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Abstract

Mathematical modelling has made significant contributions to the optimisation of the use of antimicrobial treatments. In this review we discuss the key processes that such mathematical modelling should attempt to capture. In particular, we highlight that the response of the host immune system requires quantification and illustrate this with a novel model structure.

Keywords

Mathematical modelling, pharmacokinetics, pharmacodynamics, drug resistance.

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Introduction – a brief history of PKPD modelling

There is a long history of the investigation of optimal dosing regimens [1] that form the precursor to the application of mathematical modelling (or PKPD) to the discovery and development of antibiotic therapies. An understanding of optimal dose and frequency of dosing is now informed by the understanding of pharmacokinetic and pharmacodynamic differences between drugs, between host species and species and strains of pathogens. However, like many therapy areas, antibiotic PKPD (exposure- response) modelling is often very empirical, the aim being to identify a target drug concentration to test in patients. This is driven in part by necessity and gaps in our understanding. A concern with this approach might be that important mechanisms are not addressed which may limit quantitative translation to the clinic - especially the translation of optimum dose and dosing schedule. This is because the empirical approach may not fully account for time dependent factors such drug concentration (PK), bacterial load (disease course) drug resistance and the response of the immune system. Below we exemplify that a mathematical model, developed to capture key antibiotic PKPD mechanisms, need not be complex and yet can provide further insight into the biology behind the experimental data. Many of these aspects are thoroughly reviewed already in Nielsen and Friberg 2013 [2]and Rayner et al 2021 [3] so will not be covered in detail here – the aim in this paper will be to identify the key components or “ingredients” required in a mechanistic model of antimicrobrial drug actionl, in particular the response of the immune system.

First Model Ingredient: Exposure response

The first requirement is an understanding of the exposure-response relationship and how it translates from in vitro to in vivo mouse models. This is important for dose setting in the clinic with the addition of pharmacokinetic knowledge. Demonstrating translation of the exposure-response relationship fromin vitro to in vivo allows a much wider range of species of bacteria to be considered than an in vivo resource would afford. Historically the minimum inhibitory concentration (MIC) has been used as the in vitro potency measure. To determine the aspect of drug exposure most predictive of antimicrobial activity the in vivo free AUC/MIC, Cmax/MIC and time over MIC are plotted versus the reduction in bacterial load in vivo for a range of antibiotic dose levels. Potentially this is carried out in multiple strains of bacteria with MIC being used to normalise drug exposure for inherent susceptibility. Dose-fractionation is a necessary study design element because of the inherent correlation of Cmax, AUC and time over MIC as dose is varied. A more serious consideration is that MIC is a composite potency measure influenced not only by the pharmacological effect of the drug (reduced proliferation/ killing) but also the intrinsic proliferation rate of the bacteria and background death rates [2]. It is also potentially dependent on the duration of the drug incubation and cell density used in the assessment. Typically, these metrics suggest that maintaining exposure above MIC is required for a reduction in infection – and this seems rational given that in vitro concentrations above MIC, by definition, will reduce the population of bacteria. An example of this is colistin [4], where free AUC/MIC >10 (Average concentration 10-fold that of MIC) are required to reduce the CFU count.

There are more mechanistic approaches that have been adopted to characterise the course of infection and concentration-effect relationship as thoroughly reviewed by [Nielsen and Friberg 2013]. These models, applied to time series data from in vitro and in vivo experiments, separate the intrinsic growth rate and an EC50, that relates drug concentration to effect, that that will not suffer from the potential oversimplification of MIC. This is very important given that in vivo pharmacokinetics result in significant fluctuations in the drug concentration that the infection is exposed to, compared to the constant concentrations typical of an in vitro incubation. However, it is noted that systems such as hollow fibre injection and other dynamic in vitro models can reproduce

fluctuating drug concentrations and provide a useful link between in vitro and in vivo experiments. Similarly time-kill experiments give insight into the onset of antibacterial effect.

