

Levofloxacin Pharmacokinetics/Pharmacodynamics, Dosing, Susceptibility Breakpoints, and Artificial Intelligence in the Treatment of Multidrug-resistant Tuberculosis

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Background. Levofloxacin is used for the treatment of multidrug-resistant tuberculosis; however the optimal dose is unknown.

Methods. We used the hollow fiber system model of tuberculosis (HFS-TB) to identify 0–24 hour area under the concentration-time curve (AUC_{0-24}) to minimum inhibitory concentration (MIC) ratios associated with maximal microbial kill and suppression of acquired drug resistance (ADR) of *Mycobacterium tuberculosis* (*Mtb*). Levofloxacin-resistant isolates underwent whole-genome sequencing. Ten thousands patient Monte Carlo experiments (MCEs) were used to identify doses best able to achieve the HFS-TB-derived target exposures in cavitary tuberculosis and tuberculous meningitis. Next, we used an ensemble of artificial intelligence (AI) algorithms to identify the most important predictors of sputum conversion, ADR, and death in Tanzanian patients with pulmonary multidrug-resistant tuberculosis treated with a levofloxacin-containing regimen. We also performed probit regression to identify optimal levofloxacin doses in Vietnamese tuberculous meningitis patients.

Results. In the HFS-TB, the AUC_{0-24}/MIC associated with maximal *Mtb* kill was 146, while that associated with suppression of resistance was 360. The most common *gyrA* mutations in resistant *Mtb* were Asp94Gly, Asp94Asn, and Asp94Tyr. The minimum dose to achieve target exposures in MCEs was 1500 mg/day. AI algorithms identified an AUC_{0-24}/MIC of 160 as predictive of microbiologic cure, followed by levofloxacin 2-hour peak concentration and body weight. Probit regression identified an optimal dose of 25 mg/kg as associated with >90% favorable response in adults with pulmonary tuberculosis.

Conclusions. The levofloxacin dose of 25 mg/kg or 1500 mg/day was adequate for replacement of high-dose moxifloxacin in treatment of multidrug-resistant tuberculosis.

Keywords. tuberculous meningitis; hollow fiber system model; artificial intelligence; Monte Carlo experiments; *gyrA* mutations.

The treatment of multidrug-resistant (MDR) tuberculosis relies on latter generation quinolones, considered group A drugs by the World Health Organization. Moxifloxacin has been most commonly used, and doses of 800 mg/day have been proposed to minimize acquired drug resistance (ADR) [1, 2]. However, at this higher dose, moxifloxacin could be associated with higher rates of QT segment prolongation, which could progress to polymorphic ventricular tachycardia, an important concern especially when coadministered with other anti-tuberculous agents associated with this same

adverse event [3]. Levofloxacin is felt to be safer in this regard; thus, it is important to determine if levofloxacin can replace high-dose moxifloxacin and, if so, to identify the optimal dose for MDR tuberculosis.

While pharmacokinetic-pharmacodynamic (PK/PD) studies of moxifloxacin and the earlier-generation ofloxacin and ciprofloxacin have been performed, levofloxacin as of yet has not been examined [1, 4, 5]. Clinical studies have identified the population PK parameters of levofloxacin in patients with tuberculosis and the penetration of levofloxacin into tuberculosis lesions [6–8]. Since moxifloxacin binds the *Mycobacterium tuberculosis* (*Mtb*) gyrase better than levofloxacin, in theory, it could be a better anti-tuberculosis agent [9]. However, moxifloxacin had lower early bactericidal activity in patients than levofloxacin [10]. On the other hand, murine studies demonstrated that standard-dose moxifloxacin was superior to high-dose (human equivalent 1000 mg) levofloxacin when used in combination with ethionamide,

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amikacin, and pyrazinamide after 4 and 5 months of therapy [10, 11]. Thus, it is as yet unclear if levofloxacin could be a sufficient replacement for moxifloxacin and, if so, what the dose equivalent to moxifloxacin 800 mg/day would be. Here, we performed a PK/PD study in the hollow fiber system model of tuberculosis (HFS-TB). We utilized the results and population PK parameters in Monte Carlo experiments (MCEs) to evaluate levofloxacin doses for patients with tuberculosis and identify the minimum inhibitory concentration (MIC) above which levofloxacin ceases to effectively kill *Mtb*. Next, we utilized machine learning (ML) methods to identify the magnitude of the PK/PD index associated with clinical outcomes in Tanzanian patients who were treated for pulmonary MDR tuberculosis [12]. We also compared the MCE dose-vs-outcome results in Vietnamese patients treated for tuberculous meningitis [13].

METHODS

Materials, Organisms, and Reagents

Mtb H37Ra (American Type Culture Collection 25177) was used for HFS-TB experiments, as described elsewhere [14]. Levofloxacin was purchased from the Baylor University Medical Center Pharmacy (Dallas, TX) and from Sigma (St. Louis, MO). Moxifloxacin-13Cd3 was purchased from Santa Cruz Biotechnology (Dallas, TX). We utilized the BACTEC MGIT 960 Mycobacterial Growth Indicator Tube system (MGIT; Franklin Lakes, NJ) to determine time-to-positivity (TTP) as a measure of bacterial burden.

MICs and Screening for Extracellular and Intracellular Effect

MICs were identified using the standard macrobroth dilution reference method, the MGIT assay, and Epsilon meter test (E test) on Middlebrook 7H10 agar, as described previously [15, 16]. The concentrations tested in the MGIT assay were 0, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1, and 2 mg/L. The macrobroth dilution assay tested the same concentration series, but up to 16 mg/L. Next, we examined the microbial kill of levofloxacin co-incubated for 7 days with extracellular and intracellular *Mtb* in test tubes and 24-well plates, as described previously [15, 16].

