March 20, 2025

Dear Professor Lambert,

Thank you for taking the time to review our manuscript again.

As before, we have attached a tracked changes version of the manuscript (compared to the previous revision, not the original submission). We also respond point by point below.

Yours sincerely,

Oscar Delaney, Andrew Letten, and Jan Engelstädter

Response to Recommender

- line 97 "if antibiotic treatment is started early by the human host before pathogenic bacteria have reached the resource limits of their niche, our model could be approximately accurate." I am not sure a model can be 'accurate' and I don't think anything can be 'approximately accurate', please reformulate

Good point, replaced with 'more realistic'.

- lines 85 and 100-101: can you specify that total resistance is equivalent to $E_{M_A}(C_A) = E_{M_B}(C_B) = 0$, which is basically equivalent to z_{AA} and z_{BB} infinite?

We have added an explanation on line 87:

Here, complete resistance ($E_i(C_j) = 0$ for any C_j) would arise with $z_{M_A,A}, z_{M_B,B} \to \infty$.

- lines 107-110, I understand your reasoning concerning the gates AND and OR, but I am not sure I am convinced by their translation in terms of birth and death rates. I think I can understand (in terms of competing exponential clocks) that it is equivalent to assume that the effects of drugs on death rate are summed and to say that 'it is sufficient for either drug to cause its death'. But it does not seem equivalent (to me) to assume that the effects of drugs on division rate are multiplied and to say that 'for a cell to divide both pathways impacted by the drugs must remain functional'.

Yes, we think the logical gates analogy isn't perfect, but might help some readers so we would prefer to leave it in. We have added a clarification (lines 108-10) that this isn't the only reasonable modeling choice. Not all drugs would work like this (perhaps some drugs target interacting pathways, and cannot be simply modelled as the product of their combined effects) but for a simple model we prefer this easy additive/multiplicative distinction.

- line 123 'to make evolving this stochastic system computationally feasible': I'm not sure it is the right way to justify using tau-leaping – the system is really simple to simulate with a classical Doob-Gillespie algorithm. It's more that you speed up simulations this way.

Exactly, we suppose it would have been feasible in some theoretical sense to do full Gillespie algorithm simulations, but with our modest compute setup and limited software optimization and cloud computing expertise we wanted a fast/simple option. We rephrased to 'less computationally intensive'.

- line 136, $z_{S,A}$ and $z_{S,B}$ are specified, but nothing is said of the 4 other z's. Also please give the units of μ and δ.

Thank you, we have now specified the other z values (complete resistance means they are infinite in the resistance case) and noted that μ and δ are per replication and per hour respectively.

- lines 147-149, 'The more important point for this simple theoretical model is to use parameter values that highlight biologically relevant phenomena, rather than using maximally likely parameter values'. I'm not sure to see what you mean. What are parameter values that 'highlight biologically relevant phenomena' if these parameter values are not 'likely'? Do you mean parameter values do not need to be finely tuned, but only need to be in the right ballpark?

Good point, we have reworded that explanation (lines 148-51) to note that the key factors in choosing parameters are 1) making interesting things happen in the model, 2) being roughly plausible, 3) preferring nice round numbers like 1 where possible over and above 4) finely tuning parameters to the latest empirical observations for a particular experimental setup.

- line 171, you should explain the link between gambler's ruin and probability of extinction (or just explain that (7) can be obtained directly by saying that extinction does occur iff at each division event, we don't have both a mutation event and the survival of this mutant)

True, not everyone will be familiar with Gambler's Ruin, so we have explained the setup and the connection to our case a bit more in lines 173-76.

- line 216, what do you mean by 'directionally plausible'?

Reworded to "plausible simplifications". We just meant that something in the direction of the simplifying assumptions we make is often true, though of course not as elegantly as in the equations.

- Equation 14, I'm not sure the three dots (forming a triangle) are canonical to symbolize logical implication

Sure, the therefore symbol doesn't add much, we removed it.

- line 226, 'if only A or B is used', please add 'that is, if C_A or C_B is zero'… (makes more clearly the link with the previous case when $C_A = C_B$)

Good idea, done.

- line 228, alternative formulation: 'the survival probability of a mutant is more sensitive to an increase in concentration of the drug to which resistance mutations arise more frequently'

Thank you for this suggestion, we have used this clearer phrasing.

