November 21, 2024

Dear Professor Lambert,

Our condolences regarding your difficult summer.

Thank you for your, and the two anonymous reviewers', thoughtful comments on our manuscript, which helped us make our work more thorough and clearly explained. We have attached a tracked changes version of the manuscript (note though that equations do not work well with the changes package, so equations are shown in normal text even if they are new, and deleted equations are not visible at all). In what follows we will respond to each point in the three reviews, with reviewer comments in black and our responses in blue.

Yours sincerely,

Oscar Delaney, Andrew Letten, and Jan Engelstädter

Response to Recommender

You have made a noticeable effort in relaxing some simplifying assumptions, except for one which seems particularly important. In the manuscript, the growth rates of bacterial populations are always constant - there is neither competition between populations nor time inhomogeneity due to drug elimination (see comments by Referee 1). Please assess the domain of validity of your results in relation to (at least one of) these assumptions.

Note that in our original submission the results shown in Figure S5 (now S6) did include pharmacokinetics, and therefore had non-constant growth rates. Regarding resource constraints to growth, this is a good point. We could not think of a way to extend our analytical approach to include resource constraints, but we have added this to our simulation, with results in Figure S7 and text in lines 319-25 and 390-400.

This point seems to be addressed in another preprint of yours cited in the manuscript. Since both manuscripts are rather short, you might consider merging them, as proposed by Referee 2.

Thanks for this suggestion. We have ultimately decided to keep the manuscripts separate. The main reason is that this paper deals with quite a focused, tightly-scoped question – the optimal dosing ratio of two drugs applied in combination when mutation rates vary – while our other paper considers monotherapy, cycling therapy, and combination therapy, and focuses on drug mode of action. Furthermore, after incorporating many of the useful suggestions in this review process, this manuscript has expanded notably in terms of the text, the figures, and the reference list, which makes us think it is better suited to present independently.

Please be more diligent in citing and comparing your work with existing literature on the topic of antibiotic resistance evolution (e.g., works cited by Referee 2). Also please provide more references from the "significant body of empirical literature" on combination therapy (line 28).

We have added more references throughout, including those recommended by Referee 2, and in the line you mention. In particular, we consider several theory papers relevant to drug mode of action in lines 43-50, and discuss the competitive release literature in lines 390-400, among other added citations.

You could easily address several comments of both referees, and at the same time improve clarity, by expanding a little bit your presentation of the model and of the consequences of your simplifying assumptions regarding parameter values : First, assuming that the β's and the ϕ's are all equal to 1, $G_S = g/((1 + C_A)(1 + C_B))$. Then your assumption that S and M_B both have zero net growth rate when $C_A = 0$ and $C_B = c$ implies $g = \delta(1+c)$ and $G_A = g/(1+C_B)$ (similarly with M_B). You should also say that under these assumptions, whenever $C_A + C_B = c, G_S < D_S$ and $G_A > D_A$ (similarly with M_B);

Thank you for these suggestions. We agree that pointing this out improves clarity, and we have added this in lines 210-12.

Second, you should write the calculations that lead to (13) and emphasize in particular (see comment by Referee 1) the intermediate step showing that (up to the dividing factor $1+c$) you have that $\mu(1-P_D) = \mu_A C_A + \mu_B C_B$ and that (the expectation of) $N_r = S_0/(C_A C_B)$.

This is an excellent point, we now use Mathematica only for the more complicated bactericidal case, and manually show some key steps in the bacteriostatic case (these are now equations 12-14).

The division (or birth) rate of bacterial cells is called growth rate and denoted G. It is more common to define the growth rate as the difference between birth and death rate.

We now use G for 'net growth rate', R for the replication rate (as B is already one of the drugs), and D for death rate.

Note that (11) can be obtained directly by saying that extinction does occur if at each division event, we don't have both a mutation event and the survival of this mutant;

Excellent point, this is a far simpler derivation, we have updated the manuscript accordingly in lines 180-88 and equation 10.