Combination therapy is one way that antimicrobial resistance might be circumvented. Typically two or more antimicrobials are investigated in a concentration dependent manner, similar to the determination of MIC, and the data tested for evidence of greater or less than additive effect using the concepts of Bliss independence and Loewe additivity[5]. An more general approach to modelling combination effects has been proposed [6]. Doern [7] has argued that in vitro combination assays are so diverse that assessing synergy and guiding dosing of patients with these assays is a non-starter until a gold standard is agreed upon. As argued against MIC, these static approaches may not pull apart the contributing factors that contribute to combination pharmacology and the dependence on the test system such as the population growth rate. More mechanistic approaches have been taken, usually borrowing assumptions of combination effects familiar in other therapy areas such as an additive effect of the total bacterial kill. A review of these models [8] concluded that there was a benefit to mechanistic approaches in being able to not only disentangle the contribution of components but also to incorporate host associated effects, e.g., the immune response.

Second Model Ingredient: The development of drug resistance

A second requirement is an understanding of the kinetics of drug resistance – especially important given the growing issues of AMR. Key resistance mechanisms include: (i) changes to the structure of the drug target; and (ii) increased expression of proteins that alter the intracellular PK of the antibiotic (drug transporters and drug metabolising enzymes). The former tends to be irreversible, requiring an alteration at the gene level, however the latter can be reversible if an environmental adaption occurs. MIC, taken after a particular time, may well have these aspects folded in. However, resistance, and its impact on the time course of an infection will be time dependent. Models based on time series data can incorporate these mechanisms and to some extent again separate them out from inherent potency and population growth rate. Key phenotypes to incorporate are [9] : resistance from the start, tolerance - whereby cells adapt to a reversibly resistant phentotype, and persisters - which have a lower rate of proliferation and so are less vulnerable to typical mechanisms of antimicrobial treatments[10]. These can all be incorporated [2] and permit the prediction of unique time-kill curves so that the underlying phenotypes might be inferred. Distinguishing between mechanisms from a numerical point of view suggests that this might be possible based upon bacterial counts only however challenging if attempting to distinguish between resistance phenotypes simultaneously [11]. Experimental approaches to aid in this identification have been suggested including measuring the MIC and MDK (minimum duration for killing) in the resulting resistant populations[9].

An issue for ongoing research in this area is the lack of diverse data sets considered. Niewiadomska et al [12] reviewed the literature and found a lack of diversity in pathogenic organisms in which AMR had been mathematically modelled and calibrated on experimental data, so clearly further work is required to fully validate the above mathematical mechanisms. It is also noted that these mathematical modelling exercises have not considered the combination of multiple treatments.

Third Model Ingredient: The contribution of the immune system to cure

The impact of the immune system has previously been considered in other therapy areas, for example in oncology. Here models have attempted to capture the immune system's recognition of malignant cells, the onset of response and, in some cases, the attempts of the tumour to escape or adapt to this immune response [13-15]. It is evident that the immune response's contribution to the

clearance of an infectious agent is important. Studies in animal infection models [16, 17] have, to some extent, quantified the immune component of clearance. Clearly then, there is an analogy with oncology, suggesting the extension of antibiotic PKPD to this approach could be of use in the development and optimisation of therapies.

Developing a model of within-host antibiotic resistance which accounts for the role of the immune system is cursed by the overall complexity of the biological system and the inherent multiscale nature of the systems (molecular to whole-body scale). However, it is possible to create a generic description of each aspect of the system – bacterial loading and the immune system response to it whilst accounting for the administration of an antibiotic. We describe here a within-host population mathematical model which accounts for bacterial loading and clearance via the immune system and an antibiotic. Here we take a generalised view of the immune system accounting for the speed and magnitude of the immune response, which we assume responds to the bacterial infection, both in terms of increasing the response and its magnitude. Our model formulation accounts for the local and global effects of antibiotic dosing as summarised in Figure 1. Mathematically our model is represented by the three nonlinear ordinary differential equations given by

$$\frac{dA}{dt} = \overbrace{\alpha(t)}^{Antibiotic\ dosing} - \overbrace{\lambda_A AB}^{Antibiotic\ removal\ by\ bacteria} - \overbrace{\delta_A A}^{Antibiotic\ elimination},$$

$$\frac{dB}{dt} = \overbrace{\rho_B B\left(1-\frac{B}{K_B}\right)}^{Bacterial\ growth} - \overbrace{\frac{\lambda_B AB}{K_R + A}}^{Antibiotic\ removal} - \overbrace{rIB}^{Immune\ system\ removal},$$

