Evaluation of bacterial kill when modelling the bronchopulmonary pharmacokinetic profile of moxifloxacin and levofloxacin against parC-containing isolates of *Streptococcus pneumoniae*

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Objectives: The increasingly recognized prevalence of first-step parC mutants in *Streptococcus pneumoniae* and the development of de novo resistance while on fluoroquinolone therapy are of concern. Previous work by our group demonstrated the ability of moxifloxacin, but not levofloxacin, to eradicate parC mutants. The objective of this experiment was to determine whether these fluoroquinolone antibiotics provided equivalent bacterial kill when similar AUC/MICs were examined.

Methods: An in vitro pharmacodynamic model was used to simulate the epithelial lining fluid (ELF) concentrations following oral administration of levofloxacin 500 mg once daily and moxifloxacin 400 mg once daily in older adults. In addition, a range of AUC/MICs were also modelled, including levofloxacin 750 mg once daily. Five different *S. pneumoniae* containing first-step parC mutations and one isolate without mutations were tested for 48 h and time–kill curves were constructed. Samples at 0, 24 and 48 h were collected for phenotypic and genotypic profiling. HPLC was used to verify that target exposures were achieved.

Results: The isolate without a parC mutation displayed a 4 log reduction in cfu after treatment with levofloxacin 500 mg and did not select for resistance. In all five isolates containing first-step parC mutations, resistance emerged within 48 h with a >16-fold increase in MIC and the acquisition of a gyrA mutant. Increasing the exposure of levofloxacin to ~750 mg dose still led to >16-fold increase in MIC at 48 h in two of the four isolates containing parC mutations. On the other hand, moxifloxacin 400 mg sustained bacterial killing against the two isolates tested without the selection of resistant mutants. It appears that the critical AUC/MIC necessary to prevent the acquisition of resistance for levofloxacin is 200 and ~400 for moxifloxacin.

Conclusions: Due to suboptimal exposures, once-daily oral regimens of levofloxacin at both 500 and 750 mg inconsistently led to bactericidal activity and the frequent acquisition of a second-step gyrA mutation in *S. pneumoniae* isolates already containing a first-step parC mutation. Conversely, once-daily moxifloxacin 400 mg provides exposures that vastly exceed the apparent efficacy breakpoint and did not select for second-step mutants until exposures were decreased 4-fold. As a result of these data and the emerging literature involving mutations in the pneumococcus, caution should be exercised when the respiratory fluoroquinolones are used to treat patients infected with *S. pneumoniae* suspected of having parC mutations.

Keywords: area under the curve, fluoroquinolones, DNA topoisomerase IV, DNA gyrase, drug resistance, bacterial
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Introduction

In the past decade, interest in the use of the ‘respiratory’ fluoroquinolones (levofloxacin, moxifloxacin, gatifloxacin and gemifloxacin) for lower respiratory tract infections (LRTIs) has heightened due to steadily increasing resistance of Streptococcus pneumoniae to many of the currently available antibacterials. Current resistance rates for β-lactams obtained during the respiratory season of 2001–2002 have increased to 18.4% for penicillin and 1.7% for ceftriaxone, as reported by the TRUST (Tracking Resistance in the United States Today) surveillance programme.1 Macrolide resistance is estimated to be 25% among clinical isolates.1,2

While current resistance to levofloxacin is low (<2%) in the United States,1,3 clinical failures have been reported.4–8 Resistance to fluoroquinolones in S. pneumoniae is primarily due to mutations in the genes encoding DNA gyrase and topoisomerase IV.3 Davidson et al.9 described four patients infected with S. pneumoniae who failed levofloxacin therapy; two of these patients had S. pneumoniae resistant to levofloxacin at the beginning of therapy and in two patients resistance developed during therapy. Furthermore Anderson et al.10 reported four episodes of re-infection with S. pneumoniae due to the development of de novo resistance while on levofloxacin therapy in immunocompromised adults.