Hollow Fiber System Model of Tuberculosis

HFS-TB construction, technical specifications, and inoculation with log-phase growth *Mtb* have been described in detail elsewhere as well as in this supplement [1, 14, 17–20]. Seven HFS-TB units were treated for 28 days via computerized syringe pumps that achieved the following levofloxacin 0–24 area under the concentration-time curve (AUC_{0-24}): 0, 1.0, 1.25, 2.0, 10.5, 21, and 42 mg*h/L. We confirmed these exposures by sampling the central compartment at 0, 1, 4, 8, 12, 22, and 23.5 hours after the last dose. Since the levofloxacin half-life in epithelial lining fluid and alveolar macrophages is 8.1–14.3 hours [21], we mimicked the midway half-life of 11 hours. Levofloxacin concentrations were measured using the assay described in detail in the [Supplementary Methods](#). The peripheral compartments were sampled on days 0, 3, 7, 10, 14, 21, and 28 for TTP and colony-forming unit (CFU) counts, as described previously [16, 22]. The levofloxacin-resistant isolates, which were captured by culturing on agar supplemented with 8 times the levofloxacin MIC, underwent whole-genome sequencing (WGS); identified mutations were confirmed by Sanger sequencing, as described elsewhere [16, 23].

PK modeling was performed in ADAPT 5, and both AUC_{0-24} and AUC_{0-24}/MIC were calculated as described elsewhere [1, 16, 24]. The relationship between AUC_{0-24}/MIC and total bacterial burden was examined using the inhibitory sigmoid maximal kill (E_{max}) model, and that for ADR using the quadratic function [1, 14–20, 22–25].

Monte Carlo Experiments Using HFS-TB Data

The EC_{80} (exposure associated with 80% of maximal kill) and the AUC_{0-24}/MIC associated with ADR suppression, whichever was the higher value, was defined as the target exposure [20]. The PK parameter estimates and variability shown in [Table 1](#), based on prior work by others, were entered in subroutine PRIOR of ADAPT 5 [6, 26]. We used a levofloxacin AUC cavity penetration ratio of 1.33 for pulmonary tuberculosis and a cerebrospinal fluid (CSF)-to-serum penetration ratio of 0.7 for tuberculous meningitis, based on the work of others [7, 27, 28]. The following levofloxacin doses were examined in each 10000 subject MCE for pulmonary disease and tuberculous meningitis: 750, 1000, 1250, and 1500 mg. Target attainment probability

Table 1. Levofloxacin Pharmacokinetic Parameters and Variances

Pharmacokinetic Parameters	Domain of Input (Mean ± SD) [6, 29]	10 000 Simulated Subjects (Mean ± SD)	US Food and Drug Administration Package Insert (Mean ± SD)
Clearance, L/h	7.63 ± 3.55	7.61 ± 1.88	8.58 ± 1.74
Volume, L	81.21 ± 41.66	81.33 ± 6.50	100 ± 16
Absorption constant, per hour	5.96 ± 2.38	5.95 ± 1.54	...
0–24 hour area under the concentration-time curve (mg*h/L) for 750 mg	...	87.13 ± 15.6	90.7 ± 17.6
Peak (mg/L) for 750 mg	...	8.56 ± 2.55	8.6 ± 1.9

Abbreviation: SD, standard deviation.

(TAP) was calculated at each MIC, ranging from 0.0625 mg/L to 16 mg/L, based on the MIC distribution of 243 *Mtb* isolates by Rodriguez [29]. Since fluoroquinolone MIC susceptibility breakpoint and distribution in *Mtb* isolates are dependent on the media used and the MGIT is commonly used for MIC distribution, we performed a sensitivity analysis using the MGIT-derived MIC distribution in 30 wild-type isolates reported by Kambli et al [30]. The overall cumulative fraction of response (CFR) was then calculated for each dose weighted over this MIC distribution, as described elsewhere in this supplement [20].

Analyses of Clinical Data Using Machine-learning and Probit Regression

We used random forests and classification and regression tree (CART) analyses, which are described in full in the Supplemental Methods and elsewhere, to identify levofloxacin PK/PD parameters predictive of outcomes in MDR tuberculosis patients [20]. Outcomes examined were sputum conversion, ADR, and death in 41 Tanzanian patients with MDR tuberculosis treated with 750 mg levofloxacin, in combination with kanamycin, cycloserine, ethionamide, and pyrazinamide, who had levofloxacin concentrations measured 2 hours after dose 4 weeks after starting therapy [12]. First, we performed compartmental PK modeling, as previously described, and compared results with prior studies that used intensive sampling (Table 1) [31, 32]. We then implemented random forests and CART to identify and rank the most important predictors of sputum conversion [32, 33]. Next, we determined the probability of favorable outcomes, given a specific levofloxacin dose, in the Tanzanian patients with pulmonary MDR tuberculosis, as well as in Vietnamese patients with tuberculous meningitis, using separate probit regression models [12, 13].

RESULTS

Activity of Static Levofloxacin Concentrations

The levofloxacin MICs for *Mtb* H37Ra were 0.0625 and 0.125 mg/L in broth macrodilution on 2 separate occasions, 0.125 mg/L in the MGIT on 2 separate occasions, and 0.16 mg/L on E-test. We adopted an MIC of 0.125 mg/L. The inhibitory sigmoid E_{max} relationship between static concentration and extracellular log-phase growth *Mtb* burden in test tubes is shown in Figure 1A. The EC_{50} was 0.25 (95% confidence interval [CI], 0.22–0.28) times MIC and E_{max} was 6.62 (95% CI, 6.09–7.16) \log_{10} CFU/mL. Figure 1B shows results for intracellular *Mtb* in 24-well plates. The EC_{50} was 0.44 (95% CI, 0.43–0.51) times MIC and E_{max} was 4.71 (95% CI, 4.38–5.08) \log_{10} CFU/mL.

