Optimal antimicrobial dosing combinations when drug-resistance mutation rates differ

Oscar Delaney [1*] Andrew D. Letten [1] Jan Engelstädter [1]

[1]School of the Environment, The University of Queensland

[*] correspondence addressed to o.delaney@uq.net.au

May 4, 2024

Abstract

Given the ongoing antimicrobial resistance crisis, it is imperative to develop dosing regimens optimised for avoiding the evolution of resistance. The rate at which bacteria acquire resistance-conferring mutations to different antimicrobial drugs spans multiple orders of magnitude. By using a mathematical model and computer simulations, we show that knowledge of relative mutation rates can meaningfully inform the optimal deployment of two drugs in a treatment regimen. We demonstrate that under plausible assumptions there is a linear relationship in log-log space between the optimal drug A:drug B dose ratio and the ratio of their mutation rates. This power law relationship holds for bacteriostatic and bactericidal drugs. If borne out empirically, these findings suggest there might be significant room to further optimise antimicrobial dosing strategies.

Keywords: microbial evolution, mutation rates, mathematical modelling, antimicrobial resistance, combination therapy, evolutionary rescue.

1 Introduction

One of the key goals of designing antimicrobial treatment regimens must be to minimise the probability that resistance develops, alongside striving to rapidly clear the patient's infection and avoid excessive toxicity. One valuable approach is to use multiple drugs, either in combination [1, 2] or sequentially [3, 4, 5, 6] such that even if a mutation conferring resistance to a single drug occurs, the mutant is still impacted by the other drug(s). Using multiple drugs, rather than just a larger dose of a single drug, may also reduce toxic side effects in the patient, especially if the drugs interact synergistically and hence allow for smaller concentrations to be efficacious [7]. Combination therapy is supported by a significant body of empirical literature, with a meta-analysis involving 4514 patients from 53 studies finding an average reduction in mortality of 17% compared to monotherapy [8].

Many mathematical and computational models have been created to better understand and predict the evolution of resistance (reviewed in [9, 10]). In most models, a key parameter is the mutation rate: the probability that a cell division in a susceptible bacterium will give rise to a cell resistant to the drug in question. The higher the mutation rate, the more likely resistance is to develop. Because this relationship is trivial, in most mathematical models the mutation rate is fixed and then ignored, and other putatively more interesting phenomena are explored. Here, we show that in combination therapy, the relative mutation rates for each drug can be an important factor in choosing the optimal quantity of each drug to apply.

The rate at which resistance mutations to a given antimicrobial drug occur may depend on the bacterium that is targeted, the current resource availability or other environmental conditions. Even within one host species and constant conditions, resistance mutation rates can vary greatly by drug [11]. This is unsurprising, as different mechanisms of action may be harder or easier for the bacteria to surmount or circumvent when sampling from the space of possible mutations. Indeed, in *Mycobacterium tuberculosis*, the infectious agent responsible for the most deaths per year worldwide [12], there is an approximately 400-fold difference in the mutation rate between two of the most commonly used first-line drugs, rifampicin and ethambutol [13]. To our knowledge, there is no centralised database of mutation rate estimates, but some example values from the literature for various drugs and species are provided in Table 1. Resistance mutation rates being orders of magnitude apart could reasonably be expected to prove important when choosing optimal dosing strategies. Intuitively, it is better to use drugs with lower mutation rates, all else equal, to minimise the probability of resistance developing.

We formalise and interrogate this intuition under a variety of plausible assumptions, and develop theoretical predictions for how different resistance mutation rates should alter optimal dosing strategies. We find that there is a power law relationship between the ratio of mutation rates and the optimal drug dosing concentrations.

Table 1: Resistance mutation rates per replication for various antibiotics and three species of bacteria: *Mycobacterium tuberculosis*, *Mycobacterium smegmatis*, and *Escherichia coli*.

Drug	Bacteria	Mutation rate estimate	Reference
Isoniazid	*M. tuberculosis*	$2.6 \cdot 10^{-8}$ to $3.2 \cdot 10^{-7}$	[13, 14]
Rifampicin	*M. tuberculosis*	$2.3 \cdot 10^{-10}$ to $1.1 \cdot 10^{-8}$	[13, 14, 15]
Streptomycin	*M. tuberculosis*	$3.0 \cdot 10^{-8}$	[13]
Ethambutol	*M. tuberculosis*	$1.0 \cdot 10^{-7}$	[13]
Rifampicin	*M. smegmatis*	$2.2 \cdot 10^{-10}$ to $9.2 \cdot 10^{-8}$	[16]
Isoniazid	*M. smegmatis*	$1.2 \cdot 10^{-9}$ to $1.2 \cdot 10^{-7}$	[16]
Streptomycin	*M. smegmatis*	$2.8 \cdot 10^{-8}$ to $5.3 \cdot 10^{-8}$	[16]
Kanamycin	*M. smegmatis*	$1.7 \cdot 10^{-8}$	[16]
Rifampicin	*E. coli*	$7.0 \cdot 10^{-9}$	[17]
Streptomycin	*E. coli*	$2.7 \cdot 10^{-9}$	[18]
Ciprofloxacin	*E. coli*	$3.6 \cdot 10^{-9}$	[19]

2 Methods

We modelled a simple scenario where there is one species of bacteria and two arbitrary drugs, A and B, administered at concentrations C_A and C_B that are constant over time (we later relax this assumption). At time t the sizes of the susceptible, A-resistant, and B-resistant populations respectively are $S(t)$, $M_A(t)$ and $M_B(t)$.

To model drug mode of action and pharmacodynamics, we normalised the effective drug concentration (E_i) for bacterial strain i and drug j onto the $[0, 1)$ interval using the sigmoid $\mathrm{E_{max}}$ model [20] (closely related to the more common Hill equation [21]). Here, z is the drug concentration at which the half-maximal effect is achieved (denoted $\mathrm{EC_{50}}$ in [22]) and β is the shape parameter which determines the steepness of the function around z [23]:

$$E_i(C_j) = \left(1 + \left(\frac{C_j}{z_{i,j}}\right)^{-\beta_j}\right)^{-1}. \tag{1}$$

We consider drugs that are either bacteriostatic (denoted $\phi_j = 1$) and only affect the cell division rate, or bactericidal (denoted $\phi_j = 0$) and only affect the cell death rate. We ignored resource constraints to growth, such that the growth rate of strain i is a constant g_i in the absence of drugs, and likewise δ_i is a constant intrinsic death rate term. Combining, the drug-dependent growth and death rates are

$$G_i = g_i(1 - E_i(C_A)\phi_A)(1 - E_i(C_B)\phi_B), \tag{2}$$

$$D_i = \delta_i + E_i(C_A)(1 - \phi_A) + E_i(C_B)(1 - \phi_B). \tag{3}$$

Denoting the j-resistance mutation rate as μ_j and ignoring back-mutations and the (initially negligible) chance of double-mutations, we specify our model as the following system of ordinary differential equations (ODEs):

$$\begin{aligned} \frac{dS}{dt} &= S(G_S(1 - \mu_A - \mu_B) - D_S), \\ \frac{dM_A}{dt} &= M_A(G_{M_A} - D_{M_A}) + SG_S\mu_A, \\ \frac{dM_B}{dt} &= M_B(G_{M_B} - D_{M_B}) + SG_S\mu_B. \end{aligned} \tag{4}$$

Along with the initial conditions, where only susceptible cells are present, this fully defines the mathematical model. For the computational implementation, in order to more realistically model the uncertainty inherent in growth and mutation, we used the Stochastic Simulation Algorithm to evolve the system over time, with separate birth and death events [24]. All simulations were performed using R v4.3.0 [25]. To make evolving the system of ODEs computationally feasible, we used tau-leaping to perform many transitions in one step with the *adaptivetau* package [26, 27]. We used the *future* package to parallelise simulation runs [28]. To store, analyse, and visualise the simulation data we used the *tidyverse* set of packages [29].