- Equation 15, I agree it is nice to have this relationship between C_A/C_B and μ_A/μ_B, however, this formulation does not highlight the dependence on C of the result, and suggests that there is a degree of freedom in the choice of C_A and C_B. It would be clearer to first state that at the optimal combination, we have $C_A = C/(1 + \sqrt{\mu_A/\mu_B})$ and $C_B = C\sqrt{\mu_A/\mu_B}/(1 + \sqrt{\mu_A/\mu_B})$.

That makes sense, we have now split out the equations first in the C_A and C_B absolute forms you suggest, and then afterwards give the ratio forms.

- The paragraph starting line 259 is somewhat obscure to me. We read that 'As resistance becomes weaker (from the earlier unrealistic supposition of total resistance), the two resistant strains become less perfectly adapted to their respective drugs', which means that $E_{M_A}(C_A)$ and $E_{M_B}(C_B)$ should be nonzero. It would be good to say that now $z_{M_A,A}$ and $z_{M_B,B}$ are finite (otherwise it is difficult to make the link with the case of total resistance). Also please change the caption of Figure 2 and S1, which only mentions $z_{M_A,B}$ and $z_{M_B,A}$. We read that these two values are exponentially distributed with expectation ζ, but it is not exactly true, they're equal to $1+$ an exponential rv with expectation $\zeta - 1$: this implies that they are always larger than 1; please explain why (I guess you want M_A to be more resistant to B than S, but this is not straightforward for the reader and this should maybe be justified biologically?).

Thank you for highlighting these issues with the paragraph on partial resistance. We have substantially revised this section to make the modeling approach clearer. We have now explicitly stated that we are relaxing the assumption of complete resistance (where $z_{M_A,A}$ and $z_{M_B,B}$ were infinite) to allow for partial resistance with finite EC_{50} values. We have clarified that these values are drawn from a shifted exponential distribution ($1 + \text{Exp}(1/(\zeta - 1))$) with mean ζ, ensuring they're always greater than 1 (the susceptible strain's EC_{50}). This shift is a modelling convenience that is biologically justified since mutations that increase drug susceptibility would be rapidly outcompeted. The exponential distribution shape reflects the biological reality that most resistance mutations confer weak resistance, while few confer strong resistance, as supported by the cited literature (Igler et al. 2021).

We've also revised the captions for Figures 2 and S1 to correctly indicate that all EC_{50} values for the mutant strains $z_{M_A,A}, z_{M_B,B}$ are drawn from this distribution. The earlier caption had an outright error that it was the cross-resistance values $z_{M_A,B}, z_{M_B,A}$ that were drawn from the exponential distribution. In this model, cross-resistance is still ignored.

- Figure 1, please specify that the yellow and green lines are confounded in panels A and D (yellow is not visible). It is surprising that the yellow curve of optimal dosing is always so straight (there is no reason why it should be a line a priori) and even confounded with the green line in panel A: are you sure there is no mathematical result available when the drugs are not necessarily bacteriostatic? (see also comment by Reviewer 1 in 1st round)

As we note in lines 242-44, the nice mathematical relationship for bacteriostatic drugs also holds for both bactericidal drugs, just the intermediate steps are (a lot) more complicated, which is why we put them in the Mathematica file available on Github. This does indeed mean that the yellow and green lines are expected (required) to coincide in panel A as well as panel D, and we now point this out in line 249. As for whether it is surprising the yellow line is straight (in log-log space that is), we have shown mathematically it is straight with our simplifying assumptions when both drugs have the same mode of action, and so we suppose it is not that surprising it is also straight just slightly shifted in the bactericidal-bacteriostatic case.

- lines 295-304, can you be more specific about how you model cost? Otherwise, it is difficult to understand/believe your quite clear-cut conclusion that 'too intermediate dosing strategies are sufficient to ensure $P_E \approx 1$ even for very skewed mutation rates'.

We modelled fitness costs simply by reducing the replication rate of the mutants by 10%. We have now made this explicit (lines 310-11). As for why this leads to intermediate dosing strategies working well, it is not that these strategies are unusually good, it is more that because the replication rate is lower, there is more liberty in what dosing strategy you use, as more or less anything reasonable will work for very skewed mutation rates (now explained in lines 315-18). That is, if there is e.g. a 50:1 skew in the mutation rate, then using anything above perhaps a 10:1 skew in dosing ratios is sufficient (numbers

approximate). We have added a sentence at the end of this paragraph to further explain our reasoning.

- line 318, 'This suggests that ignoring pharmacokinetics (as in the analytical solution) is not a fatal flaw'. Please reformulate.

Done, now rephrased as "[...] does not undermine its real-world applicability". We just meant that the analytical solution is still useful despite ignoring pharmacokinetics.