$$\frac{dI}{dt} = \overbrace{\rho_I(1+s_g B)\left(1-\frac{I}{K_I(1+s_I B)}\right)}^{Immune\ system\ response} - \overbrace{\delta_I I}^{Immune\ clearance}$$

where the initial conditions of the system are given by

$$A(0) = 0, \quad B(0) = B_0 \quad \text{and} \quad I(0) = I_0.$$

Here $A = A(t)$ denotes the concentration of the antibiotic, $B = B(t)$ the within host bacterial cell density and $I = I(t)$ the magnitude of the immune response. It is assumed that both the bacteria and immune system response grow logistically, the latter with growth rate $\rho_I(B) = \rho_I(1 + s_g B)$ and carrying-capacity $K_I(B) = K_I(1 + s_I B)$, where s_g and s_I describe how the speed and magnitude of the immune system response respond to the bacterial infection. Here the effect of antibiotic resistance is accounted for by modelling the antibiotic concentration effect on bacteria via a sigmoidal function with half-maximal value K_R (a large K_R means the antibiotic has reduced potency and so the bacteria will be relatively resistant).

To simplify the three-dimensional nature of equations (1) to (3) we consider the global effect of the antibiotic by ignoring the localised dependency described via the term $\lambda_A AB$, so henceforth, for this work, we set $\lambda_A = 0$. This decouples equation (1) from equations (2) and (3) thus allowing us to analyse equations (2) and (3) as a system of two coupled nonlinear ODEs, which we do so using the non-dimensionalised form of the equations as detailed in Annexe 1. Under the assumption of a constant antibiotic infusion ($\alpha(t) = \alpha$), the system exhibits four steady-states:

(i) **State 1** $(a_1^*, b_1^*, i_1^*) = (a^*, 0, 0)$: the case in which the host has died, all bacteria have been eradicated from the body and only antibiotic remains;

(ii) **State 2** $(a_2^*, b_2^*, i_2^*) = \left(a^*, 0, \left(1 - \frac{\delta_i}{\rho_i}\right)\right)$: All bacteria have been eradicated from the body, the immune system has returned to its normal functional levels and antibiotic remains in the system so long as the immune system response is greater than its clearance;

(iv) **State 3**:$(a_3^*, b_3^*, i_3^*) = \left(a^*, \left(1 - \frac{(\lambda_b^* + i^*)}{\rho_b}\right), i^*\right)$: A co-existence steady-state in which both the antibiotic and immune system work together to eradicate the bacterial loading, but the infection persists (i^*being given by the solution of equation (A.2)); and

(iii) **State 4** $(a_4^*, b_4^*, i_4^*) = \left(a^*, \left(1 - \frac{\lambda_b^*}{\rho_b}\right), 0\right)$: Here a persistent bacterial infection remains in the body along with the antibiotic, the immune system effectively having become non-functional,

where $a^* = \alpha/\delta_a$ is the steady-state antibiotic concentration and $\lambda_b^* = \lambda_b a^*/(K_r + a^*)$. State 1 is possible (stable) if the immune system clearance is more rapid than its response rate ($\delta_i > \rho_i$), whilst the reverse holds for State 2 with the additional condition that $\rho_b + \frac{\delta_i}{\rho_i} < 1$. State 3 is monotonically or damped oscillatory stable and State 4 is stable so long as bacterial growth dominates over the ability of the antibiotic to remove the bacteria ($\rho_{b>}\lambda_b^*$).

States 1 and 2 represent the worst and best health outcomes, whilst State 3 is a common scenario which is representative of antibiotic resistance. State 4 is the case of a severely immune-suppressed individual. In what follows we focus primarily on scenarios considering Cases 2 and 3 given these represent more likely health outcomes.

To demonstrate the dynamical behaviour of the system we consider numerical solutions, generated in Matlab, of equations (1) to (3) utilising the set of non-dimensional parameters stated in Table 1. We have chosen parameterisations here which allow us to reflect on different scenarios informed by real-world known outcomes. Our objective here is to demonstrate the conceptual qualitative nature of the system, and how a simplified description of the respective biological mechanisms can be used to capture the gross behaviour of the system, without needing to describe all aspects of the underlying biology. Such models allow for the overall system dynamics to be explored before understanding aspects of the lower-level detail. A non-dimensionalisation allows us to inform parameter relationships in order to reproduce known qualitative behaviour. Experimental and clinical parameterisation of the system will be the focus of future work.

Case studies of simulations are shown in Figure 2. We first consider the ability of the immune system to clear the bacterial infection in the absence of antibiotic. This allows us to parameterise the system for an individual whose immune system is strong enough to clear the infection, as detailed in Figure 2a, for the parameterisation given in Table 1. This is akin to State 2 above, albeit that no antibiotic is present.

We next consider the case of an immune system which is slower in responding to the presence of bacteria ($\rho_i = 0.07$). Here the individual is not able to effectively clear the infection and, after an initial period of oscillations between the immune system and bacterial loading, the system settles to a non-zero steady-state. This is akin to the co-existence steady-state (State 3), albeit in the absence of any antibiotic. We now consider how we can utilise an antibiotic to support the removal of the bacteria from the system and thus move it from State 3 to State 2. We do so by first introducing an antibiotic with a perceived level of effectiveness (as indicated by λ_b) in Figure 2(c) with $\alpha = 1, \rho_i = 0.07$ and $\lambda_b = 0.1$. Here we see that the antibiotic is able to decrease the bacterial loading, but is

not fully effective. Increasing the effectiveness of the anti-biotic (akin to adding additional antibiotics; $\lambda_b = 0.25$) leads to effective removal of the bacteria thus moving the system to the ideal outcome of State 2.

Conclusions

In this article, we have reviewed the three main ingredients required of a mechanistic PKPD model: exposure-response, drug sensitivity/ resistance phenotypes and the contribution of the immune system. In particular, we have highlighted how quantifying the immune system response can aid in the interpretation of in vitro to in vivo translation of disease pharmacology. Indeed, we should perhaps consider it as modelling the drug's contribution on top of the immune system: By increasing the clearance of pathogen the immune system is able to fully respond. Further work is needed in this area including informative measurement of the host immune system.

By having these three aspects it is possible that the mathematical models can account for and explain between-host variations in the time course of infection as well as the potential to be more translatable from nonclinical systems to patients. The relationship between regimen and efficacy can vary in terms of the pharmacokinetics, bacterial strain (MIC), adaption/resistance (variation of MIC with time) and the immune response. By factoring these in, the intrinsic factors determining the success of treatment can be identified by building patient baseline covariates into the model. This can aid in the optimisation of antimicrobial treatment. Key to this is the application of mechanistic models that can be applied in a nonlinear mixed effects framework to characterise between-subject variability in response – and it is possible the above model is applicable here. Most promising is the ability of the model to quantify the immune system response and perhaps here there is some overlap in the work quantifying the efficacy of vaccines. Clearly, however, full mathematical evaluation and experimental data that allow determination of model parameters to support model validation are needed for future work.

Annexe 1 – Non-dimensional governing equations

Equations (1) to (3) are non-dimensionalised according to

$$A(t) = K_B a(\tau), \quad B(t) = K_B b(\tau), \qquad I(t) = K_I i(\tau) \quad \text{and} \quad t = \frac{\tau}{rK_I}.$$

Substituting these scalings leads to the non-dimensional system of equations

$$\frac{da}{d\tau} = \overbrace{\alpha(\tau)}^{Antibiotic\ dosing} - \overbrace{\lambda_a ab}^{Antibiotic\ removal\ by\ bacteria} - \overbrace{\delta_a a}^{Antibiotic\ elimination},$$

$$\frac{db}{d\tau} = \overbrace{\rho_b b(1-b)}^{Bacterial\ growth} - \overbrace{\frac{\lambda_b ab}{K_r + a}}^{Antibiotic\ removal} - \overbrace{ib}^{Immune\ system\ removal},$$

$$\frac{di}{d\tau} = \overbrace{\rho_i(1+\varepsilon_g b)\left(1 - \frac{i}{1+\varepsilon_i b}\right)}^{Immune\ system\ response} - \overbrace{\delta_i i}^{Immune\ clearance},$$

where the initial conditions of the system are given by

$$a(0) = a_0, \; b(0) = b_0 \quad \text{and} \quad i(0) = i_0,$$

and the non-dimensional parameters by

$$\alpha(\tau) = \frac{\overline{\alpha(t)}}{K_B K_I r}, \quad \lambda_a = \frac{\lambda_A K_B}{rK_I}, \quad \delta_a = \frac{\delta_A}{rK_I}, \quad \rho_b = \frac{\rho_B}{rK_I}, \quad \lambda_b = \frac{\lambda_B}{rK_I}, \quad K_r = \frac{K_R}{K_B}, \quad \rho_i = \frac{\rho_I}{rK_I},$$

$$\delta_i = \frac{\delta_I}{rK_I}, \quad \varepsilon_g = s_g K_B \quad \text{and} \quad \varepsilon_i = s_I K_B.$$