Exposure-Response in the HFS-TB

The PK model predicted vs observed concentrations in HFS-TB, shown in Figure 2A, had a slope of 0.995 ± 0.003 ($r^2 > 0.999$), indicating minimal bias. The concentration-time profiles achieved are shown in Figure 2B. The levofloxacin clearance was 0.040 ± 0.002 L/h, indicating a 5% (minimal) technical

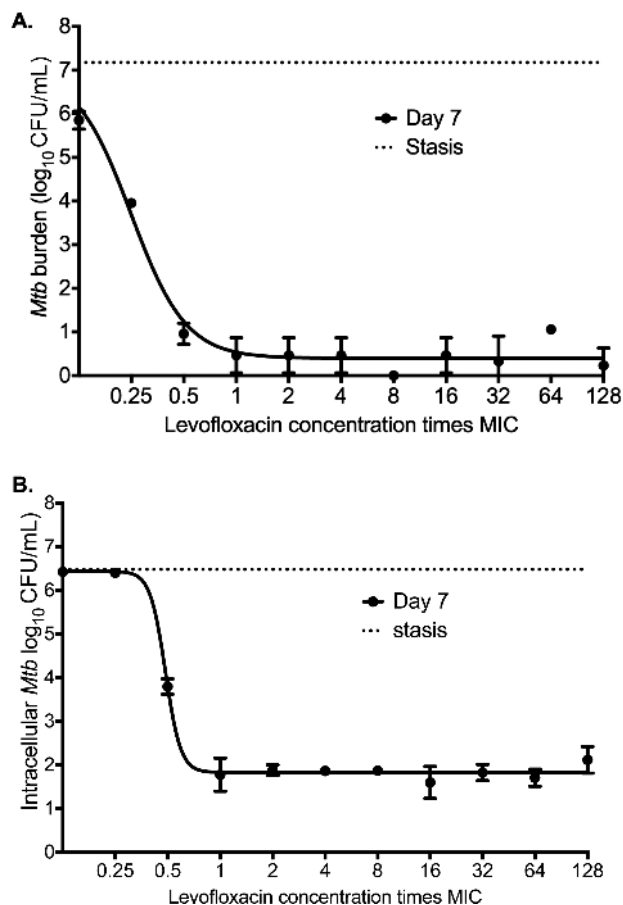


Figure 1. Levofloxacin concentration-effect against *Mycobacterium tuberculosis* (*Mtb*). *Mtb* cultures were co-incubated for 7 days, with 3 replicates for each drug concentration. Shown are mean values; error bars are for standard deviation. The stasis line indicates bacterial burden at the start of treatment. Each drug concentration is expressed as a multiple of minimum inhibitory concentration (MIC). (A) Levofloxacin effect against extracellular log-phase growth *Mtb* demonstrated a steep decline in bacterial burden below the MIC, with no increased killing at the concentration of 1 times MIC or above. Maximal kill (E_{max}) was $>7 \log_{10}$ colony-forming units (CFU)/mL below stasis. (B) We infected activated THP-1 cells and co-incubated them with levofloxacin for 7 days. The concentration-effect pattern was similar to that seen with extracellular *Mtb* co-incubation, with the difference that microbial kill below stasis was $2 \log_{10}$ CFU/mL less than against extracellular bacilli, which means less efficacy against intracellular bacilli. A lower E_{max} of intracellular vs extracellular *Mtb* has also been seen with other quinolones such as moxifloxacin. Abbreviations: MIC, minimum inhibitory concentration; *Mtb*, *Mycobacterium tuberculosis*.

variation between the HFS-TB units; the half-life was 10.84 hours, which is 98.55% accurate for the intended 11 hours. The time-kill curves, based on CFU/mL readout, are shown in Figure 2C, which demonstrates 2 clusters. Those with an $AUC_{0-24}/MIC < 20$ had effect similar to the nontreated controls, while higher exposures demonstrated considerable microbial kill. Figure 2D shows the same pattern when TTP was used as the bacterial burden readout. Inhibitory sigmoid E_{max} modeling of AUC_{0-24}/MIC vs \log_{10} CFU/mL for each day of therapy is shown in Figure 2E. Supplementary Table 1 demonstrates a remarkably consistent EC_{50} and Hill slope on all sampling days. The