The value we seek to maximise is the probability that the susceptible population is driven to extinction without resistance becoming established. This can be operationalised as the

probability that $S(t) + M_A(t) + M_B(t) = 0$ for any time t. Trivially, arbitrarily large drug concentrations are optimal for this goal. However, toxic side effects for the host mean that drug concentrations must be restricted. We use a simple toxicity model where there is some fixed maximum allowable toxicity c, and both drugs contribute equally and linearly to this maximum, that is $C_A + C_B \leq c$. To maximise the combined efficacy of the drugs the highest allowable concentrations are used ($C_A + C_B = c$). The drug concentrations are scaled to be in units standardised to the potency of the drug in question, such that $z_{S,A} = z_{S,B} = 1$.

Holding all other parameters constant, we seek a mapping $(\mu_A, \mu_B) \to (C_A, C_B)$ that maximises the probability of eventual extinction, P_E. We call a *strategy* a choice of what drug concentrations $C_A \in [0, c]$ and $C_B = c - C_A$ to apply. Drugs with lower mutation rates are preferred, however the diminishing marginal returns to increasing drug concentrations defined by the $E_i(C_j)$ function mean that it is not necessarily optimal to use only the drug with a lower mutation rate.

3 Results

3.1 Analytical solution

To find the probability P_D that a newly arisen resistant mutant cell leaves no descendants in the distant future, we can use the law of total probability with the notation that $P_{D|i}$ is the probability a strain-i mutant cell leaves no descendants in the distant future:

$$P_D = \frac{\mu_A}{\mu_A + \mu_B} P_{D|M_A} + \frac{\mu_B}{\mu_A + \mu_B} P_{D|M_B}. \tag{5}$$

Due to the stochastic nature of the model, even a mutant lineage with a positive growth rate may become extinct, and thus $P_{D|i}$ is not necessarily 0. We can again use the law of total probability, noting that the cell must either die before dividing or divide before dying, and that the probability of each occurring first is proportional to the rate of that stochastic process. If the cell successfully divides once, each of the two daughter cells will also have a $P_{D|i}$ chance of leaving no descendants, as they are functionally identical and independent. This gives

$$P_{D|i} = \frac{D_i}{G_i + D_i} \cdot 1 + \frac{G_i}{G_i + D_i} \cdot P_{D|i}^2. \tag{6}$$

This is a special case of the well-characterised Gambler's Ruin problem, and the solution known since Fermat [30] is that

$$P_{D|i} = \begin{cases} \frac{D_i}{G_i} & \text{if } D_i < G_i \\ 1 & \text{otherwise} \end{cases}. \tag{7}$$

Now, let N_m be the number of mutation events that occur before the susceptible population becomes extinct. To find $P(N_m = k)$ we can approximate the number of cell divisions in the susceptible population as a deterministic process, as it begins with a very large number of cells

so the stochasticity of individual cell divisions becomes negligible. We can also assume that $\mu_j \ll 1$ and thus use the approximation $\frac{dS}{dt} \approx S(G_S - D_S)$ and therefore $S(t) \approx S(0)e^{(G_S - D_S)t}$. Using this approximation, we can estimate the total number of replications N_r as

$$\begin{aligned} N_r &= \int_0^\infty S(t) G_S \, dt \\ &\approx \frac{S(0)}{\frac{D_S}{G_S} - 1}. \end{aligned} \tag{8}$$

Let the chance of a mutation conferring resistance to either drug occurring at each replication event be $\mu = \mu_A + \mu_B$, then noting that replication events are independent Bernoulli trials for whether a mutation occurs, we get that $N_m \sim Bin(N_r, \mu)$ and thus that

$$P(N_m = k) = \binom{N_r}{k} \mu^k (1-\mu)^{N_r - k}. \tag{9}$$

Because the survival of each mutant lineage is independent at small mutant population sizes, the overall probability of extinction is then

$$P_E = \sum_{k=0}^{\infty} P(N_m = k) P_D^k. \tag{10}$$

Substituting Equation 9 into Equation 10 we get

$$\begin{aligned} P_E &= \sum_{k=0}^{N_r} \binom{N_r}{k} \mu^k (1-\mu)^{N_r - k} P_D^k \\ &= (1 - \mu(1 - P_D))^{N_r}. \end{aligned} \tag{11}$$

Because μ is by assumption very small, we can make the approximation

$$\begin{aligned} P_E &= \exp\left(N_r \ln(1 - \mu(1 - P_D))\right) \\ &\approx \exp\left(-\mu N_r (1 - P_D)\right). \end{aligned} \tag{12}$$

The simplicity of equation 12 belies the fact that P_D and N_r are themselves nontrivial expressions. Equation 12 will be used for the computational implementation, as complicated expressions are unproblematic for numerical methods. But to make further analytical progress, it is useful to simplify the analysis by considering a small class of possible parameters that make the formulas collapse down to more manageable forms. In particular, the simplifying assumptions are:

- Resistant cells are unaffected by arbitrarily high drug concentrations ($E_{M_A}(C_A) = 0$, $E_{M_B}(C_B) = 0$ for all drug concentrations $C_A, C_B \in \mathbb{R}^+$).
- The shape parameters of the pharmacodynamic functions are unity ($\beta_A = \beta_B = 1$, equivalent to Michaelis-Menten kinetics).

- There is no cost of resistance, i.e. the drug-free growth rate and death rate of all strains are the same ($g_i = g,\ \delta_i = \delta$).
- Both drugs are bacteriostatic ($\phi_A = \phi_B = 1$).
- When only drug A is applied ($C_A = c,\ C_B = 0$), the net growth rate of the susceptible and B-resistant strains are both zero ($G_S - D_S = G_{M_B} - D_{M_B} = 0$), and vice versa for when only drug B is applied.

While restrictive, these assumptions are still plausible enough to be interesting, and will be relaxed later in the Simulations section. The final assumption is less conceptually important, but makes the computations simpler. Substituting these assumptions into equation 12 and computing using Mathematica v13.1.0.0 [31] yields

$$P_E = \exp\left(-S_0\left(\frac{\mu_A}{C_B} + \frac{\mu_B}{C_A}\right)\right). \tag{13}$$

This is a pleasingly interpretable equation: as μ_A increases relative to μ_B it is more important to use a higher dose of drug B than drug A, because A-resistant cells are still susceptible to drug B. We maximised P_E by computing its derivative with respect to C_A, setting this equal to 0, and solving for the optimal drug concentrations, denoted $\hat{C}_A$ and $\hat{C}_B$. This yields the surprisingly simple solution that

$$\begin{aligned} \frac{\hat{C}_A}{\hat{C}_B} &= \sqrt{\frac{\mu_B}{\mu_A}}, \text{ or} \\ \log\left(\frac{\hat{C}_A}{\hat{C}_B}\right) &= -\frac{1}{2}\log\left(\frac{\mu_A}{\mu_B}\right). \end{aligned} \tag{14}$$

The second version of this equation is useful as it shows that in log-log space there should be a linear relationship between the ratio of the mutation rates and the ratio of the doses. In other words, there is a power law relationship between the ratios of mutation rates and the ratio of optimal drug use, with an exponent of $-\frac{1}{2}$. This relationship exhibits the expected behaviour whereby $\mu_A \to 0$ entails $\hat{C}_B \to 0$ and $\mu_B \to 0$ entails $\hat{C}_A \to 0$. This means, as mutations become more biased towards conferring resistance to one drug, the optimal dosing strategy relies more on the other, less resistance-prone, drug. The intermediate steps are more complicated so are omitted here, but computations in Mathematica show that this same simple relationship between mutation rates and optimal dosing ratios holds when both drugs are bactericidal.

3.2 Simulations

Here, we corroborate the analytical findings computationally and explore regions of parameter space that appear inaccessible analytically.

