*J Antimicrob Chemother* 2015; **70**: 3273–3282
doi:10.1093/jac/dkv247 Advance Access publication 25 August 2015

Journal of Antimicrobial Chemotherapy

# Quantifying the impact of drug combination regimens on TB treatment efficacy and multidrug resistance probability

**Yi-Jun Lin and Chung-Min Liao***

*Department of Bioenvironmental Systems Engineering, National Taiwan University, Taipei, Taiwan 10617, Republic of China*

*Corresponding author. Tel: +886223634512; E-mail: cmliao@ntu.edu.tw

*Received 2 February 2015; returned 8 April 2015; revised 22 June 2015; accepted 21 July 2015*

**Objectives:** TB patients' non-adherence to the multidrug treatment regimen is thought to be the main cause of the emergence of drug resistance. The purpose of this study was to quantify the impacts of two-drug combination regimens and non-adherence to these regimens on treatment efficacy and drug resistance probability.

**Methods:** A drug treatment modelling strategy was developed by incorporating a pharmacokinetic/pharmacodynamic model into a bacterial population dynamic model to explore the dynamics of TB bacilli and evolution of resistance during multidrug combination therapy, with an emphasis on non-adherence. A Hill-equation-based pharmacodynamic model was used to assess the bactericidal efficacy of single drugs and to estimate drug interactions.

**Results:** Non-adherence to the treatment regimen increased treatment duration by nearly 1.6- and 3.4-fold relative to compliance with treatment. Symptom-based intermittent treatment, a form of non-adherence, might lead to treatment failure and accelerated growth and evolution of resistant mutants, resulting in a dramatically higher probability of $4.17\times10^{-3}$ (95% CI $2.10\times10^{-4}$–$1.28\times10^{-2}$) for the emergence of MDR TB. Overall, determination of the optimal treatment regimen depended on the different types of medication adherence.

**Conclusions:** Our model not only predicts evolutionary dynamics, but also quantifies treatment efficacy. More broadly, our model provides a quantitative framework for improving treatment protocols and establishing an emergence threshold of resistance that can be used to prevent drug resistance.

## Introduction

*Mycobacterium tuberculosis* is one of the world's leading killers, causing 9 million people to develop TB and 1.5 million deaths in 2013.[1] Multidrug regimens are the standard treatment for TB. The current recommended standard treatment for drug-susceptible TB is a first-line drug combination regimen for 6 months.[2] Owing to the complex drug combinations, long-term treatment durations and significant toxicities of anti-TB drugs, many patients fail to adhere to their treatment regimen, allowing naturally occurring resistant mutants to grow and evolve. WHO pointed out that single-drug-resistant TB strains have been observed in every country.[1] A particularly dangerous form of TB, MDR TB, is emerging as a serious threat to TB control, requiring up to 24 months of treatment. MDR TB is caused by bacteria that are resistant to at least rifampicin and isoniazid, the two principal first-line drugs used in combination chemotherapy.

There are four anti-TB injectable drugs, of which amikacin, kanamycin and streptomycin have been used extensively.[3] Amikacin, administered as an intramuscular injection, has been demonstrated to be less ototoxic and less painful than kanamycin and streptomycin.[3,4] The fluoroquinolones are still under development and being evaluated as first-line drugs for drug-susceptible TB; of these drugs, moxifloxacin is the most active[5–7] and may be able to reduce treatment duration.[8] Two clinical studies have reported that although regimens with moxifloxacin can rapidly decrease mycobacterial loads, they do not allow the treatment duration to be shortened to 4 months.[9,10] Bedaquiline, also known as TMC207, is the first novel anti-TB drug to be approved by the FDA in over 40 years. Based on mouse and human data, TMC207 has a long half-life and therefore can reduce the frequency of dosing to 200 mg three times a week.[11–14]

Most cases of TB are caused by drug-susceptible TB strains; however, until now no new TB drugs have been developed or approved for drug-susceptible TB since the discovery of first-line drugs between 1952 (isoniazid) and 1963 (rifampicin). Challenges with the existing standard 6 month treatment regimen remain.[7] Thus, it is imperative that research be performed to shorten the treatment duration and reduce the probability of drug resistance in TB patients. Combining new drugs with existing TB drugs provides the hope that new shorter-duration regimens might be developed that would substantially improve TB control.[7,15]

Mathematical models are helpful in examining and predicting the efficacy of drug treatment by combining *in vivo* data on

time-dependent drug concentrations [pharmacokinetics (PK)] and *in vitro* data on the effect of drugs on bacteria [pharmacodynamics (PD)].[16–19] Several PK/PD approaches have been applied to evaluate the bactericidal efficacy of TB antibiotics.[20–26] Some of these models imply that the heterogeneity of bacteria plays a crucial role in predicting drug effects because different bacteria respond differently to drugs.[20,21,26] Although it is recognized that drug combinations and drug interactions are important in assessing the effect of chemotherapy,[7,26,27] most models have not yet fully considered them. Recently, Ankomah and Levin[26] developed a two-compartment population and evolutionary dynamic model taking into account bacterial heterogeneity and drug interaction to understand how non-adherence affected the probability of drug resistance among different two-drug combination regimens. Their model, however, did not consider the influence of drug-specific PK and mutation rate on bacterial evolutionary dynamics during combination therapy.

In view of the emergence of drug resistance resulting from treatment failure due to non-adherence, we sought to extend the previously published model[26] by incorporating drug-specific PK and mutation rate for the examination of the dynamics of TB bacilli and the evolution of resistance during multidrug treatment. The purpose of this study was to quantify the impacts of two-drug combination regimens and non-adherence on treatment efficacy. The relative efficacy and the probability of resistance to two drugs among different two-drug combinations with and without non-adherence were also assessed.

## Methods

### *Study data*

Rifampicin and isoniazid are the backbone of TB treatment. Amikacin and moxifloxacin are the most effective drugs among the classes of injectable agents and fluoroquinolones, respectively.[3–8] Therefore, we selected rifampicin, isoniazid, amikacin and moxifloxacin together with the new drug TMC207 as the study drugs. A number of investigators indicated that the addition of other drugs (e.g. moxifloxacin) to isoniazid did not enhance bactericidal activity.[28–30] Consequently, the two-drug combination regimens assigned were: (i) rifampicin+isoniazid, (ii) rifampicin+amikacin, (iii) rifampicin+moxifloxacin and (iv) rifampicin+TMC207.

Three valuable datasets of *in vitro* single-drug experiments were obtained from Jayaram *et al.*,[22] Reddy *et al.*[31] and Ankomah and Levin.[26] These data can be applied to estimate parameter values in PD models. Jayaram *et al.*[22] and Reddy *et al.*[31] used drug-susceptible *M. tuberculosis* to test killing effects at different concentrations of isoniazid and TMC207, respectively. Ankomah and Levin[26] conducted a series of experiments by exposing *Mycobacterium marinum* to antibiotics containing rifampicin, amikacin and moxifloxacin to explore the relationships between drug concentrations and bacterial growth/death rates.