Note that because you assume that the exponential decrease of S is deterministic, you have that: 1) N_r has a Poisson distribution with parameter given by the rhs of (8); 2) because N_r is a Poisson variable, equation (12) holds without approximation by taking the expectation of (11) (wrt the law of N_r), which results in (12) after replacing N_r with its expectation (i.e., the parameter of its law).

Thank you for pointing this out. After careful consideration, we have retained the original approximation here, as it is very accurate given the small mutation probability at each replication. This seems simpler than using probability generating functions (but please let us know if we have misunderstood the suggested approach).

Response to Reviewer 1

All in all, I think the paper is clear and presents an interesting model on an important problem. But I am not sure of the generality of the findings. First of all, the main result depends on somewhat hidden assumptions on the interaction between the two drugs for birth and death (defined in equation 2).

This is an excellent point. The reviewer is correct that our result rests on the specific functional form we chose. We have now provided some rationale in the Methods section (lines 101-10) about why we think that particular functional form is a reasonable choice. In brief, it is reflective of Bliss independence,

whereas our impression is that the reviewer is considering Loewe additivity. We discuss the importance of this functional choice in lines 351-68 and equations 1'-3'. As such, we would argue there isn't a hidden interaction in our model, but rather it is a null model when viewed from a Bliss independence perspective.

Second, the assumption that there is no competitive release of the resistant strains is not discussed (mentioned line 68, "ignored resources constraints to growth"). That is, growth is not density limited and therefore does not depend on the total density of bacterial cells, which is expected to decline strongly in the presence of treatment. Competitive release is an important biological phenomenon and as such is most often incorporated in within-host model of AMR evolution. For example, competitive release can make resistance harder to evolve when drug dose is low, because sensitive cells are still at sufficiently high density to competitively inhibit the growth of resistant strains (see the papers by Troy Day, Andrew Read, and collaborators). Competitive release may thus change the results but this is not discussed.

Another excellent point. In lines 91-97 we explain our choice to keep the analytical model without resource constraints, and why that may reflect some realistic circumstances. And as mentioned earlier we now introduce resource constraints in the simulations component, in lines 319-25 and Figure S7. We have added some discussion of competitive release in particular in lines 390-400.

For convenience, I list below the assumptions that are made in the paper and how they are relaxed (or not):

Assumptions in main model:

0) Implicit assumption on the interaction between drugs, embedded in the function forms defined in equation (2) -> not relaxed, briefly mentioned

1) absence of competitive release -> not relaxed, not discussed

2) the mutation rates mu_A and mu_B do NOT depend on antibiotic concentration -> relaxed by exploring distributed z value.

3) the efficacy and toxicity of both drugs are the same (lines 89-90) -> discussed around line 232

4) the "toxicity function" implies that total concentration is limited to exactly c (= sharp cutoff above which it is too toxic) (discussed lines 235)

Extra-simplifying assumptions for the simple analytical result:

5) Resistant strains are UNAFFECTED by arbitrarily high drug concentration(line 129-130) -> important, relaxed later

6) Shape parameters beta are unity -> relaxed

7) No cost of resistance -> relaxed

8) Bacteriostatic (not needed I think? See comment below)

Generally, I believe it would be useful to present the outcome a null model where the drugs don't interact by default for growth (as is the case currently), and in so doing relax assumption (0). Relaxing

assumption (1), or at least some discussion of this assumption and how it limits applicability of the results, would also be necessary.

We thank the reviewer for laying out these assumptions clearly. We respond to the 'hidden interaction' assumption 0 above, and likewise have added some discussion of competitive release, as noted above.

Another, linked general comment: the probability of extinction of the bacterial population depends solely on ratios D/G, as both the P_D and the N_r only depend on ratios D/G. Is there scope to use this analytical result to build a bit more intuition about the consequences of equation (12)? In particular, if drugs A and B do not interact (a distinct assumption than that of the authors), I expect N_r to be independent of the doses of C_A/C_B (only depend on total c) and therefore effects of C_A/C_B only emerge from impact on P_D. What happens in that case? Exploring this for generic forms of the $(D/G)(C_A)$ function may be conceptually useful. In general, it would be helpful to delineate what in the result emerges from the P_D term, vs. the N_r term.