When both drugs are bactericidal or both are bacteriostatic, the relationship given in equation 14 holds (Figure 1A,D). Interestingly, the actual values of P_E differ in the two cases, but the optimal dosing strategy remains the same. A qualitatively similar relationship holds when one drug is bacteriostatic and the other is bactericidal, but the optimal dosing strategy is biased

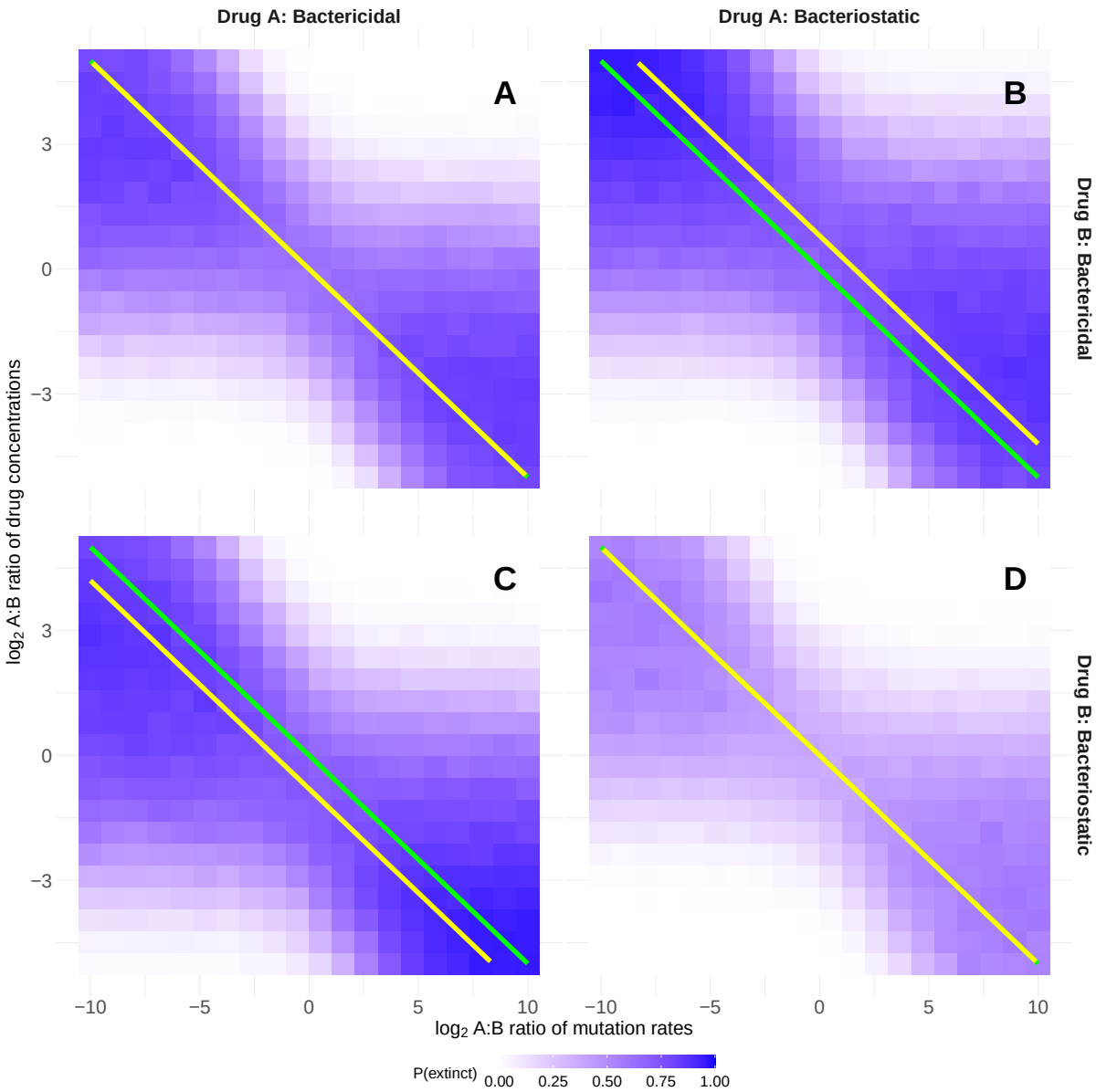

Figure 1: Compulational corroboration of basic analytical results. Each grid square shows the probability that an initial population of susceptible bacteria will be driven to extinction by that dosing strategy, averaged over 1000 simulation runs of the stochastic ODEs in equation 4. The yellow lines show the theoretically optimal dosing strategy for any given ratio of resistance mutation rates, determined by numerically evaluating P_E for many values of C_A and $C_B = c - C_A$ using Equation 12, and choosing the minimand and minimum. The green lines are the same in all panels and show the analytical result from Equation 14 for the basic scenario, for comparison. Parameter values are $\mu = 10^{-9}$, $S(0) = 10^9$, $g = 1, \delta = \frac{1}{3}, c = 2, \beta = 1$ and the drug modes of action vary in each panel. A) $\phi_A = \phi_B = 0$. B) $\phi_A = 1$, $\phi_B = 0$. C) $\phi_B = 1$, $\phi_A = 0$. D) $\phi_A = \phi_B = 1$.

towards the bacteriostatic drug (Figure 1B,C). This effect, where the coupling of mutations to replications favours the growth-inhibiting activity of bacteriostatic drugs, was recently explored in [23].

Having verified the basic analytical findings, we can begin relaxing various assumptions. As resistance becomes weaker (from the earlier unrealistic supposition of total resistance), the two resistant strains become less perfectly adapted to their respective drugs, and may even have negative growth rates. Further, in practice bacteria may acquire different mutations conferring varying extents of resistance. To incorporate this, we reran the simulations with each run having the EC_{50} of both mutant strains drawn independently from an exponential distribution with mean ζ. This reduces the effective mutation rate, as now some 'resistant' mutants have a low EC_{50} value (weak resistance) and a negative growth rate, so are evolutionary dead ends. For a fixed mutation rate ratio and two bacteriostatic drugs, as resistance becomes weaker ($z_{M_A,B}, z_{M_B,A} \to z_W = 1$) the probability of extinction tends towards 1 and the optimal strategy tends towards using equal amounts of both drugs (Figure 2). Conversely, for strong resistance as $\zeta \to \infty$, the optimal ratio of drug concentrations converges to the theoretical value given in Eq. 14 (which for the example in Figure 2 computes to $\log_2(\frac{\hat{C}_A}{\hat{C}_B}) = -\log_2(\frac{1}{8}) = \frac{3}{2}$).

The fuller results shown in Figure S1 are very similar to those in Figure 1, suggesting the simplifying assumption made in our analytical results that resistance is complete is unproblematic.

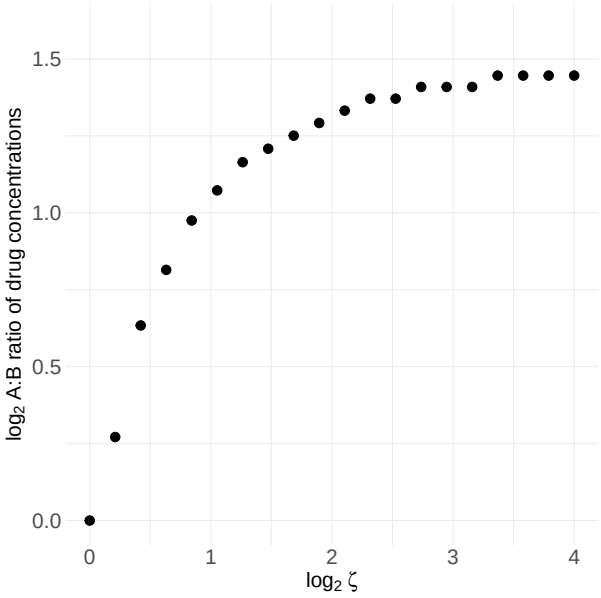

Figure 2: Optimal dosing with partial resistance. Parameters are the same as in Figure 1D except that $\mu_B = 8\mu_A$ and $z_{M_A,B}, z_{M_B,A} \sim 1 + \text{Exp}(\frac{1}{\zeta-1})$ for $\zeta > 1$ and $z_{M_A,B}, z_{M_B,A} = 1$ for $\zeta = 1$. For each dot defining a ζ value, 1000 values of z were drawn, evenly spaced from the cdf at the $0.1^{th}, 0.2^{th}, ..., 99.9^{th}$ percentiles, and the mean probability of extinction was computed over these 1000 using the approximation in Equation 12. This was done for 30 possible ratios of drug doses, and the dosing ratio which yielded the highest P_E value was plotted on the y-axis.