With increasing use of the antipneumococcal fluoroquinolones, issues surrounding the development of resistance to these agents must be addressed. The first issue involves maintaining adequate exposure of the fluoroquinolones against S. pneumoniae. Previous studies have determined that optimal fluoroquinolone efficacy is observed after achieving an AUC/MIC, the pharmacodynamic parameter that best correlates with outcome, of ≥30–40.9–12 However, much of these data have been derived from wild-type S. pneumoniae that do not express first-step mutations. Current data show that S. pneumoniae containing these first-step mutations are as high as 29.9% among clinical isolates, with the majority (67.3%) having a mutation in the parC locus only.13 In addition, Lim et al.14 determined that 59% of isolates with a levofloxacin MIC = 2 mg/L had a first-step mutation in parC. S. pneumoniae with chromosomal mutations in either the parC or parE region of topoisomerase IV or the gyrA or gyrB region of DNA gyrase have elevated MICs that are near or slightly exceed the susceptibility breakpoint, with second-step mutations involving alterations in both the parC (parE) and gyrA (gyrB) region conferring high-level fluoroquinolone resistance. Furthermore, previous work has shown moxifloxacin to be superior to the older fluoroquinolones, ciprofloxacin and levofloxacin, for restricting the selection of resistant mutants.15,16

To study this relationship, our group used an in vitro model which simulated the steady-state bronchopulmonary pharmacokinetic profile of levofloxacin and moxifloxacin to examine the rate of kill and the selection of resistant mutants against S. pneumoniae with various genotypic profiles.17 The concentrations of levofloxacin and moxifloxacin used in the model simulated those found in the epithelial lining fluid (ELF) of patients receiving conventional once-daily dosing regimens and produced AUCs of ~180 mg·h/L for levofloxacin and 208 mg·h/L for moxifloxacin.18 The most significant finding of the study involved levofloxacin against three strains of S. pneumoniae containing parC mutations. While these organisms were considered to be susceptible according to CLSI guidelines (MICs = 2 mg/L) at the onset of drug exposure, all three pneumococcal strains displayed complete regrowth and the selection of resistant mutants that had 8 times greater MICs to levofloxacin at 48 h. Despite a high exposure (AUC/MIC = 100) by conventional pharmacodynamic standards, levofloxacin was not able to eradicate any of these pneumococci. This finding is increasingly important since at least one documented clinical failure was reported to have a parC mutation and 70% of all S. pneumoniae isolates that have an MIC of 2 mg/L and 6% of those at the modal MIC (1 mg/L) of levofloxacin have been noted to contain a parC mutation.19 Simulations performed with moxifloxacin against one of the parC-containing S. pneumoniae isolates revealed complete eradication of the organism with no regrowth or acquisition of resistance.20

The objective of the present study was to evaluate whether the phenomenon seen with S. pneumoniae containing a parC mutation is a result of inadequate exposure and as such can be overcome with higher AUC/MICs or whether this is a result of mutations in DNA gyrase or topoisomerase IV that cannot be overcome by increased exposures.

Materials and methods

Bacterial strains and susceptibility testing

Five S. pneumoniae isolates with parC genotypes and one isolate with no mutations (Doern Research Laboratory, University of Iowa, Iowa City, IA, USA; Health Sciences Centre, University of Manitoba, Winnipeg, Manitoba, Canada) were selected for inclusion (Table 1). Genotypic confirmation was performed by DNA amplification and sequencing previously described by Doern and colleagues.13 Isolates containing parC + parE mutations were considered to be first-step mutants, as the parE mutation is considered to be silent when the mutation involves isoleucine to valine substitution at position 460. MICs were determined by broth microdilution according to Clinical Laboratory Standards Institute (CLSI; formerly NCCLS) methodology.19

Antibiotics

The following antibiotics were used: levofloxacin for intravenous injection, 25 mg/mL (lot E1244; expiration date, January 2005; Ortho-McNeil), and moxifloxacin standard powder (material 05517435; potency, 87.8%; lot 040000325330; expiration date August 2005; Bayer Corporation). At the time of the study all drugs were within the labelled expiration date.

| Table 1. Pre-experimental phenotypic and genotypic profiles of the S. pneumoniae isolates |
|-----------------|-----------------|-----------------|-----------------|
| **S. pneumoniae** | **Mutation** | **Levofloxacin MIC (mg/L)** | **Moxifloxacin MIC (mg/L)** |
| isolates | | | |
| 1615 | none | 2 | 0.25 |
| 1610 | parC | 1.5 | 0.25 |
| 3104 | double parC + parE | 1.5 | 0.19 |
| 18705 | parC | 2 | 0.25 |
| 59 | parC | 1 | 0.12 |
| 403 | parC + parE | 1 | 0.12 |
Organisms and treatment regimens. The models were placed in a 37°C incubator with LHB was continuously pumped into each of the models by a peristaltic pump at rates which simulated the elimination half-lives of the specific species from each model and serially diluted in normal saline. Aliquots of each diluted sample were plated in duplicate for quantitative determinations. A representative sample of the three experimental models to determine whether resistance was acquired during therapy.

