J. Math. Biol. (2009) 59:563–579
DOI 10.1007/s00285-008-0243-6

Mathematical Biology

Failure of antibiotic treatment in microbial populations

Patrick De Leenheer · N. G. Cogan

Received: 27 August 2008 / Revised: 24 November 2008 / Published online: 16 December 2008

Abstract The tolerance of bacterial populations to biocidal or antibiotic treatment has been well documented in both biofilm and planktonic settings. However, there is still very little known about the mechanisms that produce this tolerance. Evidence that small, non-mutant subpopulations of bacteria are not affected by an antibiotic challenge has been accumulating and provides an attractive explanation for the failure of typical dosing protocols. Although a dosing challenge can kill the susceptible bacteria, the remaining persister cells can serve as a source of population regrowth. We give a condition for the failure of a periodic dosing protocol for a general chemostat model, which supports the simulations of an earlier, more specialized batch model. Our condition implies that the treatment protocol fails globally, in the sense that a mixed bacterial population will ultimately persist above a level that is independent of the initial composition of the population. We also give a sufficient condition for treatment success, at least for initial population compositions near the steady state of interest, corresponding to bacterial washout. Finally, we investigate how the speed at which the bacteria are wiped out depends on the duration of administration of the antibiotic. We find that this dependence is not necessarily monotone, implying that optimal dosing does not necessarily correspond to continuous administration of the antibiotic. Thus, genuine periodic protocols can be more advantageous in treating a wide variety of bacterial infections.

Keywords Persister · Biofilm · Model · Chemostat · Tolerance

P. De Leenheer
Department of Mathematics, University of Florida, Gainesville, USA
e-mail: deleenhe@math.ufl.edu

N. G. Cogan (✉)
Department of Mathematics, Florida State University, Tallahassee, USA
e-mail: cogan@math.fsu.edu

Mathematics Subject Classification (2000) 37N25

1 Introduction

The failure of antibiotic treatments to eliminate bacterial infections has become both more evident and better understood in the past several decades. Although there is evidence that both the use and over-use of antibiotics has amplified the number of chromosomal-resistant bacteria [17,24], it is becoming increasingly clear that there are other mechanisms that protect populations of bacteria. The notion that small sub-populations of bacteria may display innate tolerance to various biocides has been proposed as a possible reason for the failure of treatment for bacterial infections [3,9,20,22]. This may depend on whether the bacteria exist in a biofilm or not [12,14,16,19,21] since bacteria within a biofilm are enmeshed in a physical gel that provides a secondary boundary that may allow small numbers of bacteria to evade the antibiotic; therefore, the failure to eliminate the entire population can allow the population to regrow.

It should be noted that populations of planktonic bacteria also contain these highly tolerant of persister cells [16,28]. Thus understanding the process of persister formation and the response of the population to biocidal application is fundamental to developing dosing protocols and treatments in both batch culture and biofilm populations.

As in many areas of biology, mathematical modeling has been used as a counterpart to experimental observations. Because there are several hypotheses regarding the mechanism of persister formation, mathematical modeling can be used to provide insight into the success of failure of treatment protocols as well as the consistency of various hypotheses. Currently, there are at least two distinct hypotheses concerning persister formation. In the first case, persister formation is attributed to senescence, and persister cells are assumed to be those that have undergone many division cycles. It is known that asymmetric division leads to degradation of parts of the cellular machinery that may be the underlying cause of persistence [26]. Mathematical analysis of a model of senescence has been described in both chemostat and biofilm settings [2,20].

A second hypothesis argues that persisters are a phenotype that is expressed at a rate that depends primarily on the growth stage. Although the biological details are not well understood, it is thought that this might be due to stochastic response to the environmental conditions [3,19,22,23,28]. This has been investigated mathematically as well [3,9,10,18,31]. In [9], a very simple model of persister formation was developed and optimal dosing protocols, that entail alternating application and resting, were described. In [10], toxin/antitoxin interaction was explicitly included and the resulting model was analyzed in a chemostