

Dear Prof Krasimira Tsaneva-Atanasova,

We thank you for your interest and comments in our work and the opportunity to revise it. We would like to thank the anonymous reviewers for the careful reading of our manuscript. Their suggestions were helpful to improve the quality and clarity of our manuscript.

All minor issues have been corrected and more detailed answers are provided below. The initial comments are in black and our response is in blue. The changes made are further outlined in italics.

We thank you again for your consideration and hope that our manuscript is now acceptable for recommendation.

Yours sincerely,  
On the behalf of authors,  
Dr. Ramsès Djidjou-Demasse.

## Reviewer 1:

1) Is it true that  $A(C) \rightarrow \text{infinity}$  as  $C \rightarrow \text{infinity}$ ? It's usually thought of as saturating at a threshold level of activity (what does it mean to have infinite antimicrobial activity?).

**Answer:** We agree that antimicrobial activity is not necessarily infinite, and there is likely a certain saturation threshold. This has been rewritten differently as  $\mathcal{A}(C) \rightarrow \mathcal{A}_{\text{sat}}$  when  $C \rightarrow C_{\text{sat}}$ , where  $\mathcal{A}_{\text{sat}}$  and  $C_{\text{sat}}$  are saturating threshold levels.

↪ *See page 1.*

2) "From this intuitive approach, it follows that there exists  $C^*$  in  $(0, \text{infinity})$  such that  $A(C^*)$  is equal to the intrinsic growth of a bacterial population, all else being equal. This threshold concentration at which a bacterial population does not grow in vitro is called the Minimum Inhibitory Concentration (MIC)." The relationship between  $A(C^*)$  being equal to the intrinsic growth rate and the MIC isn't clear to me, and this statement seems to contradict a later statement providing the precise definition of MIC. Suggest clarifying/adding details.

**Answer:** This formulation is effectively a bit confusing. It is now stated that there exists a threshold  $\mathcal{A}(C^*)$  which is equal to the intrinsic rate of increase and reverses the growth of a bacterial population, all else being equal..

↪ *See the top of page 2.*

3) I found it a curious choice to let  $x$  (the level of resistance) be a real number, such that there is "negative" resistance. The intuition behind this wasn't clear, and it leads to some difficulties understanding the meaning of various model terms. For example,  $k(x)$  goes to infinity as  $x \rightarrow -\text{infinity}$  so  $x < 0$  is highest resistance? Clarifications may be needed.

**Answer:** Note that any interval  $(a, b)$  with  $a < b$  and  $x \in (a, b)$  is also valid within the context of the model and results developed here. However, it is important to keep in mind that, intuitively there exist two threshold levels  $x_0$  and  $x_1$  (called reference 'sensitive' and 'resistant' strains) such that each strain with resistance level (labelled by  $x$ ) can be classified as 'sensitive', 'intermediate', or 'resistant' depending on whether  $x < x_0$ ,  $x_0 < x < x_1$ , or  $x > x_1$  respectively.

↪ *Such clarifications and precisions have been provided (page 3).*

4) How were parameter values determined For example, why is the intrinsic growth rate of the reference sensitive strain  $0.95p_m$ ? References are noted in the column headers of Table 1, but none are given. This comes up again in Fig. 2 and on, it is important to understand where chosen parameter values come from.

**Answer:** Thank you for this remark. Table 1 is now rewritten differently and the mention of the reference is removed. Also notice that for all simulations, we randomly set the parameters with the only purpose to illustrate our theoretical results.

↪ *Such clarification has been made in the header of Section 3 (page 6).*

5) In Eq. 3.4, should the  $x$  for the feedback of resident  $x$  be interpreted the same as level of resistance  $x$ ?

**Answer:** We recall that the quantitative descriptor  $x$  for the bacterial resistance level is also treated as the label of the bacterial strain with resistance level  $x$ .

↪ *Such precision is now mentioned in the introduction and at the beginning of the paragraph before Eq. 3.4.*

6) In Fig. 3, I suggest using a text label for  $(1 - (p_1/p_m))^{-1}$  and  $(1 - (p_0/p_m))^{-1}$  to help the reader.