Drug interactions of rifampicin+isoniazid[32] and rifampicin+TMC207[31] were quantified by the fractional inhibitory concentration index (FICI) based on *in vitro* two-drug combination experiments with drug-susceptible *M. tuberculosis*. Ankomah and Levin[26] also used *M. marinum* to carry out additional *in vitro* experiments on two-drug combinations to examine drug interactions. Here, drug interaction coefficients ($\alpha$) were estimated from experimentally observed bacterial growth/death rates based on rifampicin+amikacin and rifampicin+moxifloxacin interactions.[26]

### *Two-drug treatment model*

This study incorporated the PK/PD model into the TB bacterial population dynamic model to develop a two-drug treatment modelling strategy

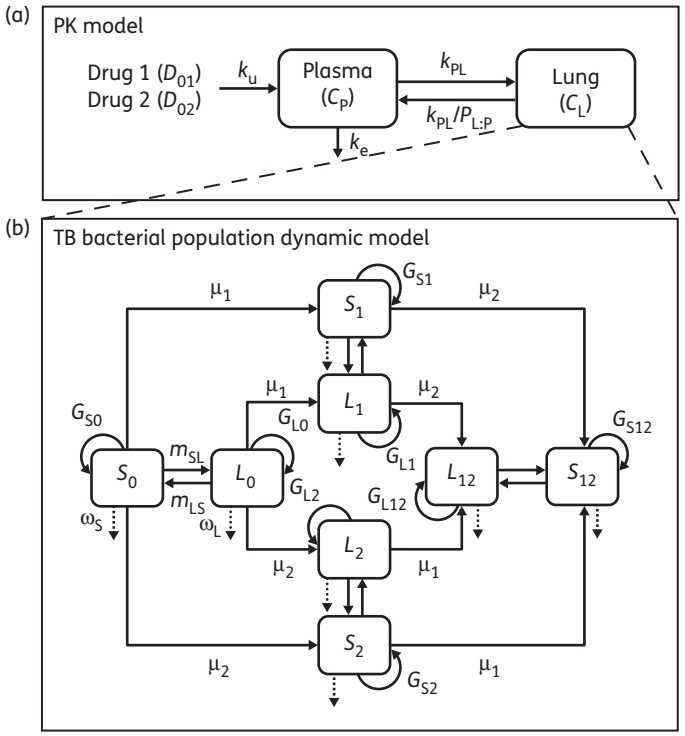


**Figure 1.** Schematic representation of the two-drug treatment model showing (a) two-compartment PK model of plasma and lung and (b) two certain types of TB bacilli in the bacterial population dynamic model. $D_0$, input dose of drug; $C_P$, drug concentrations in plasma; $C_L$, drug concentrations in the lung; $k_u$, drug uptake rate; $k_{PL}$, constant transfer rate between plasma and lungs; $P_{L:P}$, lungs-to-plasma partition coefficient; $k_e$, drug elimination rate; $S$, rapidly dividing bacterial populations; $L$, slowly dividing (latent) bacterial populations; $S_0$ and $L_0$, bacteria susceptible to both drugs; $S_1$ and $L_1$, bacteria resistant to drug 1; $S_2$ and $L_2$, bacteria resistant to drug 2; $S_{12}$ and $L_{12}$, bacteria resistant to both drugs; $G_{S0}$ to $G_{L12}$, net bacterial growth rates in the presence of drugs; $m_{SL}$, migration rate from $S$ to $L$; $m_{LS}$, migration rate from $L$ to $S$; $\omega_S$, removal rate of $S$; $\omega_L$, removal rate of $L$; $\mu_1$ and $\mu_2$, mutation rates for drugs 1 and 2.

(Figure 1) built on previously well-developed drug treatment models.[20–26] A two-compartment PK model (Figure 1a) was used to simulate the dynamics of drug concentrations in the plasma ($C_P$) and lung ($C_L$). Two types of TB bacteria, rapidly dividing ($S$) and slowly dividing ($L$) populations, were included in the TB bacterial population dynamic model (Figure 1b). $L$ represents the case of latent bacteria that can resist killing by antibiotics relatively more than $S$. In other words, $S$ is more susceptible to drugs. We further subdivided $S$ and $L$ into four groups: one that is susceptible to both drugs ($S_0$ and $L_0$) and others that are resistant to drug 1 ($S_1$ and $L_1$), drug 2 ($S_2$ and $L_2$) and both drug 1 and drug 2 ($S_{12}$ and $L_{12}$). The overall dynamics of TB bacilli (Figure 1b) can be briefly described as follows: (i) $S$ and $L$ can transform into each other at migration rates $m_{SL}$ and $m_{LS}$; (ii) single- and two-drug-resistant bacteria occur at mutation rates $\mu_1$ and $\mu_2$; and (iii) bacteria in the $S$ and $L$ states can be removed at constant rates $\omega_S$ and $\omega_L$, respectively, with an assumption of $\omega_S > \omega_L$.

To explore the effects of drugs on population and evolutionary dynamics of TB bacteria, we linked the TB bacterial population dynamic model and the PD models based on the relationship between drug concentration and bacterial growth/death rate. A four-parameter Hill-equation-based single-drug PD model[33] was adopted to optimally fit the published data[22,26,31] to describe the relationship between the concentration of a single drug and the bacterial growth/death rate. We further incorporated

drug interaction into the single-drug PD model to construct a two-drug PD model to describe the killing effect by two-drug combinations. The details of model equations, parameterization, uncertainty and sensitivity analyses are given in Appendix 1 (available as Supplementary data at *JAC* Online).

### Treatment adherence

In addition to compliance with treatment, three broadly inclusive types of non-adherence were taken into consideration based on Lipsitch and Levin,[17,20] as well as Ankomah and Levin,[26] including 20% random non-adherence, periodicity-based intermittent treatment and symptom-based intermittent treatment. Various drug uptake rates corresponding to the four medication scenarios were designed and incorporated into the two-drug treatment model to examine the relative impacts of compliance and non-adherence on treatment efficacy and the likelihood of drug resistance.

A 20% random non-adherence represents a probability of 80% that both drugs will be taken. Periodicity-based intermittent treatment was chosen to alleviate the side effects and the difficulty in accessing drugs because the drugs may be costly and medical resources are limited. Symptom-based intermittent treatment was intended to mimic a situation where patients take their drugs depending on symptom severity. The details of the model simulation scheme are specified in Appendix 2.

### Treatment efficacy assessment

In this study, there were two indicators to evaluate treatment efficacy. One was the time until the total bacterial population size was <1 cfu/mL (i.e. bacterial clearance time). Another was the probability of bacteria resistant to two drugs ($P_R$) during the course of treatment and can be estimated as:[34,35]

$$P_R = 1 - (1 - \mu_i)^{N(t)} \quad (1)$$

where $\mu_i$ is the mutation rate of single drug $i$ (mutations/bacterium/generation) and $N(t)$ is the time-dependent TB bacterial population size based on the simulation results from the two-drug treatment model. Monte Carlo (MC) simulation was used to quantify the uncertainty of $P_R$.

## Results

### PD parameter estimates

Results indicated that the four-parameter Hill-equation-based single-drug PD model provided an excellent fit ($r^2 = 0.94–0.99$; $P < 0.05$) (Table 1) and could appropriately describe the relationship between the concentration of a single drug and TB bacterial growth/death rate (Figure 2a–e). We found that the estimates of maximum growth rate of bacteria in the absence of drugs ($B_{max}$) did not significantly differ among the five drugs, with an average value of 0.04 $h^{-1}$ (Table 1). However, the shape of PD functions could be determined by the minimum growth rate of bacteria in the presence of a single drug ($B_{min}$), MIC and the Hill coefficient ($n$) ranging from −0.175 to −0.023 $h^{-1}$, 0.001 to 1.27 mg/L and 0.863 to 1.24, respectively (Table 1).