In vitro model

The in vitro model used in the study has been described previously.20 By using a central compartment model, bacteria were exposed to changing concentrations of antibiotics to simulate human broncho-pulmonary steady-state pharmacokinetic parameters previously determined in patients receiving conventional once-daily dosing regimens of levofloxacin and moxifloxacin.19 Each experiment consisted of four independent models (three antibiotic-treated models and one growth control model), which ran 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 stir 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 by a peristaltic pump at rates which simulated the elimination half-lives of the test antibiotics obtained from the human bronchopulmonary study mentioned above.18 Forty-eight hours were conducted for levofloxacin against all S. pneumoniae isolates and for moxifloxacin against isolates 1610 and 3104. A starting inoculum, prepared as four independent starting inocula, of 10⁶ cfu/mL was set up from an overnight culture of the test isolate for all model experiments. To ensure that bacteria were in logarithmic growth phase prior to anti-microbial exposure, experiments were started 0.5 h after inoculation of bacteria into the models.

In the first phase of the study, levofloxacin and moxifloxacin were added to the models to simulate concentrations found in human ELF.18 The simulated levofloxacin peak concentration and corresponding AUC were ~15 mg/L and 180 mg-h/L, respectively. Moxifloxacin simulated peak concentration and AUC were ~12 mg/L and 208 mg-h/L, respectively. In the second phase of the study, target exposures of levofloxacin and moxifloxacin were adjusted to obtain the desired AUC/MIC based on the MIC of the specific S. pneumoniae isolate tested. To confirm the desired exposures, samples were taken at various time points throughout the entire duration of the experiments and samples were stored at –80°C until they were assayed for drug concentration.

To assess bacterial density over time, samples were obtained from each model and serially diluted in normal saline. Aliquots of each diluted sample were plated in duplicate for quantitative culture. The volume of the aliquots used for bacterial counts was 100 and 300 mL, respectively, based on half-life and flow rate calculations. Trypticase soy agar plates (100 mm diameter) with 5% sheep blood were used for quantitative determinations.

Pharmacokinetic analysis

Target human bronchopulmonary pharmacokinetic parameters 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 area under the concentration–time curve (AUC), were determined for each antibiotic by non-compartmental methods. The maximum (peak) and minimum (trough) concentrations were determined directly from the actual drug concentration data from each set of experiments. The AUC values were calculated by the trapezoidal method. By using experimental pharmacokinetic and screening MIC data, the AUC₀–₂₄/MICs were determined.

Results

Susceptibility testing

Table 1 shows the genotypic profiles as well as the pre-experimental MICs of levofloxacin and moxifloxacin for the six S. pneumoniae isolates used throughout the study. For isolates 1610 and 3104, in which a range of AUC/MICs were explored for both levofloxacin and moxifloxacin, fractionated MICs were performed to more adequately determine the exact MIC rather than relying on standard broth microdilution methodology (serial 2-fold dilutions). For levofloxacin, between the MIC range of 1–2, 0.25 mg/L partitions were tested. Likewise, for moxifloxacin, 0.03 mg/L partitions were tested between an MIC range of 0.12–0.25 mg/L.

Pharmacokinetic analysis

The target levofloxacin and moxifloxacin exposures in ELF and the mean values of the experimental studies are summarized in Table 2. The average AUC achieved in experiments simulating a levofloxacin 500 mg dose was 175 ± 16 mg-h/L and that of moxifloxacin 400 mg was 192 ± 4 mg-h/L. These values closely approximated the target values of 180 for levofloxacin 500 mg and 208 of moxifloxacin 400 mg. In addition, peak and trough concentrations of each simulated dosing regimen closely approximated target values and are thus not reported.

The different exposures (AUC/MICs) obtained in these experiments were obtained by linear modification of the target concentrations at 0, 4, 8, 12 and 24 h for each 24 h period the
A conservative estimate of an oral levofloxacin 750 mg once-daily dose was achieved by simply doubling the AUC achieved following an oral 500 mg once-daily regimen. Five experiments among four isolates simulated this 750 mg dose in which the average AUC was 368–25 mg·h/L, which closely approximated the target AUC of 360 mg·h/L.