Changing the shape parameter (β) noticeably changes the basic result. If $\beta > 1$ then the pharmacodynamic function has a sigmoidal shape and thus is steeper around the z-value where the drug has half its maximal effect. This means that intermediate values of both drugs are

less beneficial than a more potent dose of just one drug, especially when both drugs are bacteriostatic. Beyond some threshold β value, using just one drug is optimal (Figure 3). At this threshold value the intermediate drug concentration ratio switches from being the global maximum of extinction probability to only a local maximum, so an underlying smooth function leads to a discontinuous result upon taking the maximand. If instead $\beta < 1$ then the pharmacodynamic function is steep initially near a drug concentration of zero, and then approaches the maximum inhibitory effect slowly. Thus, it is most valuable to use some of both drugs. And again, below some threshold, using equal quantities of both drugs is optimal. Results for different mutation rate ratios and drug types are shown in Figures S2 and S3 for $\beta = 3$ and $\beta = 0.2$ respectively.

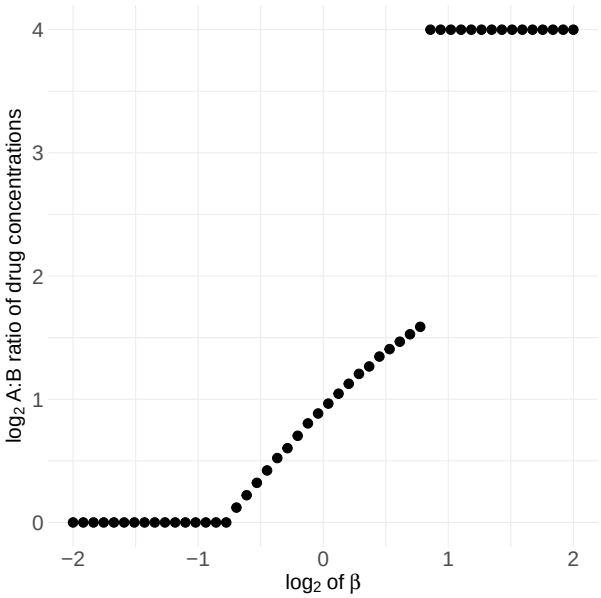

Figure 3: Optimal dosing under shape parameter variation. Parameters are the same as in Figure 1D except that $\delta = 1.1 - \left(1 + c^{-\beta}\right)^{-1}$ which ensures that regardless of the value of β the susceptible population's growth rate is always at least slightly negative. The maximum ratio tried was $2^4 = 16$, so the fact that dots clump there does not suggest that value is special, instead that arbitrarily large ratios are optimal, but cannot readily be plotted on a finite y-axis.

Thus far cost-free resistance has been assumed, whereas in reality mutations that confer resistance often reduce the maximum growth rate or cause other fitness costs. For the basic model, in the limit as $C_B \to 0$, δ was chosen such that $G_{M_B} - D_{M_B} \to 0$, whereas once resistance costs are introduced the net growth rate of mutants can become negative. There is a probability of 0 that a mutant with a negative growth rate survives in the long term, and so all negative growth rates are equally good from the perspective of minimising resistance evolution. Thus, here too intermediate dosing strategies are sufficient to ensure $P_E \approx 1$ even for very skewed mutation rates (Figure S4).

We also extended our basic model to include pharmacokinetics, and found that introducing a drug decay rate of $0.15h^{-1}$ left the basic results roughly unchanged (Figure S5), suggesting that the fact the analytical solution ignores pharmacokinetics is not a fatal flaw.

4 Discussion

Antimicrobial combination therapy is justified partly on the basis that it reduces the probability of infectious pathogens evolving resistance [1, 2]. To date, however, the design of optimal dosing regimens in combination therapy has given little consideration to drug-specific variation in pathogen resistance mutation rates. Here we have shown that as two drugs have increasingly different mutation rates, the optimal dosing strategy entails using an increasingly large fraction of the drug with a lower resistance mutation rate, according to a simple power law relationship. This is an intuitive result. This result is relatively robust to changing the drugs' modes of action. Across various alterations to the basic scenario — such as changes to the shape parameter β, making resistance costly or incomplete, increasing the death rate, or adding pharmacokinetics — the relationship between a skewed mutation rate and skewed optimal dosing strategy persists, but in several cases the dosing skew should never be raised above some maximum value.

Antibiotic resistance is particularly concerning in tuberculosis, where as of 2022 12% of all cases worldwide involved multidrug-resistant (MDR) strains of *M. tuberculosis* [32]. A key component of an MDR containment strategy is to minimise the incidence of already resistant strains acquiring resistance to another drug which was previously efficacious. Clinical data from Georgia indicates that for MDR patients being treated with second-line antibiotics, 9% acquire resistance to ofloxacin during treatment, and 10% to kanamycin [33]. To our knowledge, there is no empirical data linking the probability of tuberculosis patients acquiring resistance to a particular drug with the rate at which resistance mutations to that drug arise in the laboratory, however there is a strong prima facie reason to expect such a connection. Mutation rate differences among strains of *M. tuberculosis* have been investigated, and indeed a strain with more frequent mutations in the laboratory had elevated levels of MDR in clinical infections [34].

In our analysis and simulations, apart from the dosing concentration and resistance mutation rate, the two drugs had identical properties. This need not be the case. If drug A has a higher rate at which mutations conferring resistance to it arise, but it is also more potent per unit of toxicity, it may still be preferable to use a larger dose of it than drug B. Moreover, the toxicity model used here is unrealistic: in reality, there is no sharp cutoff beyond which further increases in drug doses have catastrophic consequences and before which toxicity is zero. Instead, negative side effects are likely to be a smooth monotonically increasing function of drug concentration [35], and it could be that the two drugs have additive, antagonistic, or synergistic combined effects on total toxicity. Allowing for this greater subtlety in drug toxicity would be a valuable avenue for further research, but could complicate the mathematical analysis considerably.

One of the key weaknesses of the analytical solution presented here is that it relies on constant growth and death rates over time for all strains, whereas in reality drugs decay over time in the patient's body. It appears that this simplification does not change the core result, however, given the introduction of pharmacokinetics in Figure S5 left the main trend unchanged. Our analytical results relied on assuming mutations conferring complete resistance, that is an infinitely wide mutant selection window, where for arbitrarily large drug concentrations the mutant still achieves a positive growth rate. This is clearly unrealistic. Our simulation results in Figures 2 and S1 show that relaxing this assumption to allow for a realistic mutant selection

window weakens but does not drastically change the result. The simulations could be extended in many ways, such as including resource-constrained growth, and multiple species of commensal or pathogen bacteria.

Antimicrobial resistance is often conferred not by *de novo* mutations but through horizontal gene transfer (HGT), e.g. through the transfer of plasmids (conjugation), or the uptake of free DNA from the environment (transformation) [36, 37]. Whilst our model does not incorporate HGT of resistance genes, we believe that in some situations our results may still be applicable, at least approximately. For example, consider a scenario in which a drug-susceptible pathogen co-occurs but is not in competition with resistant commensal bacteria, and that the resistance genes can be transferred to the pathogen. In this situation, one would expect per capita rates of HGT to be roughly constant over time. Therefore, within our model framework, the process of HGT would be equivalent to the process of mutation, and our results would extend to mutations acquired through HGT or a combination of both mutation and HGT. Depending on the bacteria and mechanism of HGT, rates of HGT are potentially orders of magnitude greater than mutation rates. Thus, using a drug to which the commensal bacteria is susceptible could make resistance considerably less likely to evolve. More complex scenarios where the donor populations are also affected by the drug or interact with the pathogen population (e.g., through competition or cross-feeding) would require a new model incorporating these effects.