We further fitted the two-drug PD model to the experimental data for two-drug combinations to obtain the estimates of α for rifampicin + amikacin ($r^2 = 0.83$; $P < 0.05$) and rifampicin + moxifloxacin ($r^2 = 0.97$; $P < 0.001$) (Figure 2f and g and Table 2). We showed that the combination of rifampicin + amikacin had a synergistic drug–drug interaction (α = 3.602), whereas rifampicin + moxifloxacin had an antagonistic effect with a value of α = −2.181 (Table 2). On the other hand, drug interactions of rifampicin + isoniazid and rifampicin + TMC207 could be estimated based on the published FICI, revealing that rifampicin + isoniazid and rifampicin + TMC207 had no interaction effect ($0.5 < FICI \leq 4.0$) with the corresponding value of α = 0 (Table 2).

### Treatment adherence impacts

Simulation results revealed that from 20% random non-adherence to symptom-based intermittent treatment, the frequency of drug discontinuation increased gradually (Table S1 and Figure S1). Figure 3 shows that in the cases of compliance, 20% random non-adherence and periodicity-based intermittent treatment, bacteria could be cleared by all combination regimens during a 6 month treatment. We found that intermittent drug use could cause a continual rise in bacterial populations (Figure 3 and Figure S2), thereby increasing the treatment time needed (Table 3). For compliance and 20% random non-adherence, rifampicin + amikacin had the shortest clearance times, of 31 and 50 days, respectively, whereas under periodicity-based intermittent treatment the shortest clearance time was 65 days, observed for rifampicin + moxifloxacin (Table 3). Overall, periodicity-based intermittent treatment took approximately 1.6–3.4 times longer to clear bacteria compared with compliance with treatment (Table 3). Furthermore, our results

**Table 1.** Fitted coefficients (mean ± SEM) of Hill-equation-based single-drug PD model for the relationship between concentration of single drug and bacterial growth/death rate

| Drug | $B_{max}$ ($h^{-1}$) | $B_{min}$ ($h^{-1}$) | MIC (mg/L) | $n$ | $r^2$ | $P$ |
|---|---|---|---|---|---|---|
| Rifampicin[a] | 0.0453 ± 0.0018* | −0.125 ± 0.0072* | 1.27 ± 0.22 | 0.925 ± 0.17 | 0.99 | * |
| Amikacin[a] | 0.0457 ± 0.0012** | −0.145 ± 0.0019*** | 0.38 ± 0.029** | 1.23 ± 0.12* | 0.99 | ** |
| Moxifloxacin[a] | 0.0478 ± 0.0015** | −0.166 ± 0.0052** | 0.461 ± 0.055* | 0.863 ± 0.091* | 0.99 | * |
| Isoniazid[b] | 0.040 ± 0.0215* | −0.175 ± 0.009*** | 0.001 ± 0.0007* | 0.886 ± 0.287* | 0.94 | ** |
| TMC207[c] | 0.043 ± 0.0016*** | −0.023 ± 0.011 | 0.39 ± 0.03*** | 1.24 ± 0.52* | 0.99 | *** |

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.
[a]Adopted from Ankomah and Levin.[26]
[b]Estimated based on Jayaram *et al.*[22]
[c]Estimated based on Reddy *et al.*[31]

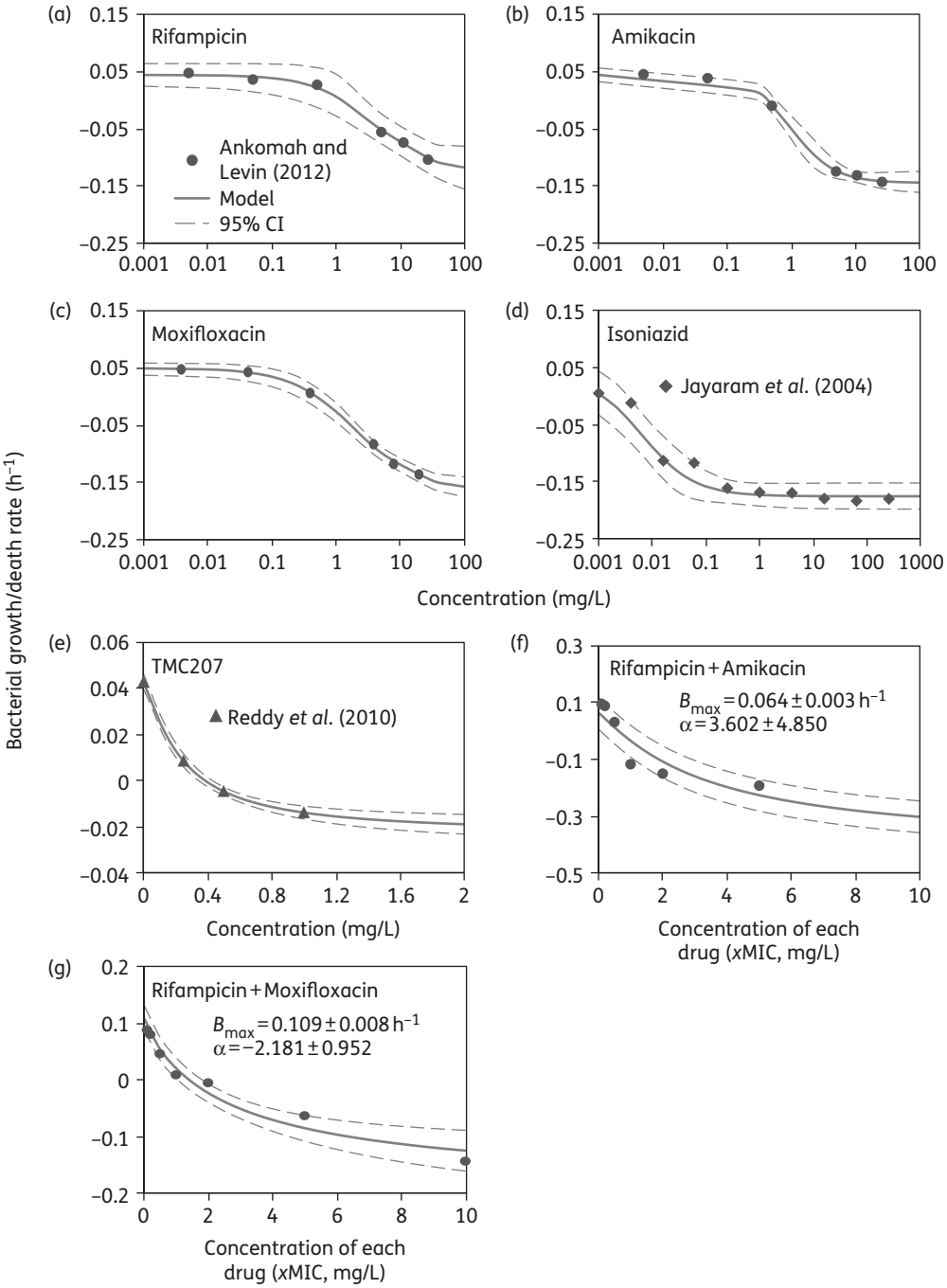


**Figure 2.** Fitting the single-drug PD model to describe the relationship between drug concentrations and bacterial growth/death rates for (a) rifampicin, (b) amikacin, (c) moxifloxacin, (d) isoniazid and (e) TMC207. Estimating the drug interaction coefficient (α) for (f) rifampicin + amikacin and (g) rifampicin + moxifloxacin by fitting the two-drug PD model to the published experimental data on the drug combinations.

also revealed that latent bacterial populations played an important role in clearance (Figure S2).