**Bactericidal activity**

The average bacterial density of the starting inoculum was $2.09 \times 10^6 \pm 0.14 \times 10^6$ cfu/mL. Figure 1 summarizes the resultant killing curves when simulating human exposures obtained after oral administration of levofloxacin 500 mg once daily and moxifloxacin 400 mg once daily. For the treatment models, data are plotted as the means of the three models; for the growth control, the mean of all the isolates tested were used.

Levofloxacin produced complete killing of isolate 1615, which had no mutations. In all isolates containing a parC–parE mutation, an approximately 3 log reduction occurred at 12 h, with subsequent regrowth starting at 12 h and approaching the level of control at 24 and 48 h. A corresponding ±16-fold increase in MIC at 48 h due to the acquisition of a second-step gyrA mutation was observed in all situations. Because all the isolates tested had an MIC of levofloxacin between 1 and 2 mg/L, the AUC/MICs obtained were 126, 112, 75, 166 and 173 for isolates 1610, 3104, 18705, 59 and 403, respectively.

Moxifloxacin was observed to have rapid bactericidal activity (>3 log reduction) against all isolates tested. Since regrowth did not occur in any of the parC–parE isolates with the highest initial MICs, 0.25 and 0.1875 mg/L, corresponding to an exposure of 756 and 1040 for isolates 1610 and 3104, respectively, additional experiments against isolates with MICs of >0.125 mg/L were not deemed necessary. Resistance did not develop (MIC did not increase) in any isolate tested at human exposures following a simulated 400 mg daily regimen.

**Table 2. Pharmacokinetic results and pharmacodynamic endpoints achieved following various exposures of levofloxacin and moxifloxacin**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Drug/regimen</th>
<th>Target AUC/Actual AUC$_{0–24}$ (mg·h/L)</th>
<th>Original MIC (mg/L)</th>
<th>Actual AUC$_{0–24}$/MIC</th>
<th>Phenotypic resistance$^a$</th>
<th>Acquisition of second-step mutation$^b$</th>
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<tr>
<td>1615</td>
<td>Levofloxacin</td>
<td>189$^a$</td>
<td>2</td>
<td>95</td>
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<td>–</td>
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<tr>
<td></td>
<td>Levofloxacin</td>
<td>350$^d$</td>
<td>1.5</td>
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<tr>
<td></td>
<td></td>
<td>793</td>
<td>1.5</td>
<td>529</td>
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<td>–</td>
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<td>Levofloxacin</td>
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<td>1.5</td>
<td>43</td>
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<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>168$^b$</td>
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<td>112</td>
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<td>746</td>
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<tr>
<td>403</td>
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<tr>
<td></td>
<td>Moxifloxacin</td>
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<td></td>
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<tr>
<td>1610</td>
<td>Moxifloxacin</td>
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<td>188</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>189$^c$</td>
<td>0.25</td>
<td>756</td>
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<td>245</td>
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<td>+</td>
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<td></td>
<td>195$^c$</td>
<td>0.1875</td>
<td>1040</td>
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</table>

$^a$ > 8-fold increase in MIC.
$^b$ gyrA mutation in all instances.
$^c$ Simulating oral levofloxacin 500 mg daily.
$^d$ Simulating oral levofloxacin 750 mg daily.
$^e$ Simulating oral moxifloxacin 400 mg daily.
LVX and MXF versus *S. pneumoniae* with *parCs*

Figure 1. Antimicrobial efficacy of levofloxacin and moxifloxacin against *S. pneumoniae* isolates simulating concentrations in human epithelial lining fluid. ×, control; closed circles, 1610 (levofloxacin); open circles, 1610 (moxifloxacin); closed triangles, 3104 (levofloxacin); open triangles, 3104 (moxifloxacin); closed squares, 18705 (levofloxacin); filled diamonds, 59 (levofloxacin); +, 403 (levofloxacin); asterisks, 1615 (levofloxacin).