While these results will take time to become clinically applicable, the potential of using the (often well-characterised) resistance mutation rate in deciding on a treatment strategy is unreasonably underexplored. Even if theoretical models as abstract and (compared to reality) simple as this one cannot be directly applied in clinical settings, our results could motivate experimental efforts to corroborate them, which could in turn lead to in-vivo tests. Our findings should in principle be straightforward to test in the laboratory. This would require assembling a set of drugs with considerably different mutation rates in some model bacteria, and challenging parallel susceptible populations with different pairs of these drugs in a variety of concentration ratios. Including resistance mutation rates in clinical deliberations regarding what combination of drugs to use has the potential to clear more infections and minimise resistance evolution.

Acknolwedgements

We would like to thank the Engelstädter and Letten groups for discussions on this topic, in particular James Richardson and Christopher Brown. GPT-4 was used for coding advice (but did not write any part of the manuscript).

Funding

OD received a Vice-Chancellor's Scholarship and Harriett Marks Bursary at the University of Queensland. ADL is supported by Australian Research Council grants DP220103350 and DE230100373. JE is supported by Australian Research Council grant DP190103039.

Conflict of Interest Disclosure

The authors declare that they comply with the PCI rule of having no financial conflicts of interest in relation to the content of the article.

Data Availability

All R and Mathematica code used to generate the figures and perform the symbolic manipulations, respectively, is available at https://doi.org/10.5281/zenodo.11112244

References

1. Angst DC, Tepekule B, Sun L, Bogos B, and Bonhoeffer S. 2021 Comparing treatment strategies to reduce antibiotic resistance in an in vitro epidemiological setting. Proceedings of the National Academy of Sciences of the United States of America. 118 :e2023467118. (doi:10.1073/pnas.2023467118)
2. Leekha S, Terrell CL, and Edson RS. 2011 General Principles of Antimicrobial Therapy. Mayo Clinic Proceedings. 86 :156–67. (doi:10.4065/mcp.2010.0639)
3. Nyhoegen C and Uecker H. 2023 Sequential antibiotic therapy in the laboratory and in the patient. J R Soc Interface. 20 :20220793–20220793. (doi:10.1098/rsif.2022.0793)
4. Imamovic L and Sommer MOA. 2013 Use of Collateral Sensitivity Networks to Design Drug Cycling Protocols That Avoid Resistance Development. Science Translational Medicine. 5 :204ra132–204ra132. (doi:10.1126/scitranslmed.3006609)
5. Kim S, Lieberman TD, and Kishony R. 2014 Alternating antibiotic treatments constrain evolutionary paths to multidrug resistance. Proceedings of the National Academy of Sciences. 111 :14494–9. (doi:10.1073/pnas.1409800111)
6. Baym M, Stone LK, and Kishony R. 2016 Multidrug evolutionary strategies to reverse antibiotic resistance. Science. 351 :aad3292. (doi:10.1126/science.aad3292)

7. Rao GG, Li J, Garonzik SM, Nation RL, and Forrest A. 2018 Assessment and modelling of antibacterial combination regimens. Clinical Microbiology and Infection. 24 :689–96. (doi:10.1016/j.cmi.2017.12.004)

8. Schmid A, Wolfensberger A, Nemeth J, Schreiber PW, Sax H, and Kuster SP. 2019 Monotherapy versus combination therapy for multidrug-resistant Gram-negative infections: Systematic Review and Meta-Analysis. Scientific Reports. 9 :15290. (doi:10.1038/s41598-019-51711-x)

9. Temime L, Hejblum G, Setbon M, and Valleron AJ. 2008 The rising impact of mathematical modelling in epidemiology: antibiotic resistance research as a case study. Epidemiology & Infection. 136 :289–98. (doi:10.1017/S0950268807009442)

10. Birkegård AC, Halasa T, Toft N, Folkesson A, and Græsbøll K. 2018 Send more data: a systematic review of mathematical models of antimicrobial resistance. Antimicrobial Resistance & Infection Control. 7 :117. (doi:10.1186/s13756-018-0406-1)

11. Martinez JL and Baquero F. 2000 Mutation Frequencies and Antibiotic Resistance. Antimicrobial Agents and Chemotherapy. 44 :1771–7

12. Centres for Disease Control and Prevention. Tuberculosis. 2023 Jun 5. Available from: https://www.cdc.gov/globalhealth/newsroom/topics/tb/index.html

13. David HL. 1970 Probability Distribution of Drug-Resistant Mutants in Unselected Populations of Mycobacterium tuberculosis. Applied Microbiology. 20 :810–4. (doi:10.1128/am.20.5.810-814.1970)

14. Bergval IL, Schuitema ARJ, Klatser PR, and Anthony RM. 2009 Resistant mutants of Mycobacterium tuberculosis selected in vitro do not reflect the in vivo mechanism of isoniazid resistance. Journal of Antimicrobial Chemotherapy. 64 :515–23. (doi:10.1093/jac/dkp237)

15. Werngren J and Hoffner SE. 2003 Drug-Susceptible Mycobacterium tuberculosis Beijing Genotype Does Not Develop Mutation-Conferred Resistance to Rifampin at an Elevated Rate. Journal of Clinical Microbiology. 41 :1520–4. (doi:10.1128/jcm.41.4.1520-1524.2003)

16. Nyinoh IW. 2019 Spontaneous mutations conferring antibiotic resistance to antitubercular drugs at a range of concentrations in Mycobacterium smegmatis. Drug Development Research. 80 :147–54. (doi:10.1002/ddr.21497)

17. Krašovec R, Belavkin RV, Aston JAD, Channon A, Aston E, Rash BM, Kadirvel M, Forbes S, and Knight CG. 2014 Mutation rate plasticity in rifampicin resistance depends on Escherichia coli cell–cell interactions. Nature Communications. 5 :3742. (doi:10.1038/ncomms4742)

18. Spagnolo F, Rinaldi C, Sajorda DR, and Dykhuizen DE. 2016 Evolution of Resistance to Continuously Increasing Streptomycin Concentrations in Populations of Escherichia coli. Antimicrobial Agents and Chemotherapy. 60 :1336–42. (doi:10.1128/aac.01359-15)

19. Huseby DL, Pietsch F, Brandis G, Garoff L, Tegehall A, and Hughes D. 2017 Mutation Supply and Relative Fitness Shape the Genotypes of Ciprofloxacin-Resistant Escherichia coli. Molecular Biology and Evolution. 34 :1029–39. (doi:10.1093/molbev/msx052)

20. Meibohm B and Derendorf H. 1997 Basic concepts of pharmacokinetic/pharmacodynamic (PK/PD) modelling. International Journal of Clinical Pharmacology and Therapeutics. 35 :401–13

21. Regoes RR, Wiuff C, Zappala RM, Garner KN, Baquero F, and Levin BR. 2004 Pharmacodynamic Functions: a Multiparameter Approach to the Design of Antibiotic Treatment Regimens. Antimicrobial Agents and Chemotherapy. 48 :3670–6. (doi:10.1128/AAC.48.10.3670-3676.2004)

22. Neubig RR, Spedding M, Kenakin T, and Christopoulos A. 2003 International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. XXXVIII. Update on Terms and Symbols in Quantitative Pharmacology. Pharmacological Reviews. 55 :597–606. (doi:10.1124/pr.55.4.4)

23. Delaney O, Letten AD, and Engelstädter J. 2023 Drug mode of action and resource constraints modulate antimicrobial resistance evolution. bioRxiv. (doi:10.1101/2023.08.29.555413)

24. Gillespie DT. 1976 A general method for numerically simulating the stochastic time evolution of coupled chemical reactions. Journal of Computational Physics. 22 :403–34. (doi:10.1016/0021-9991(76)90041-3)

