parC* and *parE* mutations were considered first-step mutants, as the *parE* mutation is considered silent when the mutation involves an isoleucine-to-valine substitution at position 460. MICs were determined by use of a microdilution technique by the method of the National Committee for Clinical Laboratory Standards (22).

Antibiotics. The following antibiotics were used: levofloxacin for intravenous injection (25 mg/ml; lot E1213; expiration date, December 2004; Ortho-McNeil) and moxifloxacin standard powder (BAY 12-8039; potency, 87.8%; lot 661093E; expiration date, September 2004; Bayer Corporation). All drugs were used before the labeled expiration date.

Bacterial growth medium. Cation-adjusted Mueller-Hinton broth (CAMHB; Becton Dickinson, Sparks, Md.) supplemented with 2.5 to 5% lysed horse blood (LHB; Remel, Lenexa, Kans.) was used as the bacterial growth medium in all in vitro model experiments. The volumes of growth medium used in each of the models with levofloxacin and moxifloxacin were 1,000 and 300 ml, respectively, on the basis of half-life and flow-rate calculations. Trypticase soy agar plates (diameter, 100 mm) with 5% sheep blood were used for quantitative determinations. Mueller-Hinton agar plates (diameter, 100 mm) with 2.5 to 5% LHB containing levofloxacin and moxifloxacin at concentrations of two, four, or eight times the MIC or no drug were used for quantitative determinations of resistance.

In vitro model. The in vitro model used in this study has been described previously (9). By using a central compartment model, bacteria were exposed to changing concentrations of antibiotics to simulate the pharmacokinetic parameters of the drugs in the human bronchopulmonary region previously determined with patients receiving conventional once-daily dosing regimens of levofloxacin and moxifloxacin (4). Each experiment consisted of four independent models (three models of antibiotic treatment and one growth control model), which were run simultaneously for all organisms and treatment regimens. The models were placed in a 37°C temperature-controlled circulating water bath for optimal temperature control, and magnetic stirring bars were used in each model to ensure adequate mixing of all contents. Fresh CAMHB supplemented with LHB was continuously pumped into each of the models with a peristaltic pump at rates which simulated the elimination half-lives of the test antibiotics obtained in the study of pharmacokinetics of the drugs in the human bronchopulmonary region mentioned above (4). The levofloxacin half-lives from 0 to 12 and 12 to 24 h were 6 and 10 h, respectively. The moxifloxacin half-life was 20 h.

Studies were conducted over 48 h with levofloxacin and all *S. pneumoniae* isolates and with moxifloxacin and *S. pneumoniae* isolates 53, 1911, 93, and 1312. A starting inoculum, prepared as four independent starting inocula, of 10^6 CFU/ml was prepared from an overnight culture of the test isolate for all model experiments. To ensure that the bacteria were in logarithmic growth phase prior to antimicrobial exposure, experiments were started 0.5 h after inoculation of the bacteria into the models.

Levofloxacin and moxifloxacin were added to the models at concentrations that simulated the concentrations found in human epithelial lining fluid (4). The simulated peak and trough levofloxacin concentrations were 15.23 ± 4.53 and 2.94 ± 1.74 $\mu\text{g/ml}$, respectively. The simulated peak and trough moxifloxacin concentrations were 11.66 ± 11.90 and 5.71 ± 6.3 $\mu\text{g/ml}$, respectively. To confirm the simulation of the pharmacokinetic parameters in the human bronchopulmonary region, samples were taken throughout the duration of the model experiment, and samples were stored at -80°C until they were assayed for their drug concentrations.

To assess bacterial density over time, samples were obtained from each model and serially diluted in CAMHB. Aliquots of each diluted sample were plated in duplicate for quantitative culture. The volume of the aliquots used for determination of bacterial counts was either 10 or 100 μl , depending on the dilution used. After 24 h of incubation at 37°C, the change in the \log_{10} number of CFU per milliliter over the 48-h interval was calculated, and time-kill curves were constructed by plotting the \log_{10} number of CFU per milliliter against time. The limit of quantification was 10^1 CFU/ml.

To assess the development of resistance over time for each of the test organisms, samples were obtained from each model at 0, 8, 24, and 48 h and plated in duplicate for quantitative culture on Mueller-Hinton agar with 2.5 to 5% LHB containing levofloxacin or moxifloxacin at concentrations of two, four, and eight times the MIC or no drug. The development of resistance was further examined by postexperimental MIC and genotypic profile determinations for *S. pneumoniae* isolates 1386 and 1610.

TABLE 2. Simulated dosing regimens, target human epithelial lining fluid drug concentrations, and corresponding pharmacokinetic profiles observed in in vitro simulations

Antibiotic (regimen), parameter, and isolate	Peak concn ($\mu\text{g/ml}$) ^a	Trough concn ($\mu\text{g/ml}$) ^b	AUC ₀₋₂₄ ($\mu\text{g} \cdot \text{h/ml}$) ^c
Levofloxacin (500 mg every 24 h)			
Target value ^c (mean [\pm SD])	15.23 (4.53)	2.94 (1.74)	180
Actual value (mean [\pm SD]) ^d			
53	13.79 (2.11)	4.38 (0.50)	268 (34)
1911	17.52 (1.58)	3.33 (0.50)	203 (22)
93	15.82 (0.20)	3.52 (0.20)	193 (4)
1312	16.75 (1.52)	3.83 (0.15)	200 (15)
1386	18.19 (0.21)	3.20 (0.58)	202 (18)
1610	17.88 (0.66)	2.80 (0.39)	194 (5)
Moxifloxacin (400 mg every 24 h)			
Target value ^c (mean [\pm SD])	11.66 (11.9)	5.71 (6.3)	208
Actual value (mean [\pm SD]) ^d			
53	11.47 (0.17)	5.62 (0.23)	201 (1)
1911	10.97 (0.29)	5.76 (0.55)	198 (9)
93	12.51 (0.16)	6.21 (0.46)	222 (4)
1312	11.73 (0.52)	5.92 (0.35)	199 (17)

^a Values were obtained at 4 h after dose administration.

^b Values were obtained at 24 h after dose administration.

^c Adapted from reference 4.

^d Values are presented as the means for the three treatment models.

Antibiotic concentration determinations. Samples of CAMHB supplemented with LHB taken from each of the treatment models were assayed for levofloxacin and moxifloxacin concentrations. Samples were analyzed by a validated ion-paired high-performance liquid chromatography (HPLC) method as described previously (20), with modifications. CAMHB with LHB was used to prepare standards, check samples, and dilute samples as required. For the moxifloxacin assay, a Waters Associates (Milford, Mass.) 515 pump was equipped with a Nucleosil 100 C₁₈ column (10 μm ; 4.6 by 250 mm; Alltech Associates, Deerfield, Ill.) and a $\mu\text{Bondapak C}_{18}$ Guard-pak precolumn (Waters Associates). A programmable fluorescence detector (emission, 418 nm; excitation, 295 nm; range, 0.1 absorbance units, full scale; model 980; Applied Biosystems, Foster City, Calif.) was used to detect the analytes. The mobile phase consisted of a mixture of 0.01 M sodium phosphate buffer with 0.01 M tetrabutylammonium hydrogen sulfate and acetonitrile (80:20) filtered through a 0.22- μm -pore-size filter. The flow rate was 1.3 ml/min. A 150- μl aliquot of a standard, quality control, or unknown sample and a 50- μl aliquot of an internal standard (gatifloxacin at 2.0 $\mu\text{g/ml}$) were placed in a labeled tube. A 600- μl volume of acetonitrile was added to each tube, and the tubes were vortexed for 30 s. The supernatants were

transferred to a clean tube and dried under a stream of nitrogen at 40°C. The residual material was reconstituted in a 200 μl of 0.01 N HCl. The solution was vortexed and transferred to WISP vials for injection into a WISP 717 Plus autosampler (Waters Associates). A chromatography data system (EZChrome Elite; Scientific Software, San Ramon, Calif.) was used for data acquisition. The moxifloxacin HPLC assay was linear ($r = 0.9995$ to 1.0000) over a concentration range of 1.0 to 50.0 $\mu\text{g/ml}$. The intraday quality control samples ($n = 10$) with concentrations of 2.0 and 40.0 $\mu\text{g/ml}$ had coefficients of variation (CVs) of 0.6694 and 0.8059%, respectively. The interday quality control samples ($n = 7$) with concentrations of 2.0 and 40.0 $\mu\text{g/ml}$ had CVs of 0.47 and 0.89%, respectively.