**Answer:** This has been done.

↪ *See figure 4*

7) "Our analysis emphasizes that treatment strategies that promote synergy between host immunity ( $\mu$ ) and the antimicrobial activity ( $k_0$ ) are a key component in achieving our TTO objective." Host immunity is not generally something that is known when determining treatment strategies. It would

be nice to explore more clinically-actionable scheduling guided by the results of the analysis. For example, what are the effects of intermittent dosing or non-continuous dosing given the chemotherapy's pharmacokinetics?

**Answer:** We agree with the reviewer. This sentence needs to be clarified. We now write. "Our analysis emphasizes that potential success of the treatment does not depend on the antimicrobial activity ( $k_0$ ) alone but should be assessed with respect to the level of host immunity ( $\mu$ ) as well. These results suggest that treatments with low antimicrobial activity should be limited to infections which elicit weak immune responses (e.g. respiratory infections). They also echoed earlier studies on synergy between chemotherapy and immune response.

↪ *See second paragraph, page 13.*

As for the the continuous vs discontinuous chemotherapy administration, it constitutes a very interesting extension to the present model.

## Reviewer 2:

1.  $b(t,x)$  and  $B(t)$  are sometimes denoted as population densities and sometimes as population sizes. It is necessary to define this very clearly and give the value range in Table 1.

**Answer:** Such issue has been fixed along the text. However, giving an up-front range for the density  $b$  is quite difficult. Indeed, as shown for e.g. by estimate E.12, such range mostly depends on the model parameters.

2. How is the functional form of the killing rate  $k(x)$  (p. 3) motivated?

**Answer:** For our model formulation and analysis, the killing rate function of the antimicrobial  $k(\cdot)$  is a decreasing function with respect to the resistance level  $x$ . Our primary goal is to define the function  $k(\cdot)$  with two parameters, namely,  $k_0$  and  $k_1$  representing the antimicrobial activity undergone by strains the MIC of which are exactly  $C_0$  and  $C_1$  and hereafter called reference strains 0 and 1. We then assume that the functional  $k$  takes the form at page 4. Such details were lacking and are now given above the functional form of  $k$ .

↪ *See page 4*

3. How is the functional form of the intrinsic growth rate  $p(x)$  (p. 3) motivated? What about the scenario in which population size is constant and  $p(x)=0$ ?

**Answer:** As previously, the functional form for  $p$  is not strictly important for our model formulation and analysis. The main important property is that  $p$  should be a decreasing function with respect to the resistance level  $x$ .

↪ *Such precision is now given below the functional form of  $p$  (page 4).*

**Answer:** With such a choice of the functional form of  $p$ , the scenario  $p(\infty) = 0$  corresponds to a strain  $x$  that takes an infinite concentration of antimicrobial to inhibit, and so pay an infinite cost then compromising its growth itself.

↪ *Such precision are given above functional form of  $p$  (page 4).*

4. Eq. (2.1) is the Langevin equation describing the dynamics of  $b(t,x)$ . Regarding its formulation, several questions arise: i. In the first term, which describes the influx into sub-population  $b(t,x)$  due to mutations from sub-populations  $b(t,y)$ , what is the role of the intrinsic growth rate  $p(y)$ ? This somehow makes sense for  $y=x$ , which corresponds to the influx to  $b(t,x)$  due to growth of sub-population  $x$  (i.e. when no mutations happen). However, otherwise it appears that mutations from  $y \neq x$  can only occur proportionally to the growth rate  $p(y)$ , whereas in reality even in a sub-population  $y$  of constant size mutations can be introduced (as some cells die and others divide, and the latter is associated with the mutational process).

**Answer:** We have derived System (2.2) that we think helps to better understand Model (2.1). Indeed, System (2.2) describes the dynamics of two bacterial populations: sensitive  $B_S$  and resistant  $B_R$ . Sensitive bacteria  $B_S$  growth at effective rate  $p(0)/(1 + B_S + B_R)^\alpha$ . Furthermore, while a proportion

$\varepsilon_0$  corresponds to a mutant growth (*i.e.* mutations away from the sub-population  $B_S$ ), the remainder  $(1-\varepsilon_0)$  corresponds to a faithful growth. Next, the sensitive population  $B_S$  is cleared at rate  $\mu(0)+k(0)$  accounting for actions of the immune response  $\mu(0)$  and antimicrobial  $k(0)$ . The same interpretation holds for the resistant population  $B_R$ . Such details were lacking and are now given below System (2.2).  
 $\hookrightarrow$  See page 6

ii. The equation does not contain a term that accounts for mutations away from sub-population  $x$  (proportional to  $J(y-x)*b(t,x)$ ).

