Optimal Control Methods for Controlling Bacterial Populations with Persister Dynamics

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Abstract. Bacterial tolerance to antibiotics is a well-known phenomena; however, only recent studies of bacterial biofilms have shown how multifaceted tolerance really is. By joining into a structured community and offering shared protection and gene transfer, bacterial populations can protect themselves genotypically, phenotypically and physically. In this study, we collect a line of research that focuses on phenotypic (or plastic) tolerance. The dynamics of persister formation are becoming better understood, even though there are major questions that remain. The thrust of our results indicate that even without detailed description of the biological mechanisms, theoretical studies can offer strategies that can eradicate bacterial populations with existing drugs.

Keywords: Persisters, biofilms, tolerance, modeling
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Introduction

Populations of bacteria exhibit multiple methods to evade killing by antibiotics, antimicrobials, environmental, and biological challenges. Developing methods to enhance the action of *existing* technology/medicine, would be a very useful contribution. Our main focus is on developing biologically relevant mathematical models that we can use to pose *optimal control* methodology to predict effecting dosing protocols.

We begin the discussion with a short discussion of the current understanding of the dominant tolerance mechanisms: physiological, phenotypic and physical. In biofilm settings, all of these are connected while in planktonic populations we can often control the physical and physiological mechanisms to focus on the most novel (and least well-understood) persister dynamics. We then describe results of a few recent investigations and close with a brief demonstration of experimental validation of a key mathematical prediction.

Previous Studies:

A simple model of persister dynamics was proposed in [5] and extended to a chemostat in [6, 8]. Spatial variations were neglected and the bacterial population was separated into susceptible and persister types. Susceptible bacteria consume nutrient, reproduce and are killed by antibiotics at a rate proportional to their growth rate. Persisters consume a negligible amount of nutrient and do not reproduce. This allows persisters to tolerate the antibiotic. Susceptible bacteria can transition into the persister population at a rate that is independent of the antibiotic concentration. Persisters can revert to susceptibles at a slower rate. Moreover, the presence of antibiotics prevents the reversion. For the chemostat system described in [6, 8], the population densities of persister and susceptible (B_p and B_s, respectively) and substrate (S) are governed by a system of ordinary differential equations:

$$\frac{dB_s}{dt} = [(1-A-k_l)\,f(S)-D]\,B_s + k_g(A)B_p \tag{1}$$

$$\frac{dB_p}{dt} = k_l f(S)B_s - [k_g(A)+D]B_p \tag{2}$$

$$\frac{dS}{dt} = D(S_0-S) - \frac{f(S)B_s}{Y}. \tag{3}$$

Nutrient enters at source concentration S_0 and the washout rate is denoted D. The function $f(S)$ denotes the growth rate. Typically Monod kinetics are used $\left(f(S)=\mu\frac{S}{k_s+S}\right)$ although the results in [8] are valid for a variety of functions.

Susceptible bacteria transition to persisters at a rate proportional to the growth rate with proportionality constant k_l. Persister bacteria can transition back to susceptible bacteria at a rate that depends on the antibiotic. In [9] it was shown that persisters either do not revert or revert very slowly unless the antibiotic concentration is zero (or possibly close to zero). In this form of the model, the killing of bacteria by the antibiotic is proportional to the product of the growth rate and the parameter A.

When $D = 0$, as in [5], there are some outcomes that can be determined easily. Constant application of antibiotic kills all susceptible bacteria, leaving persisters to repopulate once the challenge was removed. It can be shown that by cycling between biocide challenge are biocide withdrawal, both susceptibles and persisters can be eliminated [5]. The optimal dose/withdrawal protocol (e.g. the one that kills the entire population the fastest) can also be determined numerically. A more sophisticated version, that incorporates one potential mechanism for the induction (toxin/antitoxin interaction) has also been proposed [6, 10]. The former model has been shown to have similar behavior as in [5].