We agree with the reviewer's intuition that under Loewe additivity – what the reviewer is referring to as 'no interaction' – N_r (which we have replaced with $\mathcal{N}$ now to not confuse with R for replication rate) would depend only on c. We now explain this in lines 361-68.

14: optimal, please define wrt what criterion

Done, we specify this as choosing the dose ratio 'that maximises the chance of treatment success' in line 14.

27 "synergistically" -> indeed I understand that there is some form of synergy in the model, please state so.

See earlier explanation for Bliss independence, under which there is not an interaction.

28 "Combination therapy is supported..." please specify in which type/context of infections, what pathogen.

We now give citations for tuberculosis specifically, and an experimental evolution setting in lines 29-30.

Around line 33, could introduce here the caveat that in many cases, resistance is introduced not by de novo mutation, but acquired by transmission of a resistant strain, or evolved by horizontal gene transfer (the latter is discussed).

Thank you for this suggestion, we have added this point to the text in line 37.

The authors might find this reference interesting: `https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1002299`

Thanks for the suggestion, we have added this to the introduction in lines 56-57.

Line 45, the cdc webpage referenced for Mtb no longer exists as of 16th July.

Good catch, we have updated this to a WHO reference in line 58.

Table 1: please specify the unit of mutation rate estimate (per generation? genome-wide?). To what antibiotic dose does it correspond?

We have now added the drug concentrations used in Table 1, and clarified that we are using genome-wide resistance mutation probabilities per replication.

Line 66, please clarify whether ϕ can vary continuously between 0 or 1, or if it is always exactly 0 or 1.

We use $\phi \in \{0, 1\}$ only, and have clarified this in lines 90-91. Intermediate values could also be used, but this would unnecessarily complicate the model for limited analytical benefit.

Equation (2): the implicit assumption of synergy between drugs built in equation (2) should be commented more. For example, with $\beta_j = 1, z_{i,j} = 1$, the growth rate with $C_A = 1$ and $C_B = 1$ is equal to 25% of the growth rate without drug. The growth rate with $C_A = 2$ and $C_B = 0$ is equal to 33% of the growth rate without drug. Thus, there is a built-in assumption that the two drugs are not equivalent and that it is best to apply some of the two drugs (and not exclusively one drug). It would also probably be clearer to compare explicitly with a model without such assumption, that is one where applying C_A or C_B is equivalent in terms of effect on growth rate (e.g. $G_i = g_i * exp(-(C_A + C_B))$).

As discussed above, we have added justification for our choice of functional form, and an explanation about Bliss additivity vs Loewe independence.

Line 75 I was surprised to read that the ODE model is actually never used. Would be clearer to define the model in terms of individual reactions (birth and death) and then explain how this is implemented. There is no need to formulate an ODE model at all, if it is not used.

Good idea, we have now added Table 2 with the individual reaction rates for the Gillespie algorithm. However, we chose to also retain the ODE model as many readers will be more familiar with this type of model and find it useful. We are now careful to point out that the ODE represents a deterministic version of our model, but that we then move on to consider a stochastic version of this model (lines 114-22).

Could specify below equation (4) that the mutation rates mu_A and mu_B do NOT depend on antibiotic concentrations, and this assumption is relaxed later on. Again this is a critical assumption, as resistance to high drug concentration is less frequent.

Good point, we now mention this in lines 113-14.

Line 87 I suspect the actual value of c is quite important, as this will determine the domain of non-linearity of the E function in which C_A + C_B is. Would be good to test several alternative values of c (e.g. lower / higher than 2)

Excellent point, we have now added Figure S5 using a higher total drug concentration of $c = 5$ and note in lines 205-15 that the main result is unchanged.