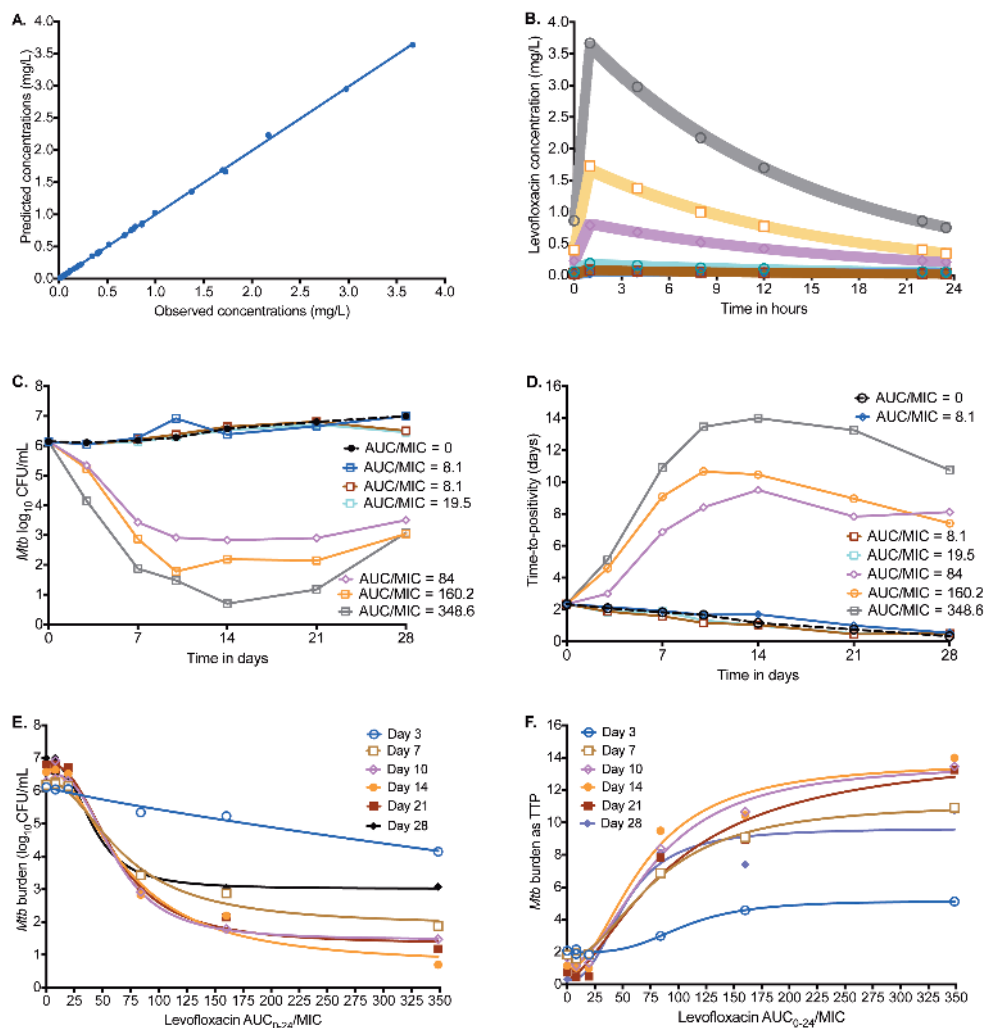


Figure 2. Levofloxacin pharmacokinetics (PKs) and time-kill curves in the hollow fiber system model of tuberculosis (HFS-TB). (A) Model-predicted vs observed levofloxacin concentrations in the hollow fiber system. (B) Levofloxacin concentrations achieved in each HFS-TB at each time point were used for PK modeling. The shaded area is the 95% confidence interval for the PK model-derived concentrations for each dose; the observed concentrations show that the model described the data well. (C) Time change in colony-forming units per milliliter (CFU/mL) with duration of therapy shows that the exposures could be separated into 2 groups. The highest exposure achieved near sterilization on day 14, followed by rebound on day 21. (D) Time-to-positivity (TTP) shows the same pattern as with CFU/mL. (E) The inhibitory sigmoid maximal kill (E_{max}) model curves are shown based on the CFU/mL readout. (F) Inhibitory sigmoid E_{max} modeling using TTP as a measure of bacterial burden demonstrated a higher exposure associated with 50% of maximal kill than was observed for CFU/mL. Abbreviations: AUC_{0-24} , 0–24 hour area under the concentration-time curve; MIC, minimum inhibitory concentration; *Mtb*, *Mycobacterium tuberculosis*.

TTP-based inhibitory sigmoid E_{max} fits are shown in Figure 2F and Supplementary Table 1. We calculated the EC_{80} averaged across all sampling days, which was an AUC_{0-24}/MIC of 146.40 (95% CI, 112.1–180.8).

Evolution of Resistance in the HFS-TB

The evolution of the levofloxacin-resistant *Mtb* subpopulation is shown in Figure 3A; ADR arose on day 21. The relationship between AUC_{0-24}/MIC and the levofloxacin-resistant subpopulation is shown in Figure 3B. The figure shows that the EC_{80} AUC_{0-24}/MIC of 146 also amplified the proportion of levofloxacin-resistant *Mtb* to near maximal,

similar to what was noted with moxifloxacin and ciprofloxacin [1, 5]. Suppression of ADR was at an AUC_{0-24}/MIC of 360. Levofloxacin-resistant isolates from the HFS-TB underwent WGS that identified the following *gyrA* mutations: Asp94Gly, Asp94Asn, and Asp94Tyr. The results were confirmed by Sanger sequencing, shown in Figure 3C. Indeed, results show that all mutations were in the quinolone resistance determining region [34].

MCE Dose Selection for Pulmonary and Meningeal Tuberculosis

The TAPs of different doses in pulmonary tuberculosis for the EC_{80} target exposure are shown in Figure 4A at each MIC. The CFR,

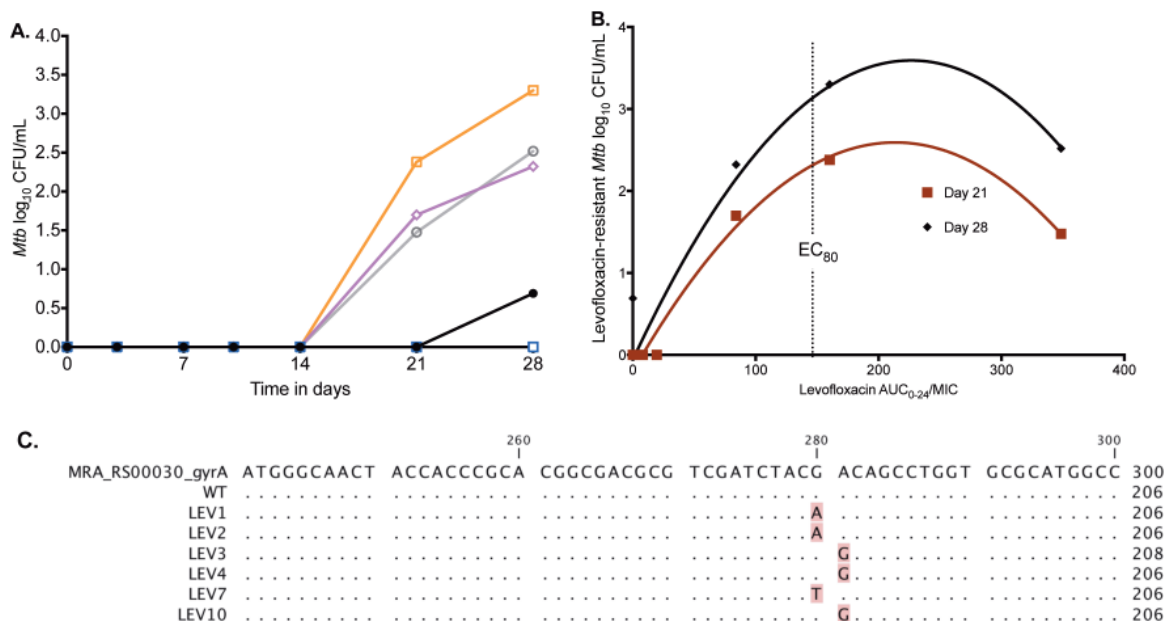


Figure 3. Acquired resistance in the hollow fiber system and whole genome sequencing. (A) The acquired levofloxacin-resistant bacterial burden (log₁₀ colony-forming units per milliliter [CFU/mL]) arose in 3 of the levofloxacin-treated systems on day 21, under the 3 highest 0–24 hour area under the concentration-time curve/minimum inhibitory concentration (AUC₀₋₂₄/MIC) exposures of 84 (magenta diamonds), 160.2 (orange squares), and 348.6 (gray open circles), which were higher than nontreated controls (black solid circles), as shown. On day 28, the proportion of the levofloxacin-resistant subpopulation to the total hollow fiber system model of tuberculosis (HFS-TB) CFU/mL was 6.56% in the HFS-TB exposed to an AUC₀₋₂₄/MIC = 84, 100% for AUC₀₋₂₄/MIC = 160.2, and 27.5% for AUC₀₋₂₄/MIC = 348.6. (B) Based on the size of the levofloxacin-resistant subpopulation, it can be seen that the exposure associated with 80% of maximal kill was associated with near maximal resistance amplification. The suppression of resistance can be read off the graph as an AUC₀₋₂₄/MIC = 360. (C) The Sanger sequence alignment compares wild-type to levofloxacin-resistant strains from the HFS-TB and shows nucleotide changes at positions 280 and 281. Abbreviations: AUC₀₋₂₄, 0–24 hour area under the concentration-time curve; EC₈₀, exposure associated with 80% of maximal kill; MIC, minimum inhibitory concentration; *Mtb*, *Mycobacterium tuberculosis*.