25. Team RC. R: A Language and Environment for Statistical Computing. Version 4.3.0. Vienna, Austria, 2023. Available from: https://www.R-project.org/

26. Cao Y, Gillespie DT, and Petzold LR. 2007 Adaptive explicit-implicit tau-leaping method with automatic tau selection. The Journal of Chemical Physics. 126 :224101. (doi:10.1063/1.2745299)

27. Johnson P. adaptivetau: Tau-Leaping Stochastic Simulation. Version R package version 2.2-3. 2019. Available from: https://CRAN.R-project.org/package=adaptivetau

28. Bengtsson H. 2021 A Unifying Framework for Parallel and Distributed Processing in R using Futures. The R Journal 13 :208–27. (doi:10.32614/RJ-2021-048)

29. Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, and Yutani H. 2019 Welcome to the tidyverse. Journal of Open Source Software 4 :1686. (doi:10.21105/joss.01686)

30. Feller W. An introduction to probability theory and its applications. 3d ed. Wiley series in probability and mathematical statistics. New York: Wiley, 1968

31. Wolfram Research Inc. Mathematica Version 13.1.0.0. Champaign, Illinois, 2022. Available from: https://www.wolfram.com/mathematica

32. Salari N, Kanjoori AH, Hosseinian-Far A, Hasheminezhad R, Mansouri K, and Mohammadi M. 2023 Global prevalence of drug-resistant tuberculosis: a systematic review and meta-analysis. Infectious Diseases of Poverty. 12 :57. (doi:10.1186/s40249-023-01107-x)

33. Kempker RR, Kipiani M, Mirtskhulava V, Tukvadze N, Magee MJ, and Blumberg HM. 2015 Acquired Drug Resistance in Mycobacterium tuberculosis and Poor Outcomes among Patients with Multidrug-Resistant Tuberculosis. Emerging Infectious Diseases. 21 :992–1001. (doi:10.3201/eid2106.141873)

34. Ford CB, Shah RR, Maeda MK, Gagneux S, Murray MB, Cohen T, Johnston JC, Gardy J, Lipsitch M, and Fortune SM. 2013 Mycobacterium tuberculosis mutation rate estimates from different lineages predict substantial differences in the emergence of drug resistant tuberculosis. Nature genetics. 45 :784–90. (doi:10.1038/ng.2656)

35. Lazarou J, Pomeranz BH, and Corey PN. 1998 Incidence of Adverse Drug Reactions in Hospitalized PatientsA Meta-analysis of Prospective Studies. JAMA. 279 :1200–5. (doi:10.1001/jama.279.15.1200)

36. Durão P, Balbontín R, and Gordo I. 2018 Evolutionary Mechanisms Shaping the Maintenance of Antibiotic Resistance. Trends in Microbiology. 26 :677–91. (doi:10.1016/j.tim.2018.01.005)

37. McInnes RS, McCallum GE, Lamberte LE, and Schaik W van. 2020 Horizontal transfer of antibiotic resistance genes in the human gut microbiome. Current Opinion in Microbiology. Host-Microbe Interactions: Bacteria 53 :35–43. (doi:10.1016/j.mib.2020.02.002)

Supplementary Figures

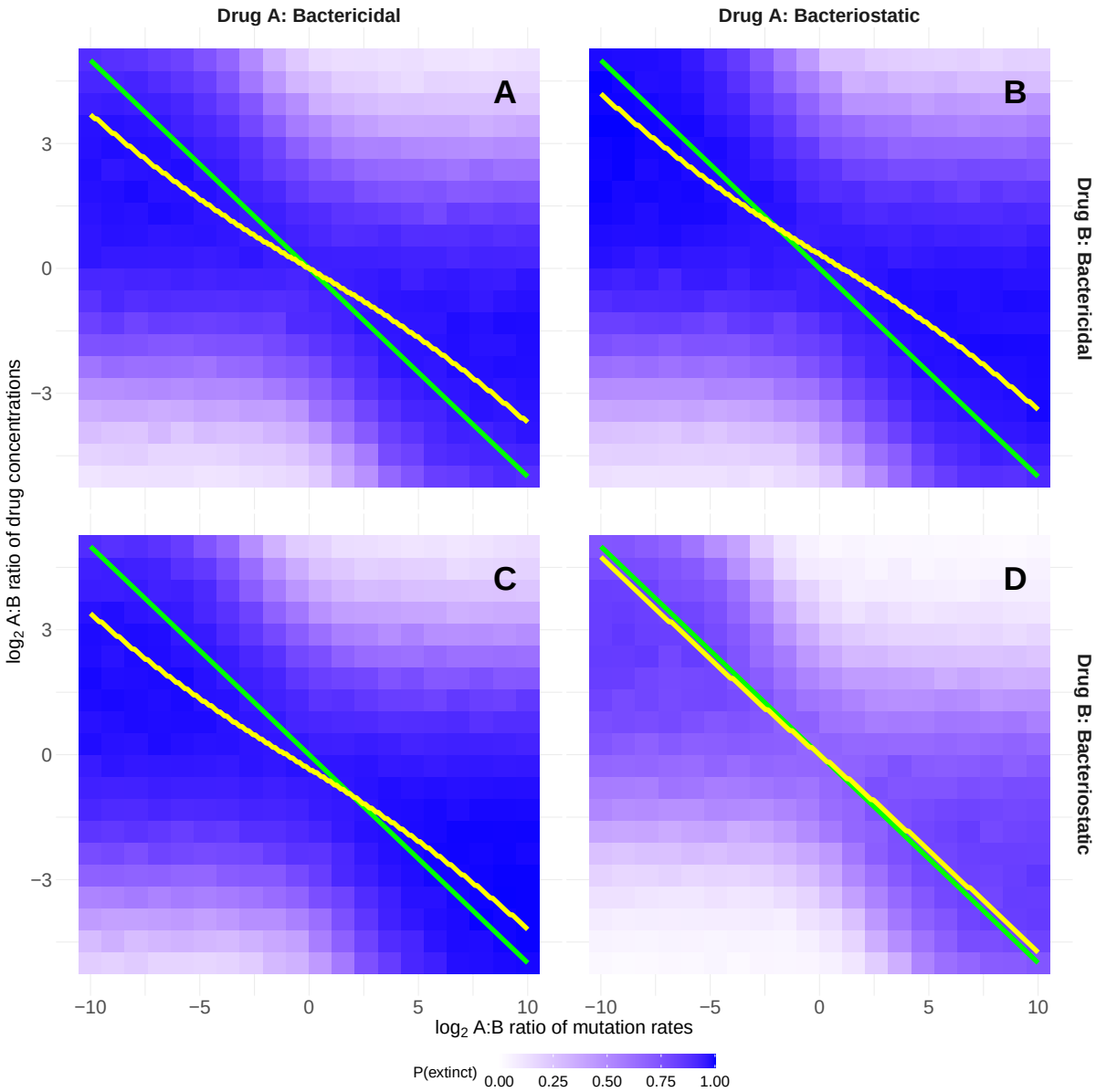

Figure S1: Optimal dosing with mutations conferring incomplete resistance. Everything is identical to Figure 1 except $z_{M_A,B}, z_{M_B,A} \sim 1 + \mathrm{Exp}(0.25)$, sampled independently for each run of the simulation.