Levofloxacin standards were prepared in a manner similar to that described above for moxifloxacin, and the concentrations were assayed by a validated HPLC procedure (23). The assay was linear ($r = 0.9995$ to 1.0000) over a concentration range of 1.0 to 20.0 $\mu\text{g/ml}$. The intraday quality control samples ($n = 11$) with concentrations of 2.0 and 18.0 $\mu\text{g/ml}$ had CVs of 1.93 and 2.38%, respectively. The interday quality control samples ($n = 7$) with concentrations of 2.0 and 18.0 $\mu\text{g/ml}$ had CVs of 1.46 and 0.84%, respectively.

Pharmacokinetic and pharmacodynamic analyses. The target values of the pharmacokinetic parameters for the human bronchopulmonary region were selected prior to initiation of the study. By using actual drug concentration data from each set of experiments, the maximum (peak) and minimum (trough) concentrations, as well as the AUCs, were determined for each antibiotic by noncompartmental methods. The AUC values were calculated by the trapezoidal method. The AUC from 0 to 24 h (AUC₀₋₂₄):MIC ratio was determined by using experimental pharmacokinetic and screening MIC data.

RESULTS

Susceptibility testing. Table 1 shows the genotypic profiles as well as the preexperimental MICs of levofloxacin and moxifloxacin for the six *S. pneumoniae* isolates used in this study.

Pharmacokinetic analysis. The target values of the pharmacokinetic parameters and the mean values of the experimental pharmacokinetic data are summarized in Table 2. The values of the pharmacokinetic parameters for levofloxacin and moxifloxacin observed in the model were similar to the target values. The actual concentrations of levofloxacin obtained in the model were, on average, 14% higher than the target concentrations. Graphical depictions of the target and actual concentrations of levofloxacin and moxifloxacin are presented in Fig. 1 and 2, respectively.

Bactericidal activity. The average bacterial density of the starting inoculum was $1.7 \times 10^6 \pm 1.9 \times 10^6$ CFU/ml. Figures 3 and 4 summarize the resultant killing curves for levofloxacin and moxifloxacin, respectively. Data are plotted as the means for levofloxacin and moxifloxacin in the three treatment mod-

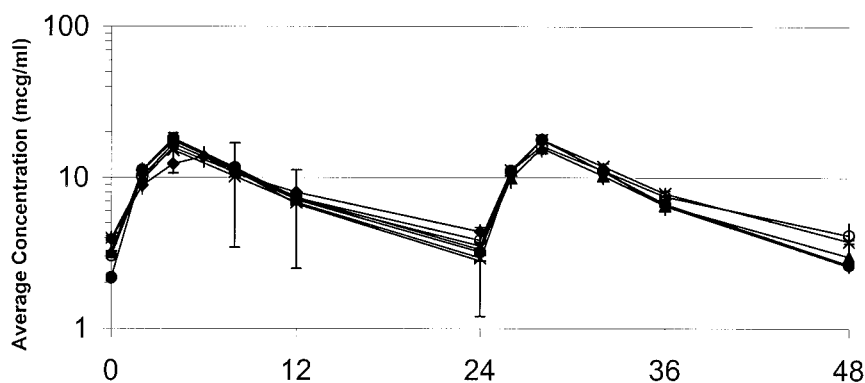


FIG. 1. Human epithelial lining fluid (ELF) levofloxacin concentrations and the corresponding pharmacokinetic profiles observed for the isolates in the in vitro simulations. Error bars represent the standard deviations for the patient epithelial lining fluid levofloxacin concentrations. The values on the x axis are times (in hours). \blacklozenge , isolate 53; \times , isolate 93; $+$, isolate 1610; \circ , isolate 1312; $*$, concentration in patient epithelial lining fluid; \blacktriangle , isolate 1911; \bullet , isolate 1386.