- line 319, 'Each replication event uses one arbitrary unit of resource, and the simulation begins with 10^9 units of resource, with a constant influx of $10^8 h^{-1}$. The maximum growth rate is now given by the Monod equation, with a resource affinity constant of 10^8'. Can you expand a little bit the paragraph starting here, explain your modeling assumptions, give symbols to these parameters (units, influx and affinity of resource), provide an equation for the effect on growth rate, explain your choice of parameter values and give an idea about how your results are robust or not to these choices? You say that 'the basic relationship between mutation rate and optimal dosing concentrations persists' but without a yellow curve, what we see is mainly the negative relationship between C_A/C_B and μ_A/μ_B, which is expected.

Thank you for suggesting this expansion. We have added more detailed explanations of our resource-based modeling approach, including the formal equations governing resource dynamics and growth rates. While we don't show the theoretical yellow curve on Figure S7 (as it was derived for resource-unlimited conditions), as you note the computational results still show the same fundamental negative relationship between optimal drug ratios and mutation rate ratios. The primary effect of resource limitation is to reduce the total number of replications before antibiotics take effect, which decreases the likelihood of resistance mutations arising and increases extinction probabilities across all scenarios.

- line 333, 'less beneficial' => more hazardous/risky

Done.

- line 338 'but in several cases the dosing skew should never be raised above some maximum value.' I must have missed something, can you specify which of your findings you are referring to here?

Good point, we have deleted this. What we were referring to is that the dosing skew need not be raised above some level for optimal results (as in figures S3 and S4). But it is not the case that it 'should not' be - all dosing skews above a certain level are equally good in those cases.

- line 351, 'Our choices of functional forms for the drug-dependent mortality and replication rates in Equations 2 and 3 were crucial for the results that followed.' Are you speaking of the mathematical results? Otherwise, it is a little worrying that these choices are crucial.

Yes exactly, the analytical/mathematical results only work with those neat functional forms, we have clarified this now.

- line 366, 'As a result, unlike with Bliss independence, skewed drug dosing ratios do not clear the infection slower.' It is not clear to me how you conclude this from the previous considerations

In essence, the Loewe additivity model does not entail the same tradeoff between maximising the bacterial death rate and minimising resistant mutants, as the two drugs are interchangeable in this model. We have improved the explanation in lines 406-09.

- line 373, 'in reality, there is no sharp cutoff', please recall here why your assumption that the total drug dose is smaller than c can be seen as a cutoff

We specified that the cutoff is at c, and that in our model using higher doses than that is not allowed (lines 418-19).

- line 408-409, if you want to interpret mutations in your model as HGT, you have to consider the rate of HGT within a host; under mass-action, the (per susceptible pathogenic bacterial cell) rate should be proportional to R, where R is the number of (commensal) bacteria carrying the resistance gene already present within the host, which itself depends in a nonlinear and time-dependent manner on the number, denoted I in this paragraph, of hosts 'infected' by this bacteria (see also comment by Reviewer 1 in 1st round). I understand that this rate is somewhat external to the dynamics you are studying, and does not interfere with it, but you should be a little more cautious when you say that it is approximately constant – it is at least time-dependent.

We completely agree that caution is warranted here, and we have added a sentence reflecting this. Also, we would like to clarify that I referred to the donor population of bacteria, not hosts infected. (Our model is a purely within-host dynamical model.) The choice of I was motivated by the fact that many readers may be familiar with the SI model of pathogen (between-host) dynamics, but we have now changed the letter to D to make it clear that we are referring to the number of resistant commensal bacteria that can act as donors of the resistance gene.

- line 414-415, 'Thus, using a drug to which the commensal bacteria is susceptible could make resistance considerably less likely to evolve.' You're basically saying that due to possible HGT, it is better to use a drug for which there are no resistance genes already present in the microbial community. I'm not sure this is worth saying.

Agreed, we have now deleted this and the preceding sentence.

- Figures S3-S4: please say a word in main text or caption how to interpret the Z-shaped yellow curves. In Figures 3 and S2, you explain that beyond a certain threshold value of mutation rate ratio, the best strategy is to use only one drug. But what's happening in Figures S3 and S4?

Good point, this was inadequately explained previously. We have added some explanatory text in the S3 caption. And also, in response to a previous suggestion, we added lines 315-18 interpreting Figure S4 and the costs of resistance case. In both cases, the issue is that extinction can be overdetermined and thus not be affected by how extreme a drug dosing ratio is used (beyond some level).