which is the proportion of patients with pulmonary tuberculosis who achieved target exposures with each dose, were as shown in Figure 4B. However, the EC₈₀ target maximally amplifies ADR. Therefore, we performed MCEs using the target exposure associated with ADR suppression, with results shown in Figure 4C and 4D, which revealed an optimal dose of 1500 mg/day, based on CFR. At that dose, the proposed susceptibility breakpoint was 0.5 mg/L. Next, we performed a sensitivity analysis utilizing MGIT-derived levofloxacin MIC distribution in which 83.33% of *Mtb* isolates had an MIC ≤ 0.38 mg/L and 16.77% had an MIC of 0.75 mg/L [30]. The 1500-mg/day TAP for resistance suppression was 99.29% at an MIC of 0.38 mg/L and 15.67% at an MIC of 0.75 mg/L; thus, the proposed susceptibility breakpoint would remain the same at 0.5 mg/L even in MGIT assays.

In tuberculous meningitis, the performance of doses in achieving the EC₈₀ target were as shown in Figure 4E. The CFRs for each dose are shown in Figure 4F. A dose of 1250 mg/day was required to achieve the EC₈₀ in >90% of patients with tuberculous meningitis. For completeness, we also examined the higher target for suppression of ADR. The results shown in Figure 4G and 4H indicate that even at a dose of 3000 mg/day (ie, 4 times the current standard dose), only 78% of patients would achieve that target. However, since tuberculous meningitis is a paucibacillary disease with low risks of ADR, the EC₈₀ target will likely be clinically sufficient.

Relationships Among Exposure, Concentration, Dose, and Outcome in Patients

Clinical details of the 41 Tanzanian patients with pulmonary MDR tuberculosis have been published [12]. MICs were performed for ofloxacin in 31 of the 41 patients, but not for levofloxacin, using Sensititre; the MIC distribution is shown in Figure 5A. The mean ± standard deviation PK model-derived levofloxacin AUC₀₋₂₄ was 83.02 ± 65.00 mg*h/L in the Tanzanian patients, which is virtually the same as those identified with more intensive PK sampling in Table 1 and elsewhere. The following unfavorable outcomes were ascertained: sputum conversion status unknown 2 (5%), defaulted 6 (15%), death 6 (15%), and development of ADR 1 (2%). There was no significant difference in the distribution of the median levofloxacin peak concentration, AUC₀₋₂₄, or AUC₀₋₂₄/MIC by treatment outcome based on the Kruskal-Wallis test (Figure 5B). However, Figure 5C shows the ranking of important variables from the 2 ML algorithms: comparing microbiologic cure vs failure and favorable vs unfavorable outcomes. For microbiologic cure vs failure, the levofloxacin AUC₀₋₂₄/MIC ratio was the most important predictor at 100% importance, followed by levofloxacin 2-hour peak concentration at 86% and body mass index (BMI) at 85%. For favorable vs unfavorable outcomes in the entire dataset, peak concentration was the primary node (100%), followed by weight at 93%,