Figure 2. Antimicrobial efficacy of levofloxacin and moxifloxacin against *S. pneumoniae* isolate 1610 simulating a range of AUC/MICs. ×, control; closed circles, levofloxacin (AUC/MIC = 126); closed squares, levofloxacin (233); closed triangles, levofloxacin (529); open squares, moxifloxacin (188); open triangles, moxifloxacin (380); open circles, moxifloxacin (756).

again, corresponding to a levofloxacin exposure of 529, bacterial resistance was suppressed. As for moxifloxacin, when the target concentrations were halved, corresponding to an exposure of 380, bacterial eradication persisted to 48 h without the acquisition of resistant mutations. However, when the target concentrations were halved again, corresponding to an exposure of 188, regrowth of the organism occurred at 48 h to \( \times 10^5 \) cfu/mL. The MIC subsequently was determined to have increased >16-fold at this 48 h time point due to the acquisition of a second-step *gyrA* mutation.

Similar results were observed with the various exposures tested against isolate 3104 (Figure 3). As the exposure of levofloxacin increased, bactericidal activity persisted for 48 h. Likewise, as the exposure of moxifloxacin was decreased, bactericidal activity was lost and organism regrowth occurred to the level of control at 48 h with a 16-fold increase in MIC due to the acquisition of a second-step *gyrA* mutation. However, an interesting finding was observed when comparing the time–kill curves for each fluoroquinolone antibiotic around an exposure of 240. At levofloxacin exposures of 232 and 237, which approximates a levofloxacin 750 mg dose, bactericidal activity was observed with no subsequent selection of resistant mutants; however, at moxifloxacin exposures of 245 and 256, regrowth began at 24 h and increased to the level of control at 48 h. An MIC increase from 0.25 to 4 mg/L was subsequently determined following phenotypic analysis due to the acquisition of a second-step *gyrA* mutation.
Pharmacodynamic analysis

The results of the pharmacodynamic analysis are summarized in Table 2 and Figure 4. Table 2 demonstrates the increase in MIC as a function of exposure and whether this increase was due to the acquisition of a second-step gyrA mutant. As shown, at AUC/MICs ≤ 256, mutation occurred with a ≥16-fold increase in MIC. Upon genotypic analysis, acquisition of a gyrA mutation accounted for the raised MIC in all experiments except for levofloxacin at an exposure of 233 in isolate 1610. When a second-step gyrA mutation was acquired, an increase in MIC was observed in all fluoroquinolones tested (levofloxacin, moxifloxacin, gatifloxacin, gemifloxacin and ciprofloxacin), regardless of the selecting agent. In this one instance in which acquisition of a gyrA mutation did not occur, the genotypic mechanism that led to the increase in MIC is unclear. In the majority of the experiments, the acquisition of the second-step mutant occurred within the first 24 h of fluoroquinolone therapy. In only two instances, both with isolate 1610, at a levofloxacin exposure of 233 and moxifloxacin exposure of 188, resistance did not occur until 48 h. Also, the only instance in which an exposure of ≤256 suppressed the selection of resistant mutants was described previously at levofloxacin exposures of 232 and 237.

Figure 4 illustrates prediction curves for the change in log cfu/mL as a function of exposure between levofloxacin and moxifloxacin.

Figure 3. Antimicrobial efficacy of levofloxacin and moxifloxacin against S. pneumoniae isolate 3104 simulating a range of AUC/MICs. ×, control; asterisks, levofloxacin (AUC/MIC = 43); filled circles, levofloxacin (112); closed triangles, levofloxacin (232); closed squares, levofloxacin (237); closed diamonds, levofloxacin (497); open triangles, moxifloxacin (245); open squares, moxifloxacin (256); open diamonds, moxifloxacin (555); open circles, moxifloxacin (1040).
LVX and MXF versus S. pneumoniae with parCs

moxifloxacin at 48 h. For levofloxacin, it is clear that an exposure of ~200 is needed to suppress growth of bacteria and prevent acquisition of second-step mutants in isolates with a pre-existing first-step parC mutation. For moxifloxacin, it appears that the critical exposure to suppress resistant mutants is between 300 and 400 as resistance developed at 48 h with exposures of 188, 245 and 256 but was suppressed at exposures ≥380.

Discussion

From a clinical perspective, growing concern has been expressed that the current in vitro susceptibility studies are unable to distinguish S. pneumoniae with first-step mutations. Concurrently, numerous studies have demonstrated a much greater likelihood for pneumococci to acquire a second-step mutation if the first-step mutation is already present. This second-step mutation leads to a dramatic increase in MIC, after which adequate exposures necessary for efficacy may no longer be obtained. The present study was performed to examine the efficacy of fluoroquinolones against isolates containing a first-step parC mutation and to determine whether an alternative AUC/MIC breakpoint exists that ensures microbiological eradication of these mutants.

