Summary:

In this paper, the authors investigate how the combination of two antibiotics, which can be either bacteriostatic or bactericidal, impacts the evolution of antibiotic resistance. Specifically, the authors focus on four parameters, namely the antibiotic concentrations and the mutation rates, and seek to quantify the values that minimize the risk of resistance evolution while fully eradicating the bacterial population. To do so, they build a mathematical model that they analyze with analytical and numerical tools. This model allows them to draw a simple relationship between the extinction probability, i.e., the probability that the treatment eradicates the population without evolving resistance, and the antibiotic concentrations and mutation rates (see Equation 12 in their manuscript). Thanks to this relationship, the authors further derive an equation giving the optimal antibiotic concentrations, which depend on the mutation rates. This equation, in practice, could help design drug treatments, given that mutation rates are known.

Overall comment:

The evolution of antibiotic resistance is a major public health issue that threatens the treatment of bacterial infections, which explains why, recently, so many studies have put much effort into deciphering the mechanisms behind the evolution and spread of resistant strains. Due to abundant literature on this topic, either theoretical or experimental, it can be difficult to be innovative. In their paper, the authors claim that the novelty of their study relies on studying the impact of mutation rates on antibiotic resistance in the context of combination therapy, which I agree with. Investigating the impact of combination theory is far from novel, but, as outlined by the authors, mutation rates are often assumed to be constant in theoretical studies, whereas their value is likely to play a significant role in antibiotic resistance. Overall, I find this work interesting. I particularly enjoyed that the authors provided a simple expression allowing one to understand the impact of mutation rates on resistance evolution in combination therapy. However, I think there is room for improvement and have some concern, which I detail below.

Major comments:

1. *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.).

2. *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.

3. *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?). 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. 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.

Minor comments / detailed comments:

Line 36: Can the authors provide examples?

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)

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

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?

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

Line 71: mutation rate or mutation probability upon division?

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?

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

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

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?

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.

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

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.

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

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

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

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

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

Equation 13: S0 or S(0)?

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

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

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