Annexe 2 – Co-existence steady-state solution

The third co-existence steady state (a_3^*, b_3^*, i_3^*) is determined by solving

$$\frac{\varepsilon_g}{\rho_b}\left(1 + \frac{\varepsilon_i}{\rho_b}\right) i^{*2} - \left[\frac{\varepsilon_i}{\rho_b}\left(2\varepsilon_g - \frac{\delta_i}{\rho_i}\right) + \varepsilon_g\left(1 + \frac{1}{\rho_b}\right) + 1 + \frac{\epsilon_i}{\rho_b}\right] i^* + (1+\varepsilon_i)\left(\varepsilon_g + 1 - \frac{\delta_i}{\rho_i}\right) = 0, \quad \text{... (A2)}$$

for i^*. We observe that positive solutions are only possible here for $i^* < \rho_b - \lambda_b^*$, i.e. the immune system levels are determined by the difference in the bacterial growth rate and its rate of eradication by the antibiotic.

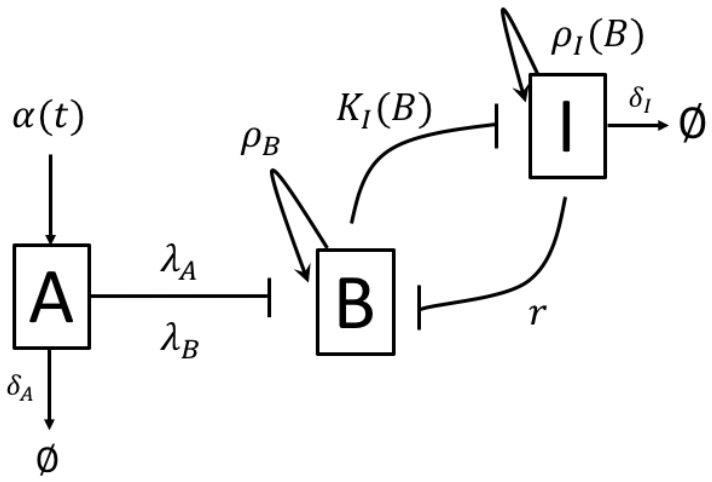

Figure 1. A schematic of the three-state model of within host antimicrobial resistance. Here an antibiotic $A(t)$ is administered at rate α(t) and cleared with rate constant δ_A. Bacteria $B(t)$ grow logistically with growth rate constant ρ_B and are removed by the antibiotic (with rate constant λ_A) and the immune system $I(t)$ (with rate constant λ_B), respectively. Bacteria seek to inhibit the immune system, which seeks to respond with growth rate $\rho_I(B)$ by increasing its capacity $K_I(B)$, to clear bacteria with rate constant r, whilst being removed with rate constant δ_I.