Line 89-90, "drug concentrations are scaled to be in units standardised to the potency of the drug in question" -> this together with C_A + C_B determines toxicity, loses some generality, correct? That is, the efficacy and level of toxicity of both drugs are the same. Would be good to state this assumption explicitly here (it is discussed around line 232).

Good point, we have now stated this assumption explicitly in line 135.

Line 94-96: I think this is the key assumption explaining the result: the shape of the E(.) function means it is not optimal to use only the less evolvable drug. But this is somewhat hidden at the end of

the Method paragraph, would be good to place it more prominently when results are derived, and in the discussion.

We agree with this analysis, and now reiterate this point in lines 241-44.

Equation 5: Would be nice to give an explanation of the $mu_A/(mu_A + mu_B)$ terms.

Good point, we now do this in lines 160-62.

Equation 11: Would be nice to give a bit of interpretation of this simple equation

As suggested by the recommender, we have now simplified the derivation of this equation (now numbered 10), and give a more intuitive explanation in lines 185-88.

Line 136-138: I do not understand the condition that when only drug A is applied ($C_A = c, C_B = 0$), then net growth rates of the susceptible and B-resistant strains are both zero: $G_S - D_S = G_{M_B} - D_{M_B} = 0$. If the antibiotic is bacteriostatic, then I expect $G_S = 0$ and $G_{M_B} = 0$ in these conditions, i.e. growth is totally inhibited. In which case $G_S - D_S$ and $G_{M_B} - D_{M_B}$ should be negative. Please clarify this condition in relation to the fact that the drugs are bacteriostatic.

Bacteriostatic drugs are not fully effective, so there can still be some replications in the presence of a bacteriostatic drug, just at a lower rate. We have added a sentence clarifying this in the manuscript (lines 210-11).

Line 154-156: I don't understand why the result on bacteriocidic drugs is deprecated (e.g. 'bacteriostatic' is mentioned as an assumption line 135): if the result also holds mathematically for bacteriocidic (and even if intermediate steps are more complicated), this is a result just a strong as that for bacteriostatic.

Good point, we have now made the bacteriostatic explanation purely analytical, and only use Mathematica for the bactericidal case. Because of this, it still makes sense to discuss them separately, even though the end result is the same.

Line 161: could explain briefly why value of P_E is lower for bacteriostatic (this build intuition for why optimal dosing strategy is biased towards the bacteriostatic drug).

We now provide some intuition regarding this in lines 253-55.

Line 176: what is z_W? Is it xi?

Yes, this was a typo, we now use the correct ζ instead (line 271).

Line 177 it was not fully clear why the optimal strategy tends to equal amounts of both drugs; if both drugs are ineffective, why would it matter to apply 50-50 of both drugs?

We have added some explanation in lines 273-75, that for increasingly ineffective drugs the overriding consideration is reducing $\mathcal{N}$ rather than increasing P_D, so using equal concentrations of the two drugs is better.

Line 214 again here, would be nice to write the intuition explicitly.

Done, lines 333-34.

Line 260 I think it is incorrect that "the process of HGT would be equivalent to the process of mutation", since mutation depends on density S but HGT from another species does not.

In population-level models, HGT is usually modelled as an epidemiological process with density-dependent transmission (also referred to as mass-action assumption) (see for example the classic paper by Levin [1] or the recent review article by Hernández-Beltrán [2]). That is, the total rate of gene transfer is a term of the form βSI, where S is the number of recipients (e.g., plasmid-free bacteria) and I is the number of donor bacteria (e.g., those carrying a plasmid). If I is constant, then βI can be merged into a single parameter quantifying the per-capita gene acquisition rate, and this parameter would then become equivalent to a mutation rate. We have added a clearer explanation of this in lines 408-11.

Figure S5: it would be still nice to show the yellow theoretical line even if the theory is for constant drug concentration

The theoretical analysis only dealt with constant drug concentrations, so the yellow line would be misleading to include here and easy to misinterpret, so we feel leaving it out is clearest. The green line is still available for reference.