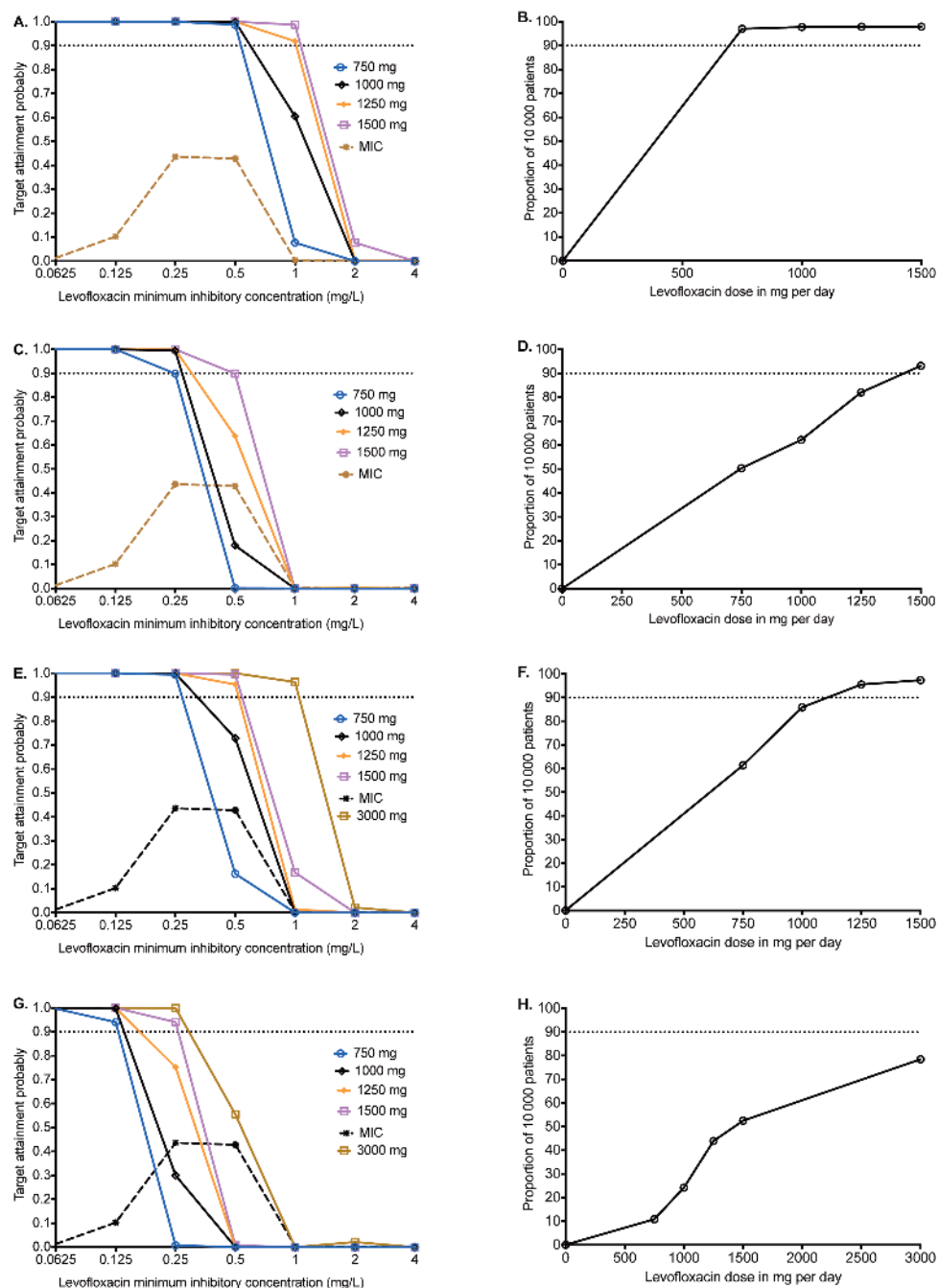


Figure 4. Monte Carlo experiments of different levofloxacin doses for pulmonary and meningeal tuberculosis. (A) Target attainment probability (TAP) for the exposure associated with 80% of maximal kill (EC_{80}) exposure in pulmonary cavities. At a dose of 1000 mg, TAP falls below 90% above a minimum inhibitory concentration (MIC) of 0.5 mg/L. (B) Proportion of 10 000 patients treated with different doses who achieved the EC_{80} in pulmonary cavities reveals an optimal dose of 1250 mg. (C) TAP of different doses for achieving 0–24 hour area under the concentration-time curve (AUC_{0-24})/MIC = 360 target exposure associated with resistance suppression in pulmonary cavities. At a dose of 1500 mg, the TAP falls below 90% above an MIC of 0.5 mg/L. (D) The levofloxacin dose that would achieve the exposure associated with resistance suppression in >90% of patients with pulmonary cavities was 1500 mg per day. (E and F) In tuberculous meningitis, the TAP for the EC_{80} target fell below 90% above an MIC of 0.5 mg/L for all doses, except the 3000 mg a day. (G and H) TAP of different doses in achieving exposure associated with resistance suppression in the cerebrospinal fluid precipitously drops above an MIC of 0.25 mg/L, demonstrating the impact of drug penetration on site of effect. Abbreviation: MIC, minimum inhibitory concentration.

BMI at 85%, and AUC_{0-24} /MIC ratio at 81%. Figure 5D shows a representative CART tree, which revealed that the AUC_{0-24} /MIC threshold predictive of microbiologic failure was 160. However, the MIC used was for ofloxacin, which is often 1 tube dilution

higher than levofloxacin, so that the threshold value would translate to a putative AUC_{0-24} /MIC of 320.

The levofloxacin serum AUC distribution in Tanzanian patients with pulmonary MDR tuberculosis is shown in

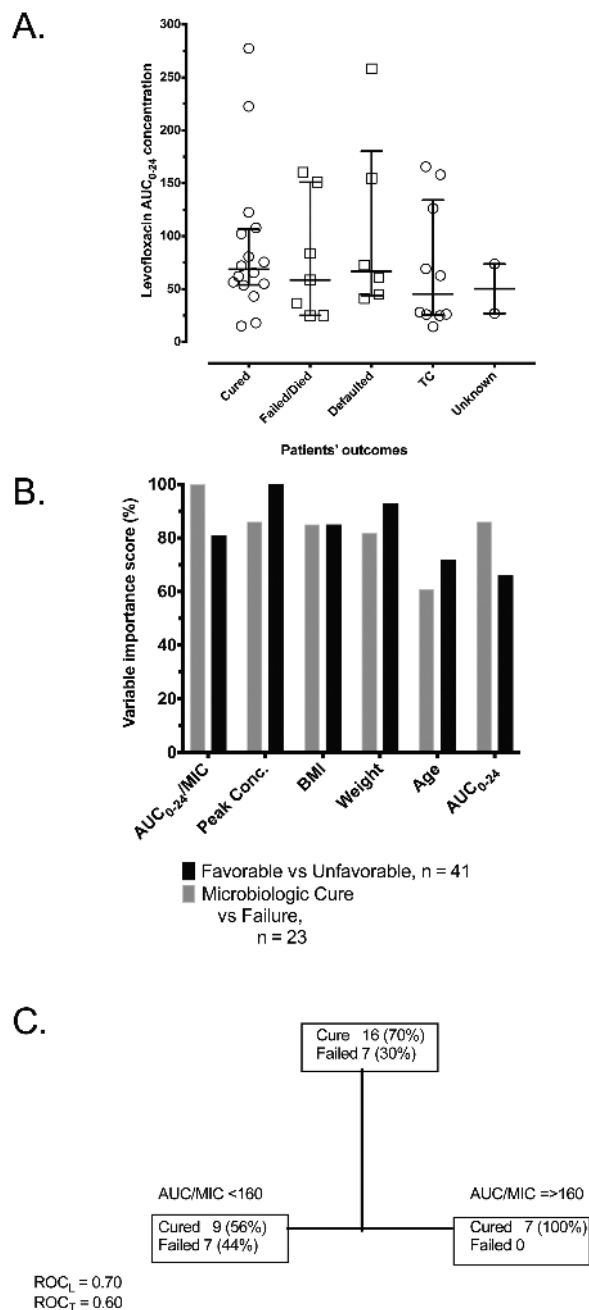


Figure 5. Artificial intelligence–derived predictors of outcomes in patients. (A) There were no statistically significant differences in the distribution of the median levofloxacin 0–24 hour area under the concentration–time curve (AUC₀₋₂₄) by treatment outcome using standard statistical inferences. (B) Ranking of important variables from 2 random forest models based on 2 definitions of outcome revealed the effect of drug concentration on outcomes. (C) Representative classification and regression tree used to generate the random forest model output shows a threshold AUC₀₋₂₄/minimum inhibitory concentration of 160. Once the threshold exposure has been identified using machine learning, it can be used in standard statistics to show an odds of failure below this threshold, which in this case shows a higher odds ratio of failure with a 95% confidence interval of 1.15 to infinity (given the 0% failure rate above the threshold value). Abbreviations: AUC₀₋₂₄, 0–24 hour area under the concentration–time curve; BMI, body mass index; MIC, minimum inhibitory concentration; ROC_L, receiver operating characteristic Learn set; ROC_T, receiver operating characteristic Test set; TC, treatment complete.