As in the case of symptom-based intermittent treatment, all 1000 MC simulations showed that treatment failed regardless of which drug combination regimen was used (Figure 4). Figure 4 demonstrates that the population sizes of bacteria resistant to drug 2 ($S_2$ and $L_2$) (i.e. isoniazid-, amikacin-, moxifloxacin- and TMC207-resistant mutants) increased significantly, whereas rifampicin-resistant mutants ($S_1$ and $L_1$) could be killed by drug 2. When these single-resistant populations grew further to a sufficiently high level, resistance to the additional drug (rifampicin) could then rapidly arise. Symptom-based intermittent treatment would lead not only to treatment failure, but also to the emergence of MDR TB (rifampicin + isoniazid) and poly-drug resistances (rifampicin + amikacin/moxifloxacin/TMC207).

### *Probability of resistance to two drugs*

Our results indicated that the probabilities of bacterial resistance to two drugs ($P_R$s) among four drug combination regimens for compliance, 20% random non-adherence and periodicity-based intermittent treatment were $<10^{-6}$, whereas symptom-based intermittent treatment was likely to be the most vulnerable to resistance (Figure 5). The results also showed that rifampicin+TMC207, which required the longest time to clear bacteria, had the highest $P_R$, of $6.25\times10^{-3}$ (95% CI $3.20\times10^{-4}$–$1.96\times10^{-2}$). The $P_R$ estimates for rifampicin+isoniazid and rifampicin+amikacin were $4.17\times10^{-3}$ ($2.10\times10^{-4}$–$1.28\times10^{-2}$) and $5.12\times10^{-3}$ ($2.48\times10^{-4}$–$1.57\times10^{-2}$), respectively, whereas the lowest $P_R$ was founded in rifampicin+moxifloxacin of $1.64\times10^{-3}$ ($7.91\times10^{-5}$–$5.30\times10^{-3}$).

Overall, estimates of $P_R$ suggest that rifampicin+TMC207 with symptom-based intermittent treatment may generate 6 poly-drug resistances in 1000 cases, whereas only 1 poly-drug resistance may occur in 1000 cases for rifampicin+moxifloxacin, with the lowest $P_R$ under the same condition. MDR TB could emerge in as many as 4 in 1000 cases during symptom-based intermittent treatment.

**Table 2.** Drug interactions among various two-drug combinations

| | Interaction[a] | |
|---|---|---|
| Drug combination | FICI | α |
| Rifampicin+isoniazid | 1.01, 0.94[b] | 0 |
| Rifampicin+amikacin | — | 3.602 (−9.864 to 17.069)[c,d] |
| Rifampicin+moxifloxacin | — | −2.181 (−4.628 to 0.625)[c,d] |
| Rifampicin+TMC207 | 2.0[e] | 0 |

α, drug interaction coefficient.
[a]Synergism (FICI ≤ 0.5; α > 0), antagonism (FICI > 4.0; α < 0) and additivity (0.5 < FICI ≤ 4.0; α = 0).
[b]Adopted from Bhusal *et al.*[32] where 1.01 and 0.94 were determined by using susceptible wild-type isolate and H37Rv laboratory strain, respectively.
[c]Estimated by fitting the two-drug PD model to the published two-drug combination experimental data (Figure 2f and g) adopted from Ankomah and Levin.[26]
[d]Median (95% CI).
[e]Adopted from Reddy *et al.*[31]

### *Sensitivity analysis*

Our sensitivity analysis showed that, with compliance and 20% random non-adherence (Figure 6a and b), a significant increase in clearance time was attributable to: (i) weakened bactericidal efficacy by decreasing *n* or α or increasing MIC; and (ii) accelerated growth of bacteria as a result of increasing relative fitness (*RF*). We found that PK parameters became progressively more important from compliance to periodicity-based intermittent treatment (Figure 6). Thereby, increased drug elimination rate ($k_e$) and volume of distribution of plasma ($V_P$) or reduced lungs-to-plasma partition coefficient ($P_{L:P}$) resulted in an increase in clearance time (Figure 6c). In general, clearance time could be shortened if the rifampicin MIC was lower (Figure 6). Results also revealed that treatment failure might result from: (i) mutants with fairly high *RF*; (ii) rifampicin concentration being too low due to

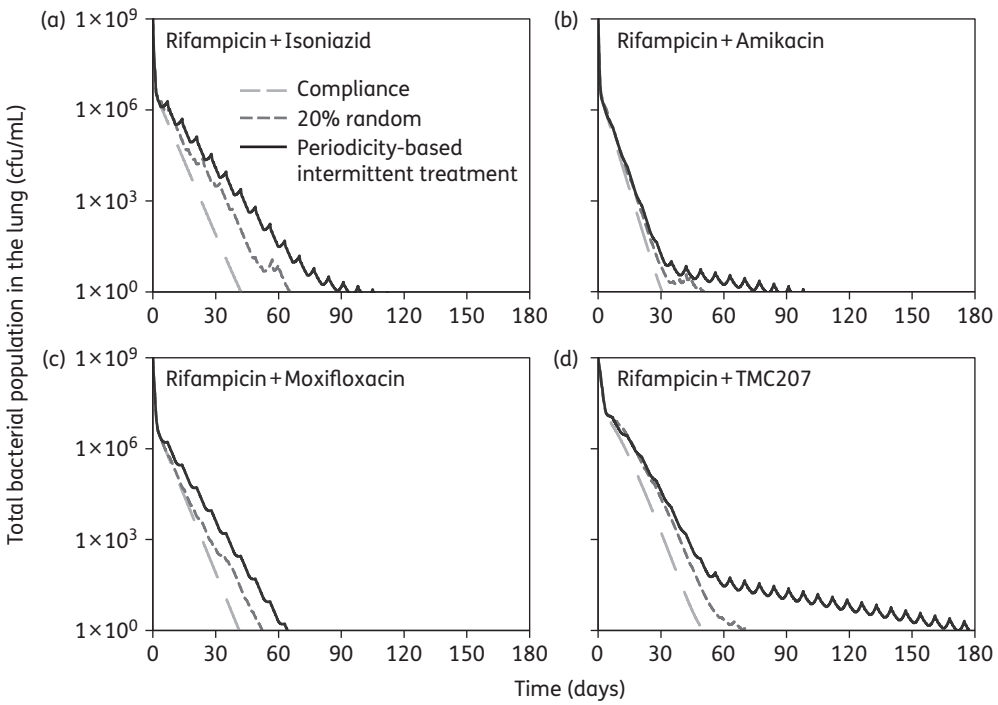


**Figure 3.** Modelling clearance dynamics of total bacterial population in the lung during 6 months of treatment with (a) rifampicin+isoniazid, (b) rifampicin+amikacin, (c) rifampicin+moxifloxacin and (d) rifampicin+TMC207 under compliance, 20% random non-adherence and periodicity-based intermittent treatment.

**Table 3.** Bacterial clearance time (days) during 6 months of treatment

| Scenario | Drug combination | | | |
|---|---|---|---|---|
| | rifampicin + isoniazid | rifampicin + amikacin | rifampicin + moxifloxacin | rifampicin + TMC207 |
| Compliance | 42 | 31 | 41 | 50 |
| 20% random non-adherence | 69 | 50 | 53 | 71 |
| Periodicity-based intermittent treatment | 93 | 80 | 65 | 172 |
| Symptom-based intermittent treatment | F | F | F | F |

F, treatment failure.

decreasing $P_{L:P}$ or increasing $k_e$; (iii) significantly weakened antibiotic activity owing to decreasing $n$ or increasing MIC; and (iv) bacteria with relatively high rates of mutation (Figure 6b and c).