When the washout rate is non-zero, as in [8], while the antibiotic is applied as a piecewise constant the optimization can be developed analytically. It was shown that there is an optimal 'bang-bang' treatment that was consistent with the dose/withdrawal protocol proposed in [5].

Optimal Control

Optimal control begins with a model for the interchange between persister and susceptible sub-populations and disinfection as described above in Equations 1-3 although it can be reformulated for optimal control where the control is the amount of antimicrobial that enters the chemostat, $A_0u(t)$, where we assume that $0 \leq u(t) \leq 1$. The objective function to be minimized is

$$J(u) = \int_{t_0}^{t_1} \frac{1}{2} K_1 (u)^2 \, dt + K_2 B_s(t_1) + K_3 B_p(t_1). \tag{4}$$

The goal is to minimize the total antibiotic applied and the bacterial populations at the end of the application cycle. The bang-bang protocol assumes periodic application of the same treatment. The optimal disinfection, for a given set of parameters and a 10-hour base period, was constant application for about 6.12 hours and withdrawal of the antibiotic the remaining time.

Pontryagin's Maximum Principle gives a method to obtain the optimal control, u^*. We find that alternating the application of antibiotic is more effective than constant dosing (see Figure 1). Additionally we can tune the timing to enhance the eradication.

PDEs In another study, we assume that the bacterial population consists of three phenotypes: susceptible cells volume fraction, B_s, persister cells volume fraction, B_p, and dead susceptible cells volume fraction, B_{sd}. We consider only one growth-limiting substrate, C, and assume that biomass constituents are incompressible. The biofilm is treated as a continuum in one spatial dimension that is fully saturated and the fluid component is neglected; therefore $B_s + B_p + B_{sd} = 1$. The spatial domain is $x \in [0, L(t)]$, the equations governing the cell volume fractions are obtained

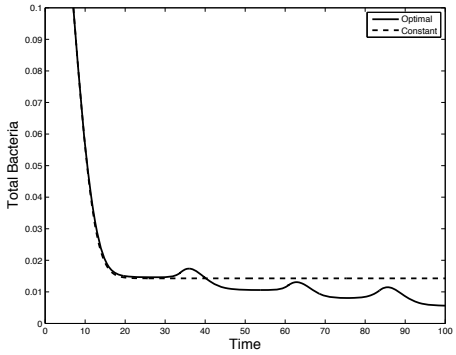

FIGURE 1. Comparison between alternating source of disinfectant with constant dosing.

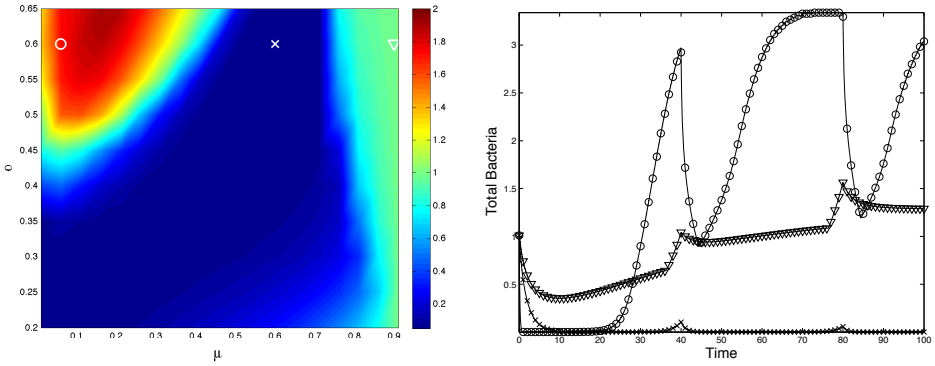

FIGURE 2. Left panel: The surface $J(\mu,\alpha)$ showing the narrowing window of successful treatment as the growth rate increases. The survival curves at particular parameter pairs are shown in the right panel.
Right panel: A comparison of survival curves for varying fractions and fixed growth rate. These three parameter pairs were chosen to be representative of an optimal protocol with a moderate fraction μ. The symbols correspond to the left panel ($\alpha = 0.6$): The circle corresponds to $\mu = 0.06$, the x corresponds to $\mu = 0.6$ and the triangle corresponds to $\mu = 0.9$. We see that parameters in the blue region are successful while the others are unsuccessful.v