Figure 6A. We utilized a probit regression model of levofloxacin dose (milligrams per kilogram) vs outcome in the same patients, with results shown in **Figure 6B**. Interestingly, the maximum probability of favorable outcomes was 60% in pulmonary disease, which is suboptimal. The dose on the flat portion of the probit curve was about 25 mg/kg or a total 1250 mg, given the weight of patients in Tanzania. Next, we analyzed data from Vietnamese patients with tuberculous meningitis on treatment with a levofloxacin-based regimen whose clinical characteristics have been published elsewhere [13]. The levofloxacin serum and CSF AUCs in these patients were as shown in **Figure 6C**. The levofloxacin penetration ratio into CSF observed was 0.71, which is close to the 0.70 used in our MCEs. Probit analyses revealed the relationship between dose and probability of disability-free survival shown in **Figure 6D**. The disability-free survival rates were also poor; however, they were in line with the current outcomes with first-line anti-tuberculosis therapy.

DISCUSSION

Aubry et al have shown that the ability of a quinolone to inhibit *Mtb* gyrase DNA supercoiling activity by 50% (IC₅₀) was 3 mg/L for gatifloxacin, 4.5 mg/L for moxifloxacin, and 5 mg/L for levofloxacin. The concentration producing 50% of the maximum DNA cleavage (CC₅₀) was 4 mg/L for gatifloxacin, 4 mg/L for moxifloxacin, but 12 mg/L for levofloxacin [35]. In **Table 2** we compare levofloxacin PK/PD parameters, MCE-derived dose, to those of moxifloxacin and gatifloxacin, derived from our separate but similarly designed studies [1, 14]. Based on speed of kill and time to ADR, the ranking would be gatifloxacin > levofloxacin > moxifloxacin. ADR arose faster with moxifloxacin than with levofloxacin, while gatifloxacin was the most effective at suppressing ADR. Indeed, the gatifloxacin-resistant subpopulation was only 5% of the total on day 28 in the systems that amplified resistance the most, as opposed to 100% for both moxifloxacin and levofloxacin (**Table 2**). Thus, based on PK/PD considerations, levofloxacin can indeed replace moxifloxacin in pulmonary tuberculosis, while gatifloxacin could be the best drug to start off with. However, in elderly patients gatifloxacin increased the rate of dysglycemia 4.3-fold compared to other antibiotics, while levofloxacin increased it 1.5-fold, and moxifloxacin did not [36]. Dysglycemia has been encountered in 2%–9% of MDR tuberculosis patients on gatifloxacin, and the incidence of this adverse event could be higher at the advocated dose of 800 mg [14, 37].

We identified the levofloxacin dose that gives equivalent effect to moxifloxacin, 800 mg/day, using HFS-TB in tandem with MCE, AI-based analyses, and probit models of clinical data. This is consistent with prior findings that show that the EC₈₀ and exposure suppressing ADR in the HFS-TB correspond closely to the exposures associated with optimal response in patients, as do susceptibility breakpoints based on these exposures [20,