We also performed the sensitivity analysis by varying single or multiple model parameters simultaneously to examine which factors determine the order in which drug resistance emerges during symptom-based intermittent treatment (Figures S3 and S4). We found that, in the cases of rifampicin + isoniazid and rifampicin + moxifloxacin, increasing the *RF* of rifampicin-resistant mutants to a maximum value of 1.12 caused the population to increase substantially (Figure S3a and c). We further decreased the *RF* of isoniazid- and moxifloxacin-resistant mutants to the 2.5 percentile, which showed that rifampicin resistance was the first to emerge (Figure S3b and d).

We also found an obvious increase in the population of rifampicin-resistant mutants if the *RF* of rifampicin-resistant mutants was increased along with a weakening of the efficacy of amikacin and TMC207 (Figure S4a and c). We further strengthened the bactericidal efficacy of rifampicin and found that the population size of rifampicin-resistant mutants could be comparable to that of amikacin-resistant mutants (Figure S4b). In contrast, further strengthening the efficacy of rifampicin had virtually no effect on either resistant population in the rifampicin-TMC207 combination (Figure S4d).

## Discussion

We developed an integrated mathematical drug treatment model that linked PK, Hill-equation-based PD and TB bacterial population dynamic models to explore the effects of non-adherence on treatment efficacy. With compliance and 20% random non-adherence, we showed that rifampicin + amikacin was the most effective regimen, which is consistent with the results of Ankomah and Levin.[26] In contrast, our results suggest that rifampicin + TMC207 will eliminate bacteria less effectively.

We further used a fixed daily dose of rifampicin (475.48 mg) in combination with different doses of TMC207 to examine treatment efficacy. Rustomjee *et al.*[12] suggested that it is safe for patients to take 100, 200 or 400 mg of TMC207 once a day. We found that, with compliance, the ability of rifampicin + 400 mg of TMC207 to clear bacteria (41 days) was equivalent to that of rifampicin + isoniazid (42 days) (Figure S5). A number of studies have demonstrated that TMC207 is a highly promising drug candidate against drug-susceptible and drug-resistant TB;[11–14,31] nevertheless, there are several caveats with the use of TMC207. A substantially higher rate of acquired drug resistance (41%) was observed in the third Phase II study.[36] An increased number of adverse side effects involving hepatotoxicity and QT prolongation are recognized to be potentially associated with cardiotoxicity.[37] Thus, monitoring of acquired drug resistance, periodic liver function testing and electrocardiography monitoring for evaluating QT prolongation, as well as avoiding alcohol and other hepatotoxic drugs, are imperative when patients are receiving TMC207.[37]

Our results showed that, if a case was treated with periodicity-based intermittent treatment, rifampicin + moxifloxacin was the best treatment regimen. Our simulations demonstrated that the latent bacterial population was an important determinant of clearance time. *RF*, one of the important parameters found in sensitivity analysis, is the only key factor determining the reproductive ability of resistant bacteria. Increase in the *RF* of resistant bacteria could promote the emergence of drug resistance. Numerous studies have highlighted the role of *RF* in affecting the disease burden of drug-resistant TB[38] and MDR TB.[39,40] Our sensitivity analysis also showed that PK parameters became more important as compliance to drug treatment decreased. As expected, PK parameters play a crucial role in maintaining the antibiotic concentration when patients are non-compliant to treatment. Overall, the shortest clearance time with rifampicin + moxifloxacin can be attributable to moxifloxacin, which has a lower $k_e$ and the lowest *RF* of mono-moxifloxacin resistance.

Rifampicin + moxifloxacin also had the lowest probability of resistance to two drugs. An *in vitro* cell model[6] and an *in vivo* murine aerosol infection model[41] both demonstrated that rifampicin + moxifloxacin displayed a mild antagonistic interaction in killing TB bacteria, but a synergistic interaction in suppressing the emergence of resistance. A strong effect of drug interaction on treatment efficacy was found when rifampicin was combined with amikacin. Our $\alpha$ estimates also showed a mild antagonism between rifampicin and moxifloxacin. Although the experimental data were adopted from Ankomah and Levin,[26] our $\alpha$ estimates for rifampicin + amikacin and rifampicin + moxifloxacin were different from their results. In this study, we used the non-linear two-drug PD model to fit the data in order to obtain optimal $\alpha$ estimates. In contrast, Ankomah and Levin,[26] reasonably, generated $\alpha$ estimates by using linear regression functions under the assumption of a two-phase interaction function, one for sub- and one for supra-antibiotic concentration, to simplify the experimentally observed concentration-dependent drug interaction. Therefore, we suggest that concentration-dependent drug interaction can be incorporated into the drug treatment model in future studies.

We used drug-susceptible TB as an initial condition to investigate the evolution of drug resistance during treatment with