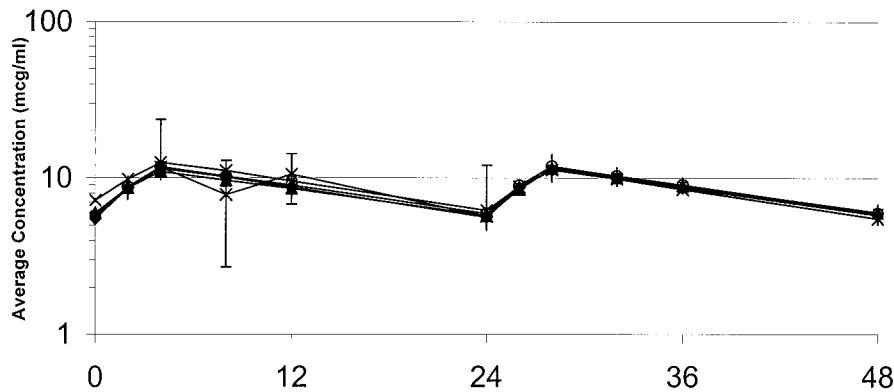


FIG. 2. Human epithelial lining fluid moxifloxacin concentrations and the corresponding pharmacokinetic profile observed for the isolates in the in vitro simulations. Error bars represent the standard deviation for the patient epithelial lining fluid moxifloxacin concentrations. The values on the *x* axis are times (in hours). ◆, isolate 53; ○, isolate 1312; ▲, isolate 1911; ×, isolate 93; *, concentration in patient epithelial lining fluid.

els as well as the mean for the growth control for all isolates tested.

Moxifloxacin was observed to have a rapid bactericidal effect (3-log reduction) against all isolates tested. The effect of moxifloxacin against isolates 1386 and 1610, which contained both the *parC* and *parE* mutations and the *parC* mutation, respectively, was not tested, as moxifloxacin produced complete eradication of isolate 1312 containing the *parC* and *parE* mutations and so testing of these isolates was not considered necessary. The densities of isolates 53 and 1312 declined to the limit of detection (10^1 CFU/ml) during the initial 8 to 12 h, with no regrowth or development of resistance observed over the remaining 48 h. For the isolate containing a two-step mutation (isolate 1911), the density declined to the limit of detection by 24 h, with no subsequent regrowth or development of resistance. The density of the isolate containing a three-step mutation (isolate 93) declined to the limit of detection by 32 h, with a regrowth of 3 logs by 48 h but no development of resistance.

Levofloxacin produced complete killing of isolate 53, which had no genotypic mutations, by 8 h, with no regrowth or the development of resistance. For those isolates containing the

parC or *parE* mutation or both mutations (isolates 1312, 1386, and 1610), there was an approximately 2.5-log reduction by 12 h, with regrowth starting at 12 h and the development of resistance to concentrations of eight times the MIC by 48 h. For the isolate containing the *parC* and *gyrA* mutations, levofloxacin produced a 3-log reduction during the first 12 h, with subsequent regrowth to the level in the control model. Levofloxacin did not produce any bacterial killing of the isolate containing a three-step mutation (*parC*, *gyrA*, *parE*), as the growth of the isolate throughout the dosing regimen was similar to that of the isolate in the control model.

Detection of resistance. None of the *S. pneumoniae* isolates tested developed resistance to moxifloxacin (Table 3). Isolates containing the *parC* or *parE* mutation or both mutations (isolates 1312, 1386, and 1610) developed resistance to levofloxacin at 24 and 48 h (Fig. 5). All three isolates grew on agar containing levofloxacin at concentrations up to eight times the MIC at 48 h. Resistant mutants of each of the three isolates emerged in all three levofloxacin treatment models during each independent run. The levofloxacin killing curves for these three isolates clearly reflected the observed changes in the MICs, since the regrowth of each isolate was noted. The pos-

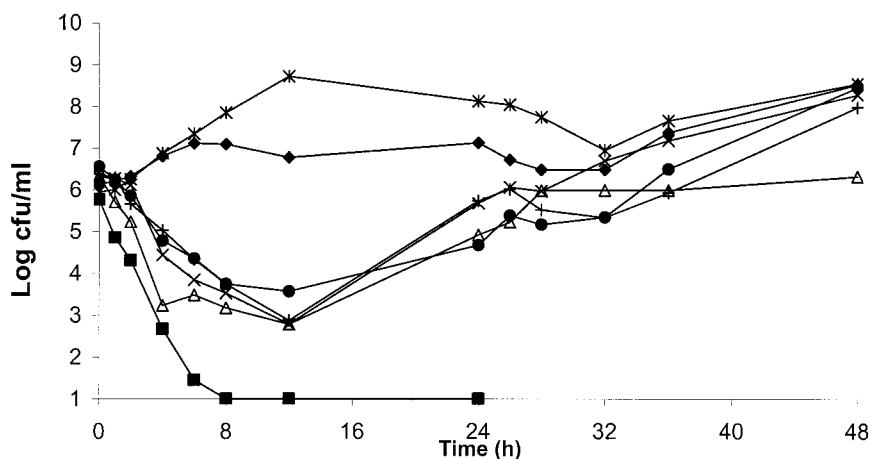


FIG. 3. Antimicrobial efficacies of levofloxacin against *S. pneumoniae* isolates after exposure to simulated concentrations in human epithelial fluid. ◆, control ($n = 6$); ■, isolate 53; △, isolate 1312; ×, isolate 1911; *, isolate 93; +, isolate 1610; ●, isolate 1386.