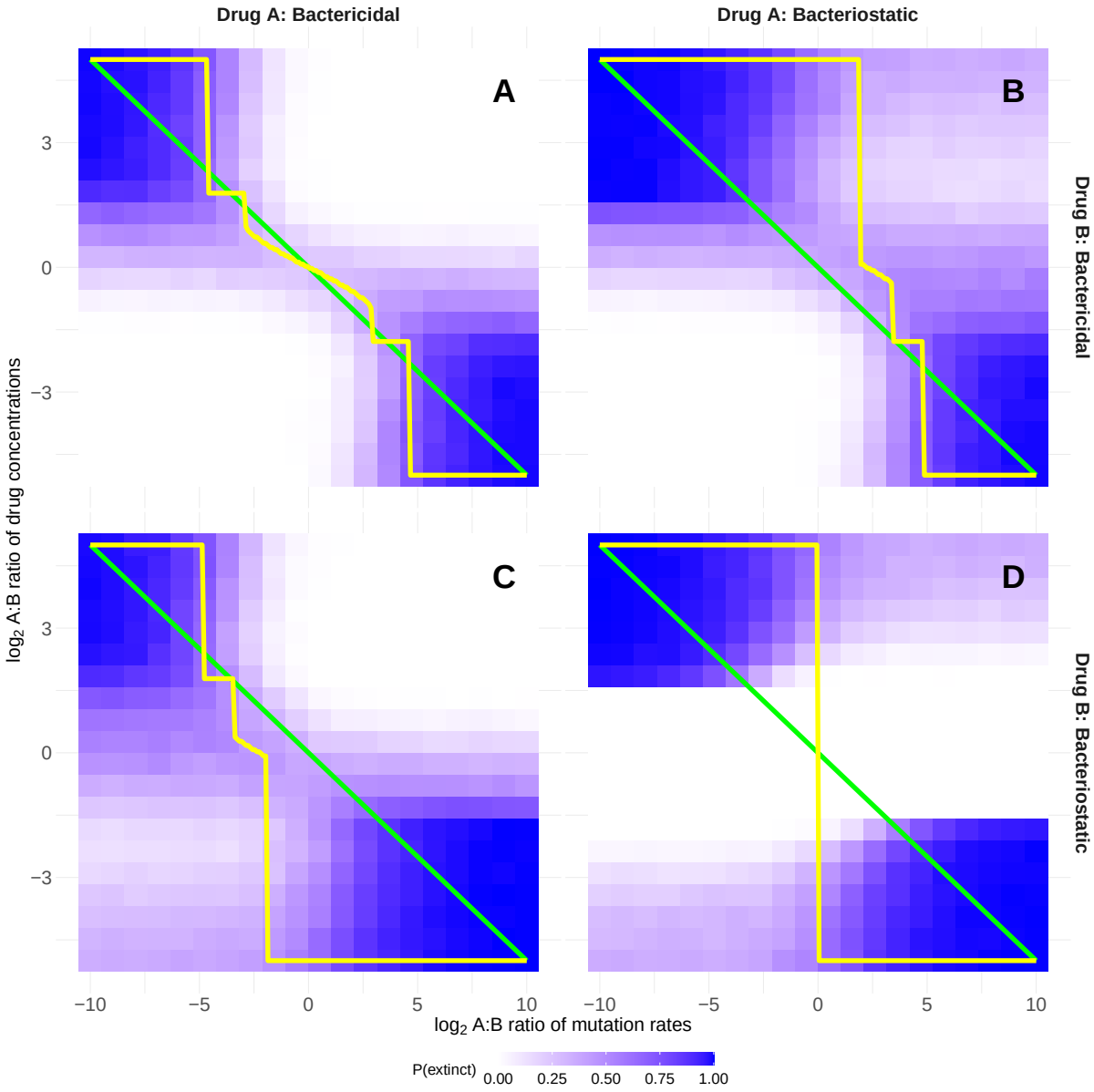

Figure S2: Optimal dosing with a larger shape parameter sometimes entails using solely one drug. Everything is identical to Figure 1 except $\beta_A = \beta_B = 3$ and $\delta = 0.19$.

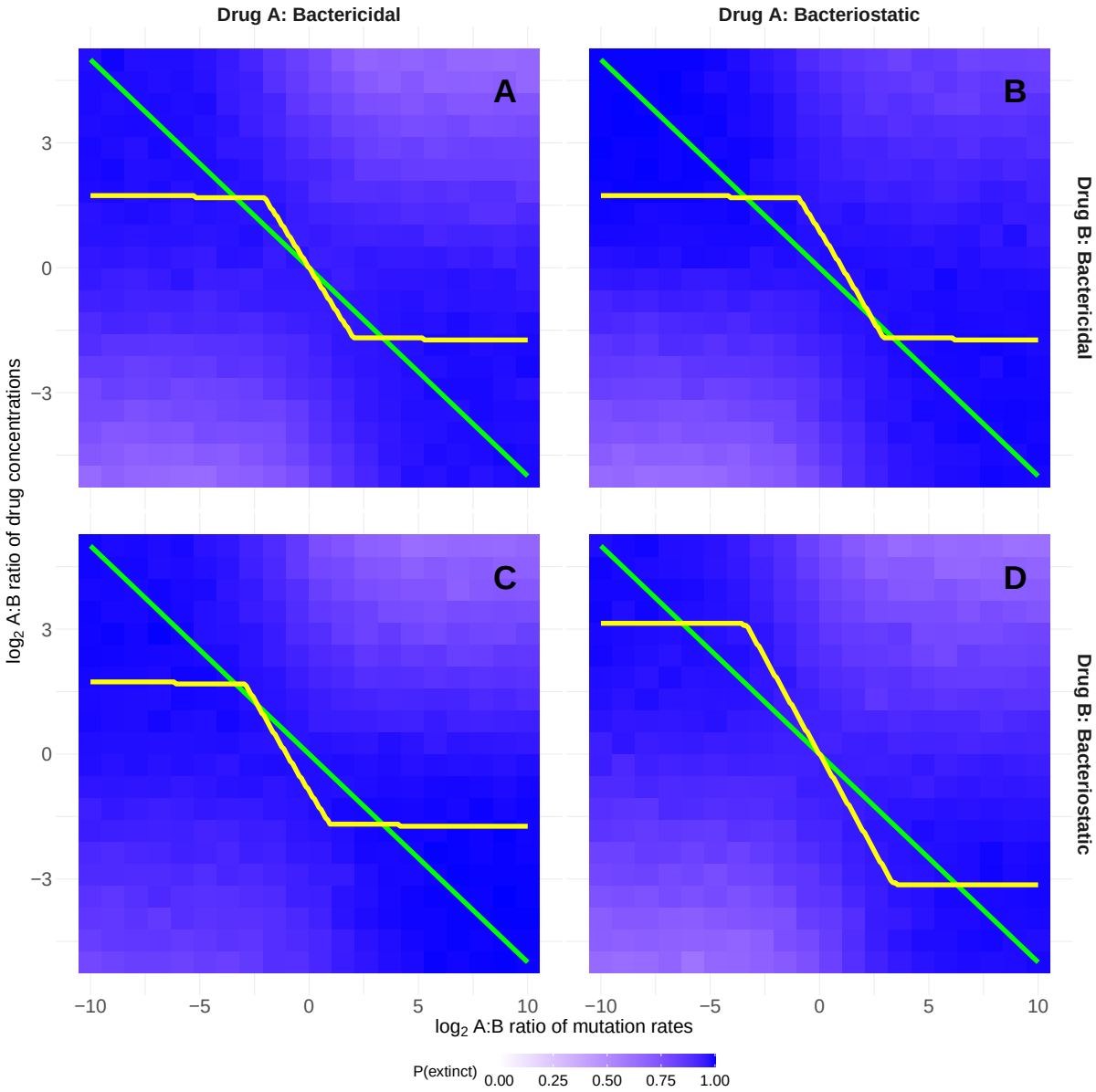

Figure S3: Optimal dosing with a smaller shape parameter always includes non-zero amounts of both drugs. Everything is identical to Figure 1 except $\beta_A = \beta_B = 0.2, \delta = 0.47$.