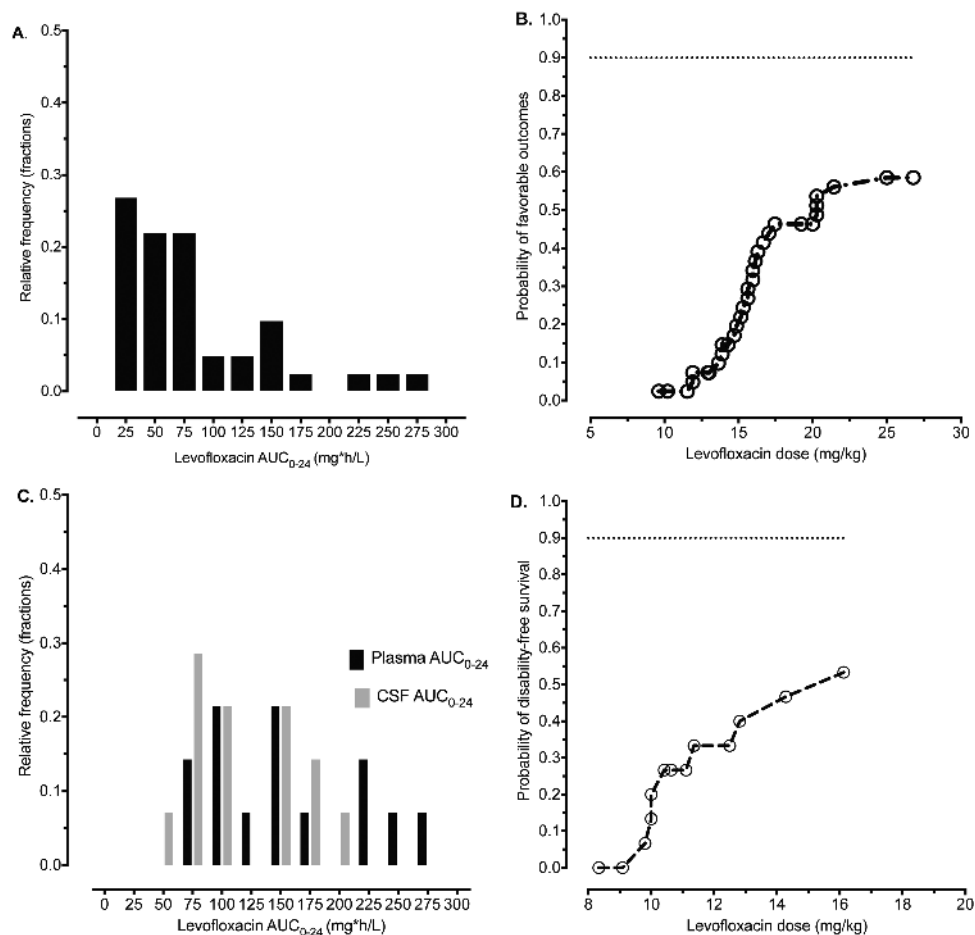


Figure 6. Probit regression in patients treated with levofloxacin-based regimens. (A) Area under the concentration-time curves (AUCs) achieved in the blood of patients with pulmonary tuberculosis in Tanzania were in the range identified with intensive pharmacokinetic sampling schemes. (B) The probit model of levofloxacin dose (milligrams per kilogram) administered to patients vs probability of good outcomes in pulmonary multidrug-resistant (MDR) tuberculosis in Tanzanian patients. The curve flattened out at 60% probability of good outcome for pulmonary MDR tuberculosis, which was a suboptimal response but in the range seen for MDR tuberculosis outcomes. (C) Distributions of AUCs in the blood and cerebrospinal fluid of Vietnamese patients with meningitis shows a distribution similar to that of Tanzanian patients for the blood. (D) Probit model in patients with drug-susceptible tuberculosis who also received rifampin for tuberculous meningitis showed a curve that was still on a steep rise and had not reached maximum, suggesting room for improvement with increased doses. Abbreviations: AUC₀₋₂₄, 0–24 hour area under the concentration-time curve; CSF, cerebrospinal fluid.

25, 38–42]. The equivalent dose should be able to minimize ADR, which is encountered for quinolones and aminoglycosides in 9%–14% of patients being treated for MDR tuberculosis at standard doses [43, 44]. We have utilized amplicon-based

next-generation and confirmatory Sanger sequencing in 158 MDR tuberculosis isolates from 4 countries and determined that 6.3% of 1811 loci examined exhibited at least some mutant population ($\geq 1\%$ of reads) in at least 1 isolate, with the largest

Table 2. Effect of Levofloxacin, Moxifloxacin, and Gatifloxacin in Hollow Fiber System Model of Tuberculosis and Monte Carlo Experiments from Different Studies With Similar Experimental Design

Variables	Moxifloxacin	Gatifloxacin	Levofloxacin
Maximal kill (\log_{10} CFU/mL/day)	0.57	0.68	0.61
Time to 1% acquired drug resistance (days)	10	21	21
% population resistant at end of experiment	100	5	100
Area under the concentration-time curve/minimum inhibitory concentration resistance suppression	53	184	360
Exposure associated with 80% of maximal kill	56	184	146
Monte Carlo experiment-derived dose (mg/day): resistance suppression			
Pulmonary tuberculosis	800	800	1500
Meningeal tuberculosis	-	1200	>3000

being *gyrA*. Thus, subpopulations of fluoroquinolone resistance are often encountered in MDR tuberculosis [45]. Mutations were within the same resistance determining codon (*gyrA* 94) identified in the HFS-TB with suboptimal levofloxacin exposures, consistent with our antibiotic resistance arrow-of-time model [46, 47]. Since resistance to quinolones reduces cure rates considerably, attaining ADR suppression targets is crucial [48, 49]. For pulmonary tuberculosis, the levofloxacin dose that is able to achieve suppression of ADR was 1500 mg/day or around 20 mg/kg/day, as shown in Figure 4. Thus, the inferiority of levofloxacin to moxifloxacin when added to amikacin, ethionamide, and pyrazinamide, as demonstrated in mice, could be due to a differential dose issue. At high enough doses, levofloxacin could be equivalent to moxifloxacin [10, 11]. In the case of tuberculous meningitis, the dose that would suppress ADR was higher than what is currently known to be tolerated by patients. However, given the lower bacterial burden in CSF compared to pulmonary cavities, the PK/PD goal of therapy in tuberculous meningitis may not be resistance suppression but rather the EC_{80} . In that case, the levofloxacin dose of 1500 mg/day could still be used for tuberculous meningitis.

We identified the MIC above which levofloxacin therapy is expected to fail in MCE. We identified this as an MIC of 0.5 mg/L for both MGIT and agar dilution using Middlebrook 7H11. At 1 dilution higher (1 mg/L), one would need to double the dose to achieve the same TAP. Since, unlike gatifloxacin, exposures associated with the EC_{50} and EC_{80} actually amplify ADR, we did not examine the effect of higher doses, such as 3000 mg/day, at higher MICs. The doses would be several times greater than the standard, and the safety is unclear, as is the effect given resistance amplification. Thus, we did not propose a susceptible dose-dependent zone for levofloxacin, as was the case with gatifloxacin and other drugs [14, 50].

Our study has a few limitations. First, the levofloxacin concentrations we utilized as an external check on PK modeling were from a retrospective study. Moreover, except for levofloxacin, concentrations of other drugs that comprised the MDR tuberculosis regimen were not measured. However, the observations of low levofloxacin concentrations associated with unfavorable outcomes in patients are similar to those derived from the modeling exercise and thus likely accurate. Second, the clinical dataset utilized for external validation of modeling for tuberculous meningitis was relatively small. However, the high rate of failure and use of the index of disability-free survival made it possible to model, despite the small sample size. On the other hand, disability-free survival cannot be solely attributable to tuberculous meningitis. The effect of other factors such as penetration of companion antituberculosis compounds into the CSF will be important to consider. Yet, both model-predicted and clinically observed unfavorable outcomes were associated with low CSF exposures and identified similar doses for success. Therefore, despite these limitations, these findings are likely to be true.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. T. G. and D. D. designed the study; T. G., D. D., and P. B. performed the hollow fiber studies; D. D. wrote the first draft of the manuscript; T. K. performed DNA extraction; T. G. and S. S. performed the WGS analysis; T. G. performed PK/PD modeling and MCE; J. P. and T. G. performed Probit and CART analysis of clinical data; S. G. M., C. P., S. B., P. A., G. T., and S. K. H. performed the clinical studies and/or analyses; D. D., J. P., S. S., and T. G. wrote the manuscript; all authors edited and contributed to the final version of the manuscript.

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