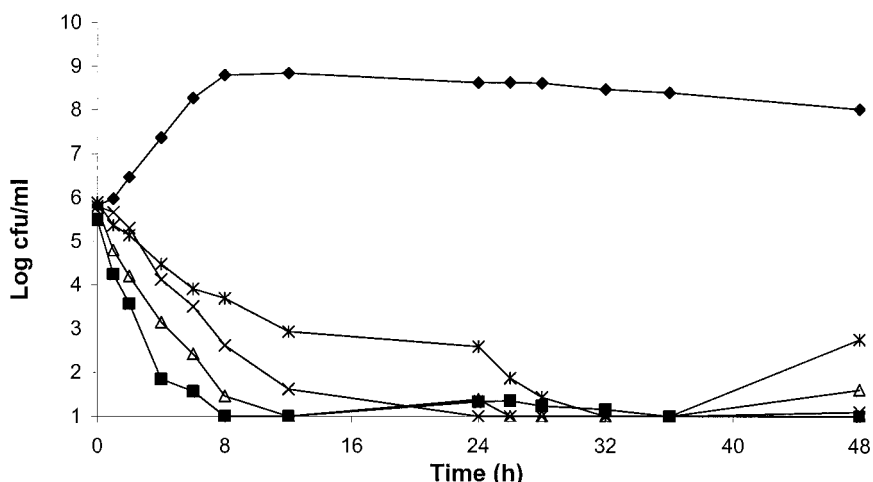


FIG. 4. Antimicrobial efficacy of moxifloxacin against *S. pneumoniae* after exposure to simulated concentrations in human epithelial fluid. ◆, control (*n* = 4); ■, isolate 53; △, isolate 1312; ×, isolate 1911; *, isolate 93.

experimental MICs and genotypic determinations for isolates 1386 and 1610 are presented in Table 4. For both isolates the ciprofloxacin, levofloxacin, moxifloxacin, and gatifloxacin MICs increased 8, 16, 16, and 32 times, respectively, in the postexposure determinations. Likewise, the genotypic profile of isolate 1386 changed from *parC parE* at 0 h to *parC parE gyrA* at 48 h. For isolate 1610, the genotypic profile changed from *parC* at 0 h to *parC parE gyrA* at 48 h. The postexposure MICs for the isolates originally containing two- and three-step mutations (isolates 1911 and 93) did not increase.

Pharmacodynamic analysis. The results of the pharmacodynamic analysis are summarized in Table 3. Moxifloxacin produced complete bactericidal activity (3-log reduction) against all isolates tested, despite the presence of two- and three-step mutations with the accompanying increased MICs and unbound AUC:MIC ratios that decreased to approximately 30. It was noted, however, that both the rate and the extent of killing increased with higher AUC:MIC ratios.

Regrowth and resistance to levofloxacin occurred with iso-

lates with the *parC* or *parE* mutation or both mutations, even though the unbound AUC:MIC ratio was 100, a level of exposure that is well above the breakpoint of 30 to 40 established in the literature for the treatment of *S. pneumoniae* infections. Exposures for the isolates containing two- and three-step mutations (isolates 1911 and 93, respectively) were well below the previously established breakpoints, and as such, bacterial killing was not observed.

DISCUSSION

The in vitro study described here was performed with the aim of determining the relationship between simulated concentrations of levofloxacin and moxifloxacin in the human bronchopulmonary region and activities against *S. pneumoniae* isolates with various genotypic profiles. We found that levofloxacin did not sustain bacterial killing and did not prevent the emergence of further resistance in mutants with a *parC* or *parE* mutation or both mutations, even though the unbound AUC:

TABLE 3. Simulated pharmacodynamics of moxifloxacin and levofloxacin in epithelial lining fluid against *S. pneumoniae* isolates with various genotypic profiles