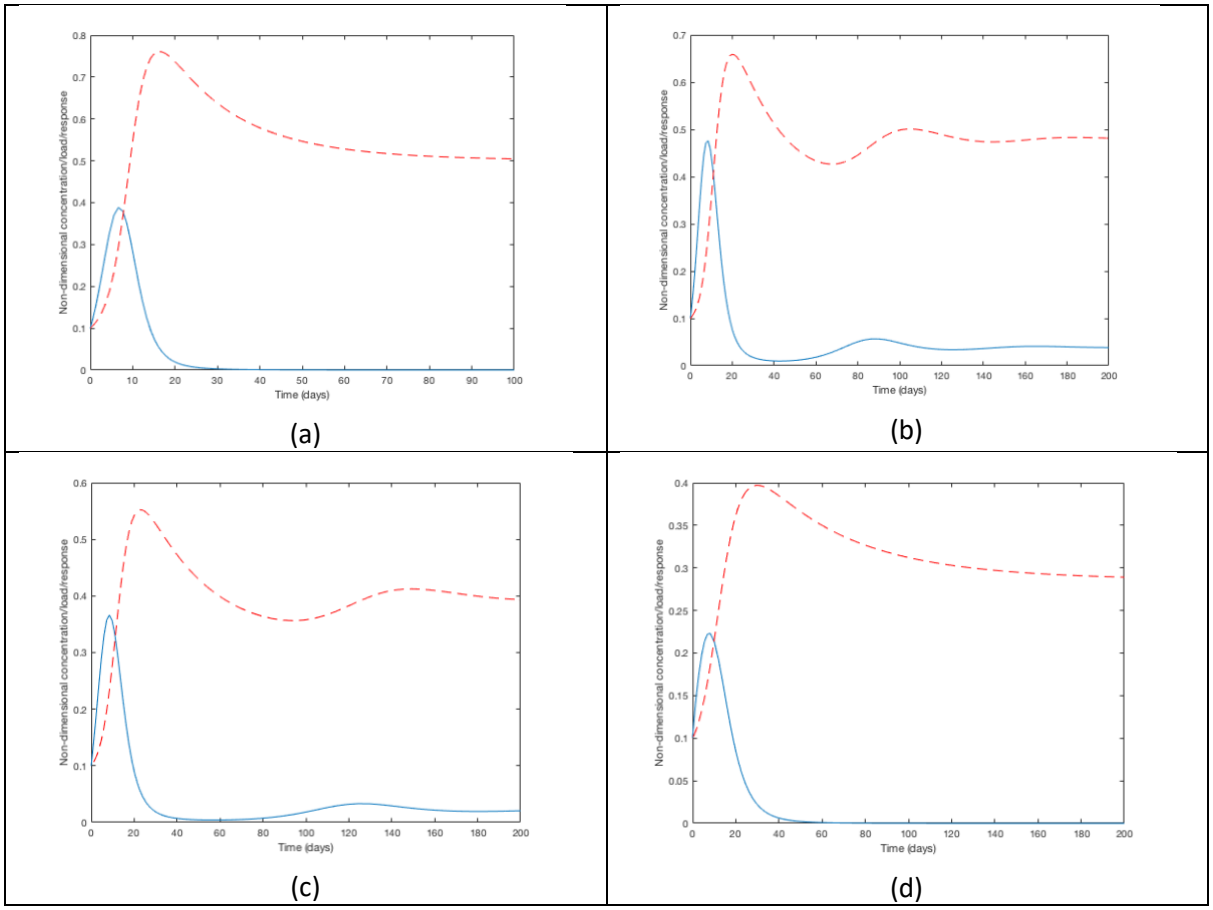

Figure 2. Case studies of the antimicrobial resistance model. Solid lines indicate the within host bacterial cell density, whilst dotted lines the immune system response. Antibiotic concentration not shown. **(a)** The case of a strong immune system, in the absence of any bacteria, being able to clear a bacterial infection ($\alpha = 0, \rho_i = 0.1$). **(b)** An immune system which responds less rapidly than (a), which leads to oscillatory damped behaviour and the bacteria not being effectively removed from the host ($\alpha = 0, \rho_i = 0.07$). **(c)** The effect of including an antibiotic for (b) to help remove the bacterial infection. Here the infection still persists after antibiotic has been included ($\alpha = 1, \rho_i = 0.07, \lambda_b = 0.1$). **(d)** In contrast to (c) a more effective antibiotic is able to remove the bacterial infection, leaving the immune system to return to its pre-infection levels ($\alpha = 1, \rho_i = 0.07, \lambda_b = 0.25$).

Table 1. The three state non-dimensional model parameters.		
Parameter	**Definition**	**Value**
α	Antibiotic dosing rate.	0
δ_a	Antibiotic clearance rate.	0.1
ρ_b	Bacterial growth rate.	0.5
λ_b	Bacterial clearance by the antibiotic.	0.1
K_r	Half-maximal antibiotic concentration level & resistance measure.	1
ρ_i	Immune system response rate.	0.1
ϵ_g	Augmented immune response rate as a result of bacterial loading.	5
ϵ_i	Augmented immune response levels as a result of bacterial loading.	5
δ_i	Immune system clearance rate.	0.05
a_0	Initial antibiotic concentration.	0/1
b_0	Initial bacterial loading.	0.1
i_0	Initial immune system response.	0.1

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