**Answer:** We think the above answer also holds here.

iii. How is the pre-factor  $(1+B(t))^\alpha$  motivated? What is the meaning of the scaling constant alpha? What is the impact of alpha on all downstream results?

**Answer:** The factor  $p(y)$  is the bacterial intrinsic growth rate,  $\frac{p(y)}{(1+B(t))^\alpha}$  is the effective growth rate, and  $\alpha > 0$  is a scaling constant. Therefore, the fraction  $\frac{p(y)}{(1+B(t))^\alpha}$  accounts for the density dependence of the reproduction rate. Such a formalism is a suitable alternative in regulating the growth of a structured population without reference to the concept of carrying capacity, which we think is not necessarily a measurable factor for this type of population. Thus, the parameter  $\alpha > 0$  is introduced only to impose the population homeostasis and does not impact our downstream results. Taking  $\alpha = 0$  leads to a population with infinite growth if no effect of the immune response nor of the antimicrobial is taken into account.

$\hookrightarrow$  Such details are been added above Eq (2.1) for clarification (page 5).

What is the range of  $B(t)$  (see point 1)?

**Answer:** Please, see the answer to point 1.

iv. The equation does not contain any noise term. However,  $b(t,x)$  can become small enough for stochasticity to matter. How robust are the results under such random fluctuations?

**Answer:** We assume stochasticity can be neglected here as infecting bacteria population sizes exceed the number at which dynamics are driven by the outcomes of few individuals. Moreover, infections with limited number of bacteria such that stochasticity does matter, should be less an issue for antimicrobial chemotherapy as they are likely to be less symptomatic and more susceptible to be controlled by the immune system.

5. It appears that Eq. (3.4) is incorrect. Do the authors mean the reproduction number of the rare mutant strategy on the LHS? Otherwise, it appears that unity should be subtracted on the RHS.

**Answer:** You are right. The unity should be subtracted from the RHS.

$\hookrightarrow$  This issue has been fixed, Eq (3.4), page 7.

6. Eq. (3.5): Based on Eq. (C.6)ff., it appears that the substitution used when going from Eq. (3.4) to Eq. (3.5) only holds when  $R_0(x) > 1$ . Is this always satisfied? For example, if  $x$  is the resistance of the reference sensitive strain, certainly its  $R_0(x) < 1$  under chemotherapy.

**Answer:** Yes, quite naturally, we assume  $\mathcal{R}_0(x) > 1$ , otherwise, the resident population  $x$  is not persistent, which a bit contradicts the concept of 'resident population'.

$\hookrightarrow$  This precision has been added above Eq (3.4), page 7.

Another question in this context is how was Eq. (C.7) derived?

**Answer:** More detail have been added for a step by step derivation of (C.7)

$\hookrightarrow$  See the text below Eq. (C.7), page 16.

7. In Figs. 4 and 5, it is a bit confusing that even though  $m_0$  is supposedly the same in all cases ( $m_0=0.05$ ), the size of the bacterial population (green filled curve) in the panels showing drug efficiency is varying.

**Answer:** This confusion is due to the fact that the range of the initial the bacterial population (green filled curve) was scaling to the y-axis (*i.e.* to the  $\mathcal{R}_0$ ).

$\hookrightarrow$  This is now fixed.

8. p. 12, paragraph 5: "This phenomenon is in accordance with the strong relationship between the rate of emergence mutant and the antimicrobial dose [10, 11]." If I interpreted the phrasing correctly, it appears that the authors here conflate the probability with which resistant mutants arise (i.e. treatment failure) with the resistance,  $x$ , conditioning on treatment failure. Figure 3 shows, however, that the probability of treatment failure monotonically decreases for increasing dosage.

**Answer:** Thank you for this remark. The sentence has been rephrased as "This phenomenon is in accordance with the strong relationship between the resistance level of the emerging bacterial population and the antimicrobial dose."

↪ *See page 13, paragraph 5.*

9. What is meant by "In addition to the death and birth rates, bacterial population resistance level mitigates the antimicrobial activity of the drug" (p. 2)? I do not quite see how resistance has effects outside of modulating birth and death rates.

**Answer:** The main idea is that bacterial population resistance level also mitigates the antimicrobial efficiency with respect to that population. This has been clarified.

↪ *See the end of page 2.*