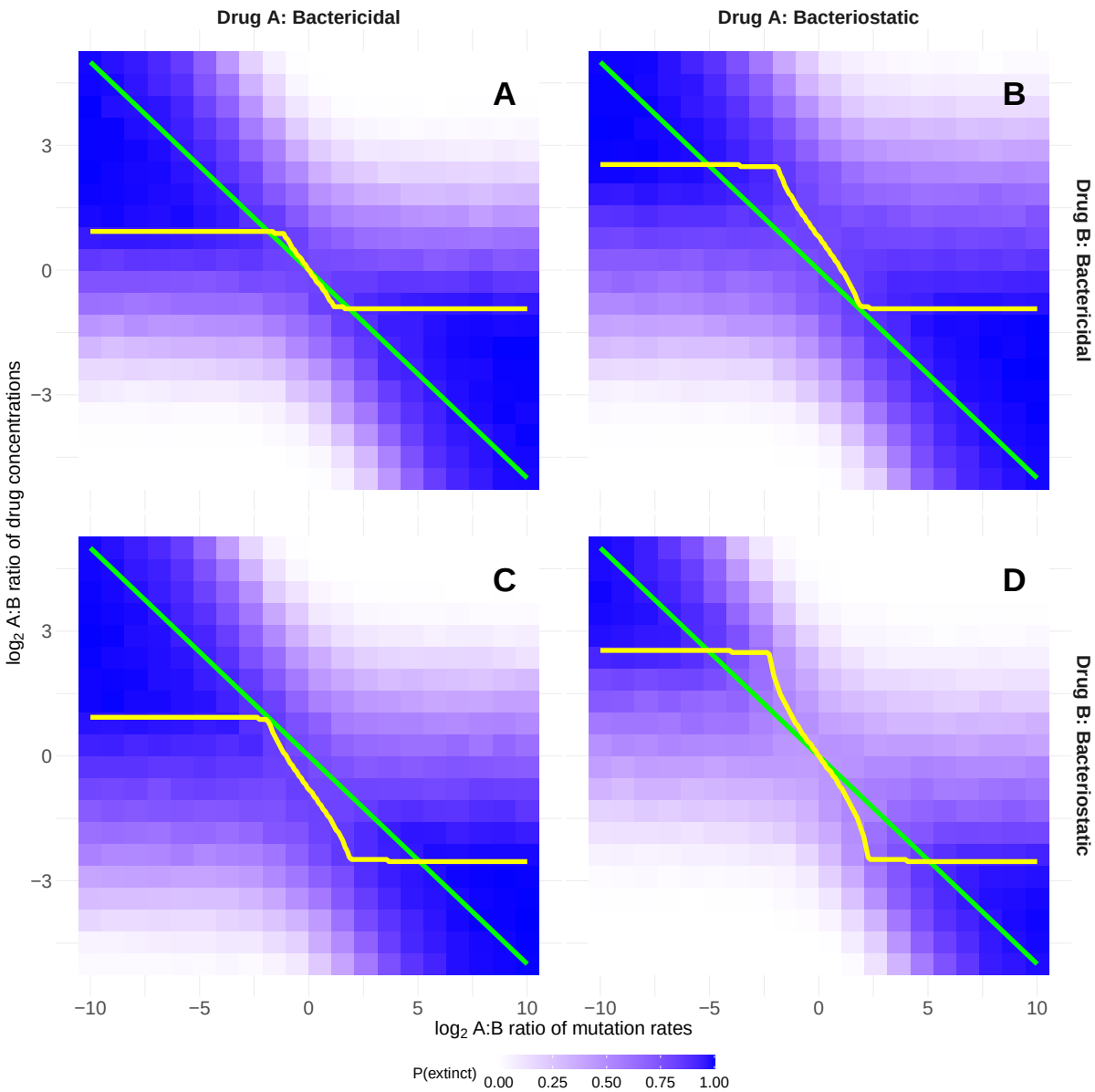

Figure S4: Optimal dosing with costs of resistance always includes non-zero amounts of both drugs. Everything is identical to Figure 1 except $g_{M_A} = g_{M_B} = g_S - 0.1 = 0.9$.

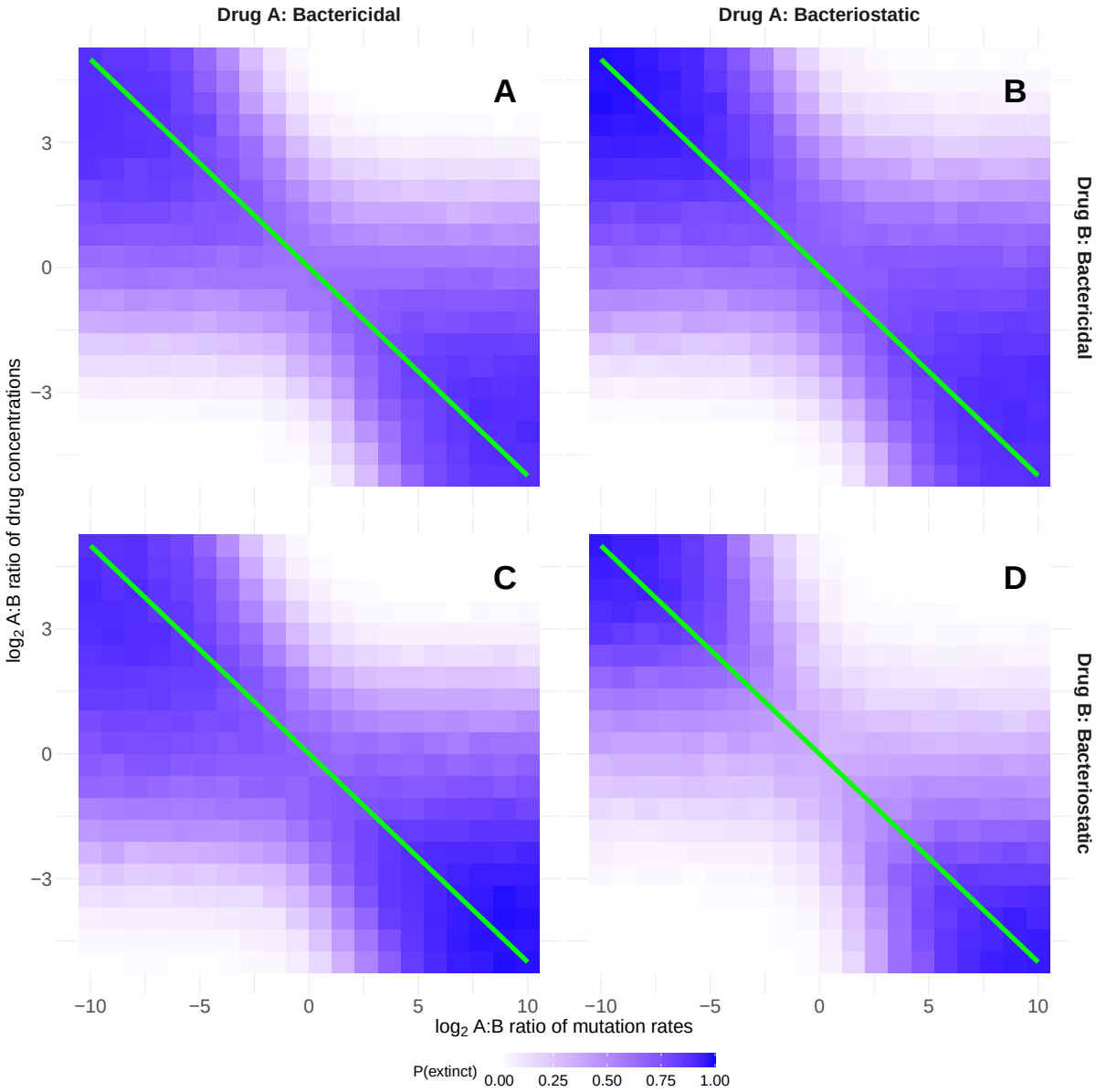

Figure S5: Optimal dosing with pharmacokinetics. Everything is identical to Figure 1 except a drug decay rate of 0.15 has been introduced with doses every 12 hours of both drugs, meaning that $e^{-0.15\times 12} = 17\%$ of the previous dose remains at the next dose. To compensate for the drug decaying, the intrinsic death rate has been increased to $\delta = 0.53$. The yellow theory lines are not shown here, as the theoretical analysis only dealt with constant drug concentrations. The original green lines are still shown for comparison.