from conservation of mass (which is equivalent to conservation of volume if the densities are constant [11]:

$$\frac{\partial B_s}{\partial t} + \underbrace{\frac{\partial}{\partial x}(vB_s)}_{\text{advection}} = \underbrace{g(C)B_s}_{\text{growth}} - \underbrace{k_d A g(C) B_s}_{\text{disinfection}} - \underbrace{k_l g(C) B_s}_{\text{loss}} + \underbrace{k_r(A) B_p}_{\text{gain}} \tag{5}$$

$$\frac{\partial B_{sd}}{\partial t} + \frac{\partial}{\partial x}(vB_{sd}) = k_d A g(C) B_s \tag{6}$$

$$\frac{\partial B_p}{\partial t} + \frac{\partial}{\partial x}(vB_p) = k_l g(C) B_s - \underbrace{k_r(A) B_p}_{\text{reversion}}, \tag{7}$$

where $x = 0$ represents the impermeable substratum the biofilm is attached to and $x = L$ represents the biofilm-bulk fluid interface. The population of susceptible cells in (5) changes due to growth, death due to killing with antibiotic, A, loss due to transition to persister cells and gain as persister cells revert back to susceptible cells. The population of persister cells in Equation (7) changes due to the net gain of transition between susceptible and persister cells. The dead cell population in Equation (6) increases due to disinfection of susceptible cells. Our model is a one-dimensional counterpart of the chemostat model of ODEs in [8] (with zero wash-out rate). The reduction of Equations (5)-(7) to ODEs along characteristics $s(t) \in (0, M]$ for some $M > 0$ gives the aforementioned ODE system in [8].

When we consider the effect of varying the growth rate of the bacteria on survival using constant total application in this set. Very small μ is rapid application of high concentration of antibiotic. The previous simulations indicate that the time the population has to recover from an antibiotic challenge is one of the key factors in success or failure. If the bacteria that revert from persister have ample time to replace the killed bacteria, the treatment will be unsuccessful. This means that if the bacteria grow faster, it will alter the distribution of success and failure. Figure 2 shows the surface $J(\mu,\alpha)$ (left panel) indicating that as the growth rate increases, the window of success shrinks. In fact, for slow growth, instantaneous application is the best course. The same figure (right panel) compares the survival curves again indicating that the optimal is in fact successful. The key results here is that the withdrawal time should be measured relative to the potential growth for reproducing bacteria and, therefore, the nutrient regime may also play a more complicated role in disinfection.

DISCUSSION

The previous sections represent the framework for building and analyzing more descriptive and specific models. Additionally, we have begun testing our predictions experimentally, which lends strong support for our theoretical

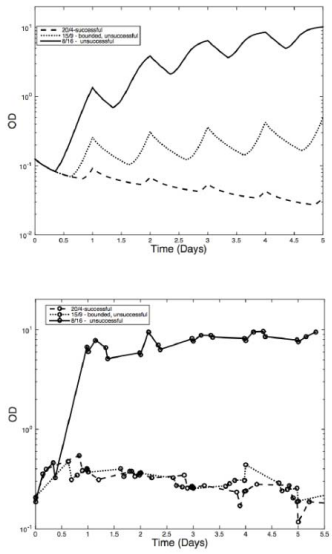

FIGURE 3. Top panel: Mathematical predictions
Bottom panel: Experimental validation of the effective timing.

approach. In Figure 3 we show model predictions that were used to determine the cut-off between effective and ineffective treatment of *E. coli* populations. Clearly the model is able to predict the timing that separates these two regimes – which is one of the most important aspects of our theoretical observations. We are currently working with experimentalists to test the optimality predictions. We hope that this will lead to novel treatments that may be effective, without necessitating the development of new drugs.

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