Antibiotic and isolate	AUC:MIC ratio	Avg (range) change in log ₁₀ CFU at:		Change in multiple of the MIC at:	
		24 h	48 h	24 h	48 h
Levofloxacin					
53	268	-4.72 (none)	-4.72 (none)	NC ^a	NC
1911	13	-0.63 (-1.94 to 0.53)	+1.97 (1.78 to 2.23)	NC	NC
93	1.5	+2.19 (2.03 to 2.25)	+2.61 (2.52 to 2.65)	NC	NC
1312	100	-1.41 (-1.47 to -1.33)	0 (-0.33 to 0.67)	2	8
1386	101	-1.88 (-2.56 to -1.0)	+1.91 (1.89 to 1.95)	8	8
1610	97	-0.62 (-0.85 to -0.37)	+1.63 (none)	4	8
Moxifloxacin					
53	804	-4.21 (-4.54 to -3.54)	-4.54 (none)	NC	NC
1911	99	-4.81 (none)	-4.71 (-4.87 to -4.57)	NC	NC
93	28	-3.31 (-3.83 to -2.92)	-3.14 (-4.83 to -2.20)	NC	NC
1312	797	-4.38 (-4.76 to -3.76)	-4.15 (-4.76 to -3.76)	NC	NC

^a NC, no change.

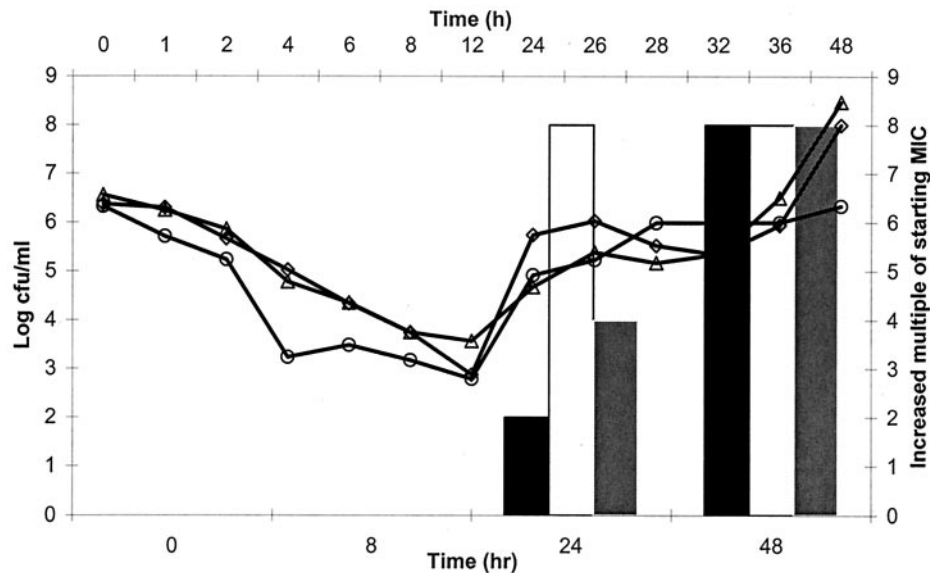


FIG. 5. Antibacterial efficacies and resistance profiles for *S. pneumoniae* containing the *parC* or *parE* mutation or both mutations after exposure to simulated human epithelial lining fluid levofloxacin concentrations. ■, MIC for isolate 1312; ○, isolate 1312; □, MIC for isolate 1386; △, isolate 1386; shaded square, MIC for isolate 1610; ◇, isolate 1610.

MIC ratio for exposure was maintained well above the breakpoint of 30 to 40 established in the literature (1, 14, 16, 21). Conversely, moxifloxacin was effective against all isolates tested, despite the presence of two- and three-step mutations with the accompanying increased MICs.

Our results highlight some important points regarding differences in the potencies of fluoroquinolones in terms of maximal effectiveness and the prevention of resistance. These findings seem to match those of Blondeau and colleagues (2), who found that moxifloxacin, but not levofloxacin, was effective at mutant prevention concentrations that were below the drug concentrations achievable in serum. Furthermore, it is becoming evident that not all fluoroquinolones are equal and, as such, should not have the same pharmacodynamic breakpoints.