Studies have been published which suggest that a free AUC/MIC breakpoint of 30 predicts microbiological efficacy against S. pneumoniae. However, these studies have routinely evaluated efficacy in the context of wild-type S. pneumoniae that did not harbour a parC mutation. In a previous study performed by our group, levofloxacin was found to be ineffective when simulating ELF concentrations of 500 mg once daily in older adults against parC mutants even though the AUC/MICs achieved, of 100–200, were much higher than 30. On the other hand, due to the much higher exposures achieved simulating moxifloxacin 400 mg once daily of 800–1600, bacterial eradication occurred with a lack of selection for resistant mutants. Because the AUC of each of the fluoroquinolones in ELF was ~200 mg·h/L, the different exposures were primarily due to the higher potency (a 3-fold lower MIC) for moxifloxacin as compared with levofloxacin against S. pneumoniae isolates.

The results of the present study reinforce what was observed previously. In all five isolates containing a parC mutation, levofloxacin, simulating ELF exposures obtained following a 500 mg dose, was ineffective in preventing resistance in isolates already containing a first-step parC mutation. Likewise, moxifloxacin, due to the much higher exposures obtained, was found to be effective against the two isolates tested, which had high initial MICs, 0.19 and 0.25 mg/L.

In an attempt to improve the probability of clinical success and decrease length of therapy, a daily dose of levofloxacin 750 mg has been suggested as being more appropriate against respiratory infections. Because data describing the AUC obtained after administration of 750 mg levofloxacin in older adults are not known, we took a conservative estimate by doubling the AUC achieved with a standard 500 mg dose. The results of experiments simulating this increased dose are mixed (Table 2). For two of the three isolates with an MIC of 1.5 or 2 mg/L (corresponding to exposures of 190–237), the MIC increased 16-fold by 48 h. However, for the isolate with an original MIC of 1 mg/L, the high exposure, 406, was adequate to suppress resistant mutants. From these data, the exposures that simulate a 750 mg dose of levofloxacin approach the critical exposure necessary to prevent the selection for resistant mutants.

One of the limitations of the Florea study was that similar exposures were not compared between the two antibiotics. This left uncertainty as to whether the difference in efficacy was entirely due to the higher potency of moxifloxacin. The results displayed in Figures 2 and 3 suggest that similar efficacy exists between these two fluoroquinolones when similar exposures are achieved. The only disparity observed between these agents occurred in isolate 3104 (Figure 3) at an exposure around 240 at which levofloxacin showed persistent bactericidal efficacy at 48 h without acquisition of a second-step gyrA mutation. However, moxifloxacin at similar exposures selected for resistance. These experiments were repeated for each drug and these findings were confirmed. At present an explanation for this discrepancy is unclear.

These findings match those reported by Allen et al. in which levofloxacin was ineffective against S. pneumoniae isolates containing a parC mutation at a therapeutic exposure of 32 and a mutant prevention concentration (MPC) directed regimen of 256. However, moxifloxacin at standard dosing remained bactericidal with no acquisition of resistant mutants. Of specific interest, when moxifloxacin exposure was decreased to match that of levofloxacin, microbiological efficacy was lost and a 12-fold increase in MIC was observed due to the acquisition of a gyrA mutant. The inability of levofloxacin to eradicate S. pneumoniae containing first-step parC mutations has also been reported by Crosier et al., who showed that levofloxacin was ineffective for S. pneumoniae isolates with an MIC = 2 mg/L. These results do differ, however, from an abstract by Zhanel et al. in which a sustained 3 log decrease in bacterial colonies and a lack of development of resistance was observed at free ELF AUC/MICs of 27 and 40 in ciprofloxacin-resistant S. pneumoniae isolates containing first-step parC mutations. These investigators concluded that levofloxacin 500 mg achieves borderline efficacy, while bacterial eradication occurs after administration of 750 mg.

Likewise, an in vitro study performed by Lister demonstrated the efficacy of levofloxacin 750 mg once daily to eradicate ciprofloxacin-resistant parC mutants of S. pneumoniae with an MIC = 2 mg/L. Although 500 mg once daily showed some regrowth at 24 h, bactericidal eradication occurred at 48 h. The reason for the disparity between these studies and our two experiments using a wide range of different S. pneumoniae isolates is unclear.