Response to Reviewer 2

Lack of literature citations: There is huge literature on modeling the evolution of antibiotic resistance. Yet, out of 36 papers cited in their work, there is only one theoretical work on resistance evolution (Nyhoegen & Uecker, 2023) and two reviews on mathematical models. In addition to not doing justice to all the colleagues working on this topic, not mentioning previous theoretical studies on modeling resistance evolution does not help the reader identify what this work brings to the field. It is a bit disappointing that the discussion contains no comparisons to previous works. For example, Equation 12 has a similar form as the rescue probability of populations facing environmental changes, which makes any comparison with results in evolutionary rescue relevant. There are other works that quantify the probability of resistance evolution, to which it might be interesting to refer (e.g., Nyhoegen & Uecker, 2023, which is cited in the manuscript; Marrec & Bitbol, 2020; Czuppon, Day, Débarre, Blanquart, 2023; etc.).

These are excellent suggestions. We have added references to the suggested works, among others, including in lines 43-50, 351-60, and 390-400, among other places. The suggested connection to the evolutionary rescue literature is particularly welcome, which we broach in lines 190-94.

Marginal contribution: The paper is pretty short (10 pages) and has, more or less, one main take-home message, namely the relationship between the optimal antibiotic concentrations and the mutation rates, which is obtained from a relatively straightforward analytical derivation. I wonder if this take-home message is substantial enough to be published without any additional results. I may have this opinion because of the lack literature citations. I came across another preprint written by the authors (Delaney O, Letten AD, and Engelstädter J. 2023 Drug mode of action and resource constraints modulate antimicrobial resistance evolution; cited in the manuscript), whose model is, if I am not mistaken, quite similar to the one presented in this work. To me, combining both papers to make a more significant contribution to the field might be a good idea, but it is my personal opinion.

An interesting suggestion, but as mentioned earlier we decided to keep the two manuscripts separate.

Lack of clarity/justification: Overall, I found the paper clear and well-written. I really had a good time

reviewing it. However, some parts of the paper can be made clearer. For example, a sketch of the model would help the reader identify its key ingredients. It is not immediately obvious that both antibiotics are applied together at the same time (is it the case by the way?).

We thank the reviewer for their kind words. We have partially rewritten the methods section to attempt to make it clearer, including clarifying this particular point on line 79. (The reviewer is correct that drugs are applied in combination.)

The parameter values used in the simulations are not biologically motivated, whereas numerous studies report pharmacodynamic parameters (e.g., Czock & Keller, 2007), fitness costs (e.g., Melnyk, Wong, Kassen), etc.

We have added a reference to this fitness costs literature in lines 296-99. The point about justifying our parameters is well taken, and we have now added a paragraph about this in the methods section (lines 135-49), citing some relevant literature and explaining that choosing values that give rise to interesting phenomena is especially important, over and above choosing maximally realistic parameters (though we note our parameters are broadly reasonable).

Although the authors made their codes available, which I really appreciate, I think it can be useful to describe the algorithm or provide a pseudo-code in the main manuscript or supplement.

We have clarified some points in the methods section, but did not feel pseudocode would add much to the clarity of the methods – there are no particularly complicated or surprising decisions to make in the code. We do however now specify the transitions that occur in the model and their associated rates in Table 2.

Line 36: Can the authors provide examples?

Done, now line 39.

Line 47: It could be interesting to discuss how tricky it is to measure mutation rates (see, e.g., https://doi.org/10.1371/journal.pbio.2005056)

Done in lines 60-63

Line 62: It may be useful to specify i=Sensitive, A-Resistant, B-Resistant, as well as j=drug A, drug B.

Good point, done in lines 83-84

Equations 2 & 3: There is no carrying capacity and, thus, no density dependence. Is it realistic? If yes, in which cases? Can the authors justify this assumption, which is quite strong?

Good point, as discussed in response to reviewer 1, we have now added more discussion of resource constraints, as well as a simulation with resource-constrained growth.