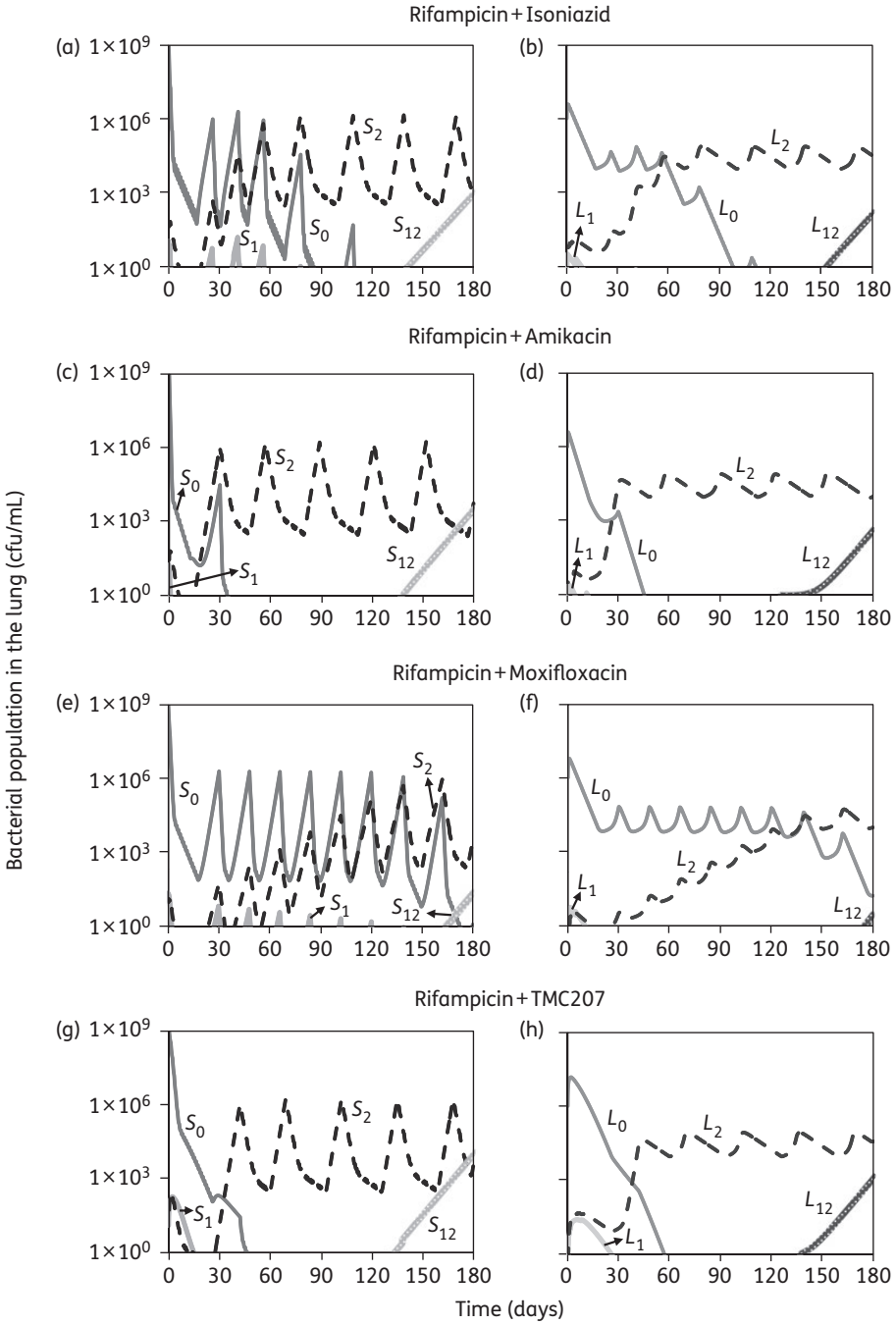


**Figure 4.** Modelling resistance dynamics of (a, c, e and g) rapidly dividing bacterial populations and (b, d, f and h) slowly dividing (latent) bacterial populations during 6 months of treatment with (a and b) rifampicin + isoniazid, (c and d) rifampicin + amikacin, (e and f) rifampicin + moxifloxacin and (g and h) rifampicin + TMC207 for symptom-based intermittent treatment. $S$, rapidly dividing bacterial populations; $L$, slowly dividing (latent) bacterial populations; $S_0$ and $L_0$, bacteria susceptible to both drugs; $S_1$ and $L_1$, bacteria resistant to drug 1 (rifampicin); $S_2$ and $L_2$, bacteria resistant to drug 2 (isoniazid, amikacin, moxifloxacin or TMC207); $S_{12}$ and $L_{12}$, bacteria resistant to both drugs.

non-adherence. Our simulations showed that, generally, isoniazid, amikacin, moxifloxacin and TMC207 resistances were observed initially before rifampicin resistance. Lipsitch and Levin[20] showed that TB bacteria were resistant to isoniazid initially and then to rifampicin in most mathematical simulation scenarios. Several epidemiological studies have demonstrated that isoniazid resistance is the first to appear and is followed by the acquisition of rifampicin resistance in drug-susceptible TB patients who acquired drug resistance during multidrug therapy.[42–44] Recent systematic reviews and meta-analyses also provide further evidence that isoniazid resistance is associated with treatment failure and an increased risk of acquiring additional drug resistance.[45,46] In fact, the global prevalence of isoniazid resistance is much higher than that of rifampicin resistance.[47]

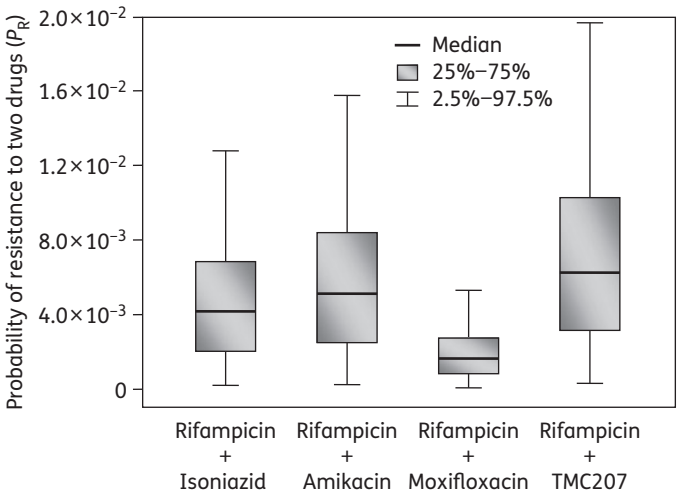


**Figure 5.** Box and whisker plot illustrating the probability of resistance to two drugs ($P_R$) among different two-drug combination regimens for symptom-based intermittent treatment.

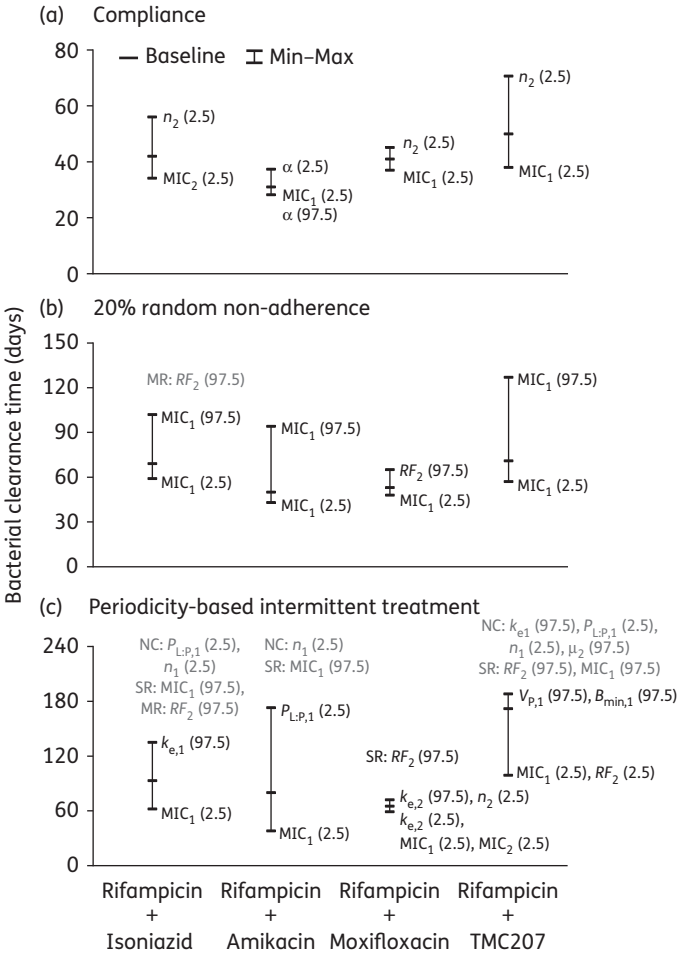


**Figure 6.** One-way sensitivity analysis of parameter contributions to bacterial clearance time during 6 months of treatment with different combination regimens under (a) compliance, (b) 20% random non-adherence and (c) periodicity-based intermittent treatment. Baseline values are given in Table 3. Parameter labelling on the right-hand side of the bars indicates the parameter which has the most influence on minimum and maximum clearance time. Parameter labelling above the bars indicates the parameter which results in treatment failure (NC, the bacteria cannot be cleared; SR, single-drug resistance; MR, multidrug resistance). Numbers in brackets represent parameter values at the 2.5 or 97.5 percentile. Subscripts 1 and 2 stand for drug 1 (rifampicin) and drug 2 (isoniazid, amikacin, moxifloxacin or TMC207), respectively. For meanings of other symbols see Table S1.