In all instances except one (isolate 1610), phenotypic resistance was associated with the acquisition of a gyrA second-step mutation presumably due to an antibiotic exposure inadequate for bacterial eradication. This second-step mutation led to a dramatic increase in MIC in all isolates and resulted in phenotypic resistance to levofloxacin, moxifloxacin and gatifloxacin irrespective of the selecting fluoroquinolone. After exposure to levofloxacin (AUC/MIC = 233), isolate 1610 expressed phenotypic resistance with a discordant genotypic profile. There are two reasons why this may have occurred. First, perhaps some mutation did occur in these isolates and genotypic analysis was not able to identify it as only the conventional quinolone-resistant-determining region (QRDR) was examined. This hypothesis appears unlikely. Another explanation is that an efflux mechanism was selected after exposure to levofloxacin; however, this appears unlikely as we repeated MIC determinations in the presence of reserpine and no alterations in these values were noted.

A disturbing trend was noted in that even when bactericidal activity was maintained over 48 h without acquisition of a
second-step mutation, limited regrowth of bacteria still was observed at the 48 h timepoint for many of the exposures tested. As displayed in Figure 2, with levofloxacin and moxifloxacin, at exposures of 529 and 380, respectively, limited regrowth of bacteria to \( \sim 10^2 \text{ cfu/mL} \) was noted. Limited regrowth was also observed in isolate 3104 for levofloxacin at bactericidal exposures of 232, 237 and 497 and for moxifloxacin at 555. Because these experiments lasted only 48 h and no mutations were noted at this timepoint, the implications of this regrowth are unknown. It is important to note that the in vitro model represents the worst-case scenario clinically due to optimal growth media for the organisms and a lack of an immune system. It is possible that a functioning immune system would lead to the clinical eradication of these isolates.

With regard to protein binding, it would be ideal in these in vitro experiments to model only free, unbound concentrations of levofloxacin and moxifloxacin as only the free drug concentration has antibacterial efficacy. One method consistently used to account for this is to assume that protein binding is the same in ELF as it is in serum and apply this percentage protein binding studies in ELF have not been undertaken it is not clear whether the concentrations reported in the BAL studies represent free or total drug. For this reason, as well as to remain consistent with the Florea article, it was decided to target the ELF concentrations as reported in older adults. However, because the majority of these experiments involved either doubling or halving the AUC, certain inferences can be made if it is assumed that these targeted concentrations are total drug concentrations. First, in all experiments simulating levofloxacin 500 mg, the AUC obtained is not adequate to prevent the acquisition of a second-step mutation; therefore, additional experiments accounting for the 30% protein binding of levofloxacin are not necessary. Similarly, no differences would be observed for moxifloxacin either. If we accounted for the 50% protein binding of moxifloxacin by halving the AUC, these results are already displayed in Table 2 and Figures 2 and 3. At these exposures (approximate AUC = 100 mg·h/L), acquisition of a second-step mutation was not observed. Results pertaining to a levofloxacin 750 mg dose are less clear, however. The exposures simulated prevented phenotypic resistance in two of four isolates tested at 48 h. If exposures were decreased by half, the 500 mg dose would be simulated and second-step mutations were observed. It is unclear whether decreasing these exposures by 30% would retain efficacy and prevent the second-step mutation in these two isolates.

In conclusion, these findings demonstrate that similar efficacy exists between levofloxacin and moxifloxacin when comparable AUC/MICs are evaluated. In the absence of host defences, levofloxacin at the standard 500 mg dose does not maintain bactericidal activity, leads to \( \geq 16 \)-fold increase in MIC and the acquisition of a second-step gyrA mutation. Levofloxacin 750 mg leads to inconsistent bactericidal activity and may select for resistance while on therapy. Due to the much higher AUC/MIC exposures obtained with standard moxifloxacin dosing, bactericidal efficacy is maintained with no selection for resistance at 48 h despite the presence of parC mutations in \( S. \ pneumoniae \). As a result of these data and the emerging literature involving mutations in the pneumococcus, caution should be exercised when the respiratory fluoroquinolones are used in patients infected with \( S. \ pneumoniae \) suspected of having parC mutations.

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Transparency declarations

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