Previous in vitro modeling studies have determined that maintenance of an unbound AUC:MIC ratio for exposure of 30 to 40 correlates with high rates of bacterial killing (14, 16). Those studies, however, were performed with *S. pneumoniae* isolates that were susceptible to the fluoroquinolones and that did not have identifiable underlying genetic mutations. Furthermore, the simulated fluoroquinolone concentrations were those achieved in serum and not the concentrations at the site of infection, the bronchopulmonary region. To our knowledge,

this is the first study to have used an in vitro model to simulate the concentrations of levofloxacin and moxifloxacin in the human bronchopulmonary region and to test their activities against *S. pneumoniae* isolates with various genetic mutations.

An in vitro study conducted by Ibrahim and colleagues (11) examined the efficacy of levofloxacin against an *S. pneumoniae* isolate containing a *gyrA* mutation and two *parE* mutations. The investigators found that levofloxacin, dosed to achieve an AUC:MIC ratio of ≥ 35 , was able to eradicate the resistant isolate. These findings may be limited in that the investigators used Todd-Hewitt broth supplemented with 0.5% yeast extract, which is not the optimal growth medium for *S. pneumoniae* and, as such, could have affected the outcome. Furthermore, the resistant isolate studied may not have been optimal for determination of the potency of levofloxacin against mutant isolates since levofloxacin is not affected as much by mutations in the *gyrA* locus as it is by mutations in the *parC* locus.

The results found in our study seem to correlate with those of an in vivo study conducted by Croisier and colleagues (5), in which levofloxacin was ineffective when mutant *S. pneumoniae* isolates with a *parC* mutation and *parC* and *gyrA* mutations and for which the MIC was ≥ 2.0 $\mu\text{g/ml}$ were used. That study also

TABLE 4. Postexperimental MICs and genotypic profiles for the *S. pneumoniae* isolates after exposure to levofloxacin at concentrations found in epithelial lining fluid

Isolate and time studied (genotypic profile)	MIC ($\mu\text{g/ml}$)			
	Levofloxacin	Ciprofloxacin	Moxifloxacin	Gatifloxacin
1386				
Preexperimental (<i>parC</i> + <i>parE</i>)	2	8	0.25	0.5
Postexperimental (<i>parC</i> + <i>parE</i> + <i>gyrA</i>)	32	>64	4	16
1610				
Preexperimental (<i>parC</i>)	2	8	0.25	0.5
Postexperimental (<i>parC</i> + <i>parE</i> + <i>gyrA</i>)	32	>64	4	16

found that resistant mutants of these isolates appeared when they had a preexisting *parC* mutation. Of further interest is an in vitro study conducted by Madaras-Kelly and colleagues (19), in which levofloxacin retained no antimicrobial effect against a *parC* mutant with phenotypic expression of fluoroquinolone efflux. Conversely, moxifloxacin exhibited greater antimicrobial effects against all mutant isolates tested in this study.

The major limitation of our study is that we did not test levofloxacin-resistant isolates with eightfold lower doses of moxifloxacin, which would have yielded the same AUC:MIC ratio produced by levofloxacin, in order to see if moxifloxacin treatment would also result in the development of resistance in these isolates. Likewise, we did not expose isolate 93 to a 20-fold higher dose of levofloxacin to see if levofloxacin would be effective at higher levels of exposure, as was the case for moxifloxacin. These studies are pending and will be useful in further elucidating the AUC:MIC ratio necessary for the fluoroquinolones.

In conclusion, our findings suggest that not all fluoroquinolones have the same AUC:MIC ratio needed to maximize antimicrobial killing and prevent the emergence of resistance. The concentrations of levofloxacin found in the bronchopulmonary region of humans did not produce bacterial eradication in the presence of first-step mutants of *S. pneumoniae*, despite exposure to high concentrations. Moreover, for these isolates inadequate levofloxacin exposures resulted in the emergence of high-level phenotypic and genotypic resistance profiles. Although an in vitro model offers the worst-case scenario for a given antimicrobial, since it does not have the benefit of an intact immune system that can aid with eradication of the infection, this technique has been widely used for pharmacodynamic profiling of the fluoroquinolones and has previously been shown to be predictive of treatment outcomes in humans. Further study is necessary to determine the magnitude of the AUC:MIC ratio for levofloxacin that is required to ensure sufficient antimicrobial effects against *S. pneumoniae* isolates containing *parC* mutations. While the conventional breakpoints of 30 to 40 for the unbound AUC:MIC ratio appear to be in question for levofloxacin, this level of exposure was found to be appropriate for moxifloxacin, as the compound displayed potent antimicrobial effects against all isolates.

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