Equations 2 & 3: Why is the impact of both drugs on the growth rate multiplicative, whereas it is additive on the death rate?

As noted above in response to reviewer 1, we now discuss in lines 101-10 how neutral interactions for bactericidal drugs are additive (under Bliss independence), but multiplicative for bacteriostatic drugs.

Line 71: mutation rate or mutation probability upon division?

Mutation probability, clarified in line 111

Equation 4: How are the division and death rates chosen? How do the mutant division and death rates compare to the sensitive rates division and death rates?

As discussed, our basic model sets costs of resistance equal to zero. Our model aims more for analytical insights than strict realism, so a replication rate of $1\ h^{-1}$ was chosen for simplicity (which is also biologically realistic). A (realistic) death rate was chosen that ensures the susceptible population crashes slowly enough that resistance mutants have a non-negligible chance of arising. These choices are now discussed in lines 135-49.

Line 74: i.e., S(0)=S0, MA(0)=0, MB(0)=0, right?

Correct, noted in lines 117-18.

Lines 76-77: What the authors call Stochastic Simulation Algorithm is basically Gillespie algorithm, right?

Correct, noted in line 121.

Line 79: The biological system the authors want to simulate does not seem very complex. Thus, why do they use an approximation rather than an exact Gillespie algorithm?

In our experience the code runs far faster with the tau-leaping approximation to Gillespie's algorithm, but still took approximately 6 hours to produce one figure. We felt tau-leaping provided the best balance between accuracy and speed. The exact algorithm can be very slow for large population sizes even if the mathematical system is not too complex.

Lines 108-109: This result has also been known in the theory of birth-death processes for a long time and is widely used in literature on evolutionary rescue.

We did not find a reference to Gambler's ruin problem in the evolutionary rescue literature, but quite possibly it is there and we missed it. We would be happy to cite an appropriate paper if one is brought to our attention.

Line 114: Is the assumption on the mutation rate biologically motivated? How robust are the authors' results if the assumption is broken down?

Mutation probabilities per replication are indeed very close to 0, as shown in Table 1 (noted now in line 176).

Line 114: I think it is worth mentioning that GS<DS in the presence of antibiotics, so that the sensitive population faces an exponential decay.

Done, lines 177-78

Equation 12: This equation is very similar to the one known in evolutionary rescue.

As noted above, we now discuss this in lines 190-94.

Lines 123-124: I do not find the expressions of PD and Nr particularly complex.

We have removed this point now (lines 195-96).

Line 133: The cost of resistance has not been defined in the manuscript.

Explained on lines 205-06 now.

Lines 136-138: So if both antibiotics are applied, the population should be eradicated without resistance evolution, right?

Correct, we now note this on lines 212-15.

Line 139: Can the authors explain why these assumptions are plausible?

Some rationale is given now in lines 216-22.

Equation 13: S0 or S(0)?

We have now defined $S(0) := S_0$ in line 117.

Equation 14: Although I find the equation super nice, I am a bit at a loss regarding whether both antibiotics are used simultaneously.

Correct, the antibiotics are used in combination, as noted now in line 79.

Lines 172-173: Why this choice? Can the authors motivate it?

Justified in lines 265-67 now.

Line 205: If I am not mistaken, it is the first occurrence of a parameter having a unit.

We have now added drug concentration units in Table 1. However, most of our quantities are in arbitrary units, or are intrinsically dimensionless, such as ϕ and β.

References

1. Levin BR. Periodic selection, infectious gene exchange and the genetic structure of E. coli populations. Genetics. 1981 Sep; 99:1–23. DOI: 10.1093/genetics/99.1.1
2. Hernández-Beltrán JCR, San Millán A, Fuentes-Hernández A, and Peña-Miller R. Mathematical Models of Plasmid Population Dynamics. Frontiers in Microbiology. 2021 Nov 4; 12. DOI: 10.3389/fmicb.2021.606396