There was an exception to these patterns of emergence of drug resistance. We observed an increased frequency of acquired mono-rifampicin resistance while increasing the *RF* of rifampicin-resistant mutants or both increasing the *RF* and weakening the efficacy of another combined drug. Rifampicin-resistant mutants that confer a high *RF* have increased growth ability, resulting in a higher probability that rifampicin resistance emerged first. Reducing the efficacy of another combined drug also provides an opportunity for rifampicin-resistant bacteria to grow vigorously. We also found that an increasing mutation rate could accelerate the evolution of drug resistance, but did not affect the pattern of emergence of drug resistance. Lipsitch and Levin[20] indicated that the killing rate of a drug is more influential than mutation rate in the emergence of resistance. *RF* and antibiotic efficacy are the most important factors in determining the order in which drug resistance emerges.

In this study, we showed that symptom-based intermittent treatment might generate MDR TB at a frequency of 4 in 1000 cases. Based on mathematical modelling, Colijn *et al.*[48] and Ford *et al.*[49] highlighted that drug resistance arises not only during the course of treatment, but also before antibiotic therapy. The probability of MDR TB occurring before treatment might be as high as 1 in 2500 cases[48] and 1 in 1000 TB cases.[49] Even during complete therapy, MDR TB could emerge in as many as 1 in 10000 cases.[48] Taken together, we found that non-adherence to treatment regimens accelerates the growth and evolution of resistant mutants, leading to as high as 40 MDR TB in 10000 cases compared with before (4–10 in 10000 cases) and during complete (1 in 10000 cases) treatment. Our probabilistic risk assessment of drug resistance is not intended to capture the exact quantitative risk, but rather to provide a concept for quantifying the potential impact of non-adherence on multidrug resistance.

A key weakness of this study is that, in many cases, the true uncertainty around parameter values may not have been captured adequately. The physiological and bacteriological parameters in the proposed two-drug treatment model cannot be easily parameterized due to data limitation. To our knowledge, well-established standard values for comparison do not exist. Additionally, our model did not specifically focus on the effect of granulomas on therapeutic efficacy. Drug concentration within TB granulomas has recently been demonstrated to vary considerably and to have spatial heterogeneity that may influence treatment outcomes.[25,50–52] We thus anticipate that granulomas can be incorporated into our model to improve predictability in future research.

Although our model did not capture the exact reality of TB chemotherapy, by considering drug-specific PK and mutation rate our model is capable of predicting the therapeutic outcomes and the evolutionary dynamics of drug resistance during multidrug treatment. For instance, knowing that a two-drug resistance will emerge at approximately month 4 using a symptom-based

intermittent treatment may help to establish the emergence threshold of resistance during the course of a single patient's treatment. We suggest that more implementations such as drug susceptibility testing, dosage adjustment and change of treatment regimens should be initiated before this moment to prevent the emergence of resistance. Further experimental and clinical studies are warranted to assess the potential use of novel drugs or combinations in shortening treatment duration.

## Acknowledgements

This study was inspired by the work of Drs Peter Ankomah and Bruce R. Levin: 'Two-drug antimicrobial chemotherapy: a mathematical model and experiments with *Mycobacterium marinum*' published in *PLoS Pathogens*. Comments by editor Dr Richard Anthony and two anonymous reviewers on this manuscript are greatly appreciated. We are also deeply grateful to Drs Yi-Hsien Cheng and Shu-Han You for providing enormously valuable comments and suggestions.

## Funding

This study was supported in part by the Ministry of Science and Technology, Republic of China under Grant MOST 100-2313-B-002-012-MY3.

## Transparency declarations

None to declare.

### *Author contributions*

Y.-J. L. and C.-M. L. designed the experiments, analysed the data and wrote the manuscript. Both authors edited and approved the final version of the manuscript.

## Supplementary data

Appendix 1, Appendix 2, Table S1 and Figures S1 to S5 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

## References

**1** WHO. *Global Tuberculosis Report*. 2014. http://apps.who.int/iris/bitstream/10665/137094/1/9789241564809_eng.pdf.

**2** WHO. *Treatment of Tuberculosis: Guidelines – Fourth Edition*. 2009. http://whqlibdoc.who.int/publications/2010/9789241547833_eng.pdf.

**3** WHO. *Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis*. 2008. http://whqlibdoc.who.int/publications/2008/9789241547581_eng.pdf.

**4** Bass JB Jr, Farer LS, Hopewell PC *et al*. Treatment of tuberculosis and tuberculosis infection in adults and children. *Am J Respir Crit Care Med* 1994; **149**: 1359–74.

**5** Peloquin CA, Hadad DJ, Molino LP *et al*. Population pharmacokinetics of levofloxacin gatifloxacin and moxifloxacin in adults with pulmonary tuberculosis. *Antimicrob Agents Chemother* 2008; **52**: 852–7.

**6** Drusano GL, Sgambati N, Eichas A *et al*. The combination of rifampin plus moxifloxacin is synergistic for suppression of resistance but antagonistic for cell kill of *Mycobacterium tuberculosis* as determined in a hollow-fiber infection model. *MBio* 2010; **10**: e00139–10.

**7** Zumla A, Nahid P, Cole ST. Advances in the development of new tuberculosis drugs and treatment regimens. *Nat Rev Drug Discov* 2013; **12**: 388–404.

**8** Wallis RS, Wang C, Meyer D *et al*. Month 2 culture status and treatment duration as predictors of tuberculosis relapse risk in a meta-regression model. *PLoS One* 2013; **8**: e71116.

**9** Gillespie SH, Crook AM, McHugh TD *et al*. Four-month moxifloxacin-based regimens for drug-sensitive tuberculosis. *N Engl J Med* 2014; **371**: 1577–87.

**10** Jindani A, Harrison TS, Nunn AJ *et al*. High-dose rifapentine with moxifloxacin for pulmonary tuberculosis. *N Engl J Med* 2014; **371**: 1599–608.

**11** Andries K, Verhasselt P, Guillemont J *et al*. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science* 2005; **307**: 223–7.

**12** Rustomjee R, Diacon AH, Allen J *et al*. Early bactericidal activity and pharmacokinetics of the diarylquinoline TMC207 in treatment of pulmonary tuberculosis. *Antimicrob Agents Chemother* 2008; **52**: 2831–5.

**13** Diacon AH, Pym A, Grobusch M *et al*. The diarylquinoline TMC207 for multidrug-resistant tuberculosis. *N Engl J Med* 2009; **360**: 2397–405.

**14** Rouan MC, Lounis N, Gevers T *et al*. Pharmacokinetics and pharmacodynamics of TMC207 and its *N*-desmethyl metabolite in a murine model of tuberculosis. *Antimicrob Agents Chemother* 2012; **56**: 1444–51.

**15** Dooley KE, Mitnick CD, Ann DeGroote M *et al*. Old drugs, new purpose: retooling existing drugs for optimized treatment of resistant tuberculosis. *Clin Infect Dis* 2012; **55**: 572–81.

**16** Holford NH, Sheiner LB. Understanding the dose-effect relationship: clinical application of pharmacokinetic-pharmacodynamic models. *Clin Pharmacokinet* 1981; **6**: 429–53.

**17** Lipsitch M, Levin B. The population dynamics of antimicrobial chemotherapy. *Antimicrob Agents Chemother* 1997; **41**: 363–73.

**18** Delacher S, Derendorf H, Hollenstein U *et al*. A combined *in vivo* pharmacokinetic-*in vitro* pharmacodynamic approach to simulate target site pharmacodynamics of antibiotics in humans. *J Antimicrob Chemother* 2000; **46**: 733–9.

**19** Nielsen EI, Friberg LE. Pharmacokinetic-pharmacodynamic modeling of antibacterial drugs. *Pharmacol Rev* 2013; **26**: 1053–90.

**20** Lipsitch M, Levin BR. Population dynamics of tuberculosis treatment: mathematical models of the roles of non-compliance and bacterial heterogeneity in the evolution of drug resistance. *Int J Tuberc Lung Dis* 1998; **2**: 187–99.

**21** Mitchison DA. Basic mechanisms of chemotherapy. *Chest* 1979; **76**: 771–81.

**22** Jayaram R, Shandil RK, Gaonkar S *et al*. Isoniazid pharmacokinetics-pharmacodynamics in an aerosol infection model of tuberculosis. *Antimicrob Agents Chemother* 2004; **48**: 2951–7.

**23** Budha NR, Lee RB, Hurdle JG *et al*. A simple *in vitro* PK/PD model system to determine time–kill curves of drugs against mycobacteria. *Tuberculosis* 2009; **89**: 378–85.

**24** Goutelle S, Bourguignon L, Jelliffe RW *et al*. Mathematical modeling of pulmonary tuberculosis therapy: insights from a prototype model with rifampin. *J Theor Biol* 2011; **282**: 80–92.

**25** Kjellsson MC, Via LE, Goh A *et al*. Pharmacokinetic evaluation of the penetration of antituberculosis agents in rabbit pulmonary lesions. *Antimicrob Agents Chemother* 2012; **56**: 446–57.

**26** Ankomah P, Levin BR. Two-drug antimicrobial chemotherapy: a mathematical model and experiments with *Mycobacterium marinum*. *PLoS Pathog* 2012; **8**: e1002487.

**27** Nuermberger E, Grosset J. Pharmacokinetic and pharmacodynamic issues in the treatment of mycobacterial infections. *Eur J Clin Microbiol Infect Dis* 2004; **23**: 243–55.

**28** Jindani A, Doré CJ, Mitchison DA. Bactericidal and sterilizing activities of antituberculosis drugs during the first 14 days. *Am J Respir Crit Care Med* 2003; **167**: 1348–54.

**29** Nuermberger EL, Yoshimatsu T, Tyagi S *et al*. Moxifloxacin-containing regimen greatly reduces time to culture conversion in murine tuberculosis. *Am J Respir Crit Care Med* 2004; **169**: 421–6.

**30** Gillespie SH, Gosling RD, Uiso L *et al*. Early bactericidal activity of a moxifloxacin and isoniazid combination in smear-positive pulmonary tuberculosis. *J Antimicrob Chemother* 2005; **56**: 1169–71.

**31** Reddy VM, Einck L, Andries K *et al*. *In vitro* interactions between new antitubercular drug candidates SQ109 and TMC207. *Antimicrob Agents Chemother* 2010; **54**: 2840–6.

**32** Bhusal Y, Shiohira CM, Yamane N. Determination of *in vitro* synergy when three antimicrobial agents are combined against *Mycobacterium tuberculosis*. *Int J Antimicrob Agents* 2005; **26**: 292–7.

**33** Regoes RR, Wiuff C, Zappala RM *et al*. Pharmacodynamic functions: a multiparameter approach to the design of antibiotic treatment regimens. *Antimicrob Agents Chemother* 2004; **48**: 3670–6.

**34** Shimao T. Drug resistance in tuberculosis control. *Tubercle* 1987; **68**: 5–18.

**35** Gillespie SH. Evolution of drug resistance in *Mycobacterium tuberculosis*: clinical and molecular perspective. *Antimicrob Agents Chemother* 2002; **46**: 267–74.

**36** Fox GJ, Menzies D. A review of the evidence for using bedaquiline (TMC207) to treat multi-drug resistant tuberculosis. *Infect Dis Ther* 2013;**2**:123–44.

**37** WHO. *The Use of Bedaquiline in the Treatment of Multidrug-Resistant Tuberculosis: Interim Policy Guidance*. 2013. http://apps.who.int/iris/bitstream/10665/84879/1/9789241505482_eng.pdf.

**38** Blower SM, Gerberding JL. Understanding, predicting and controlling the emergence of drug-resistant tuberculosis: a theoretical framework. *J Mol Med* 1998; **76**: 624–36.

**39** Cohen T, Murray M. Modeling epidemics of multidrug-resistant *M. tuberculosis of heterogeneous fitness*. *Nat Med* 2004; **10**: 1117–21.

**40** Lin YJ, Liao CM. Seasonal dynamics of tuberculosis epidemics and implications for multidrug-resistant infection risk assessment. *Epidemiol Infect* 2014; **142**: 358–70.

**41** Balasubramanian V, Solapure S, Gaonkar S *et al*. Effect of coadministration of moxifloxacin and rifampin on *Mycobacterium tuberculosis* in a murine aerosol infection model. *Antimicrob Agents Chemother* 2012; **56**: 3054–7.

**42** Elghoul MT, Joshi RM, Rizghalla T. Primary and acquired drug resistance in *Mycobacterium tuberculosis* strains in western region of Libyan Arab Jamahiriya. *Trop Geogr Med* 1989; **41**: 304–8.

**43** Nolan CM, Williams DL, Cave MD *et al*. Evolution of rifampin resistance in human immunodeficiency virus-associated tuberculosis. *Am J Respir Crit Care Med* 1995; **152**: 1067–71.

**44** Mitchison DA, Nunn AJ. Influence of initial drug resistance on the response to short-course chemotherapy of pulmonary tuberculosis. *Am Rev Respir Dis* 1986; **133**: 423–30.

**45** Menzies D, Benedetti A, Paydar A *et al*. Effect of duration and intermittency of rifampin on tuberculosis treatment outcomes: a systematic review and meta-analysis. *PLoS Med* 2009; **6**: e1000146.

**46** Menzies D, Benedetti A, Paydar A *et al*. Standardized treatment of active tuberculosis in patients with previous treatment and/or with mono-resistance to isoniazid: a systematic review and meta-analysis. *PLoS Med* 2009; **6**: e1000150.

**47** WHO. *Anti-Tuberculosis Drug Resistance in the World: Report No. 4*. 2008. http://www.who.int/tb/publications/2008/drs_report4_26feb08.pdf.

**48** Colijn C, Cohen T, Ganesh A *et al*. Spontaneous emergence of multiple drug resistance in tuberculosis before and during therapy. *PLoS One* 2011; **6**: e18327.

**49** Ford CB, Shah RR, Maeda MK *et al*. *Mycobacterium tuberculosis* mutation rate estimates from different lineages predict substantial differences in the emergence of drug-resistant tuberculosis. *Nat Genet* 2013; **45**: 784–90.

**50** Prideaux B, Dartois V, Staab D *et al*. High-sensitivity MALDI-MRM-MS imaging of moxifloxacin distribution in tuberculosis-infected rabbit lungs and granulomatous lesions. *Anal Chem* 2011; **83**: 2112–8.

**51** Prideaux B, ElNaggar MS, Zimmerman M *et al*. Mass spectrometry imaging of levofloxacin distribution in TB-infected pulmonary lesions by MALDI-MSI and continuous liquid microjunction surface sampling. *Int J Mass Spectrom* 2015; **377**: 699–708.

**52** Pienaar E, Cilfone NA, Lin PL *et al*. A computational tool integrating host immunity with antibiotic dynamics to study tuberculosis treatment. *J Theor Biol* 2015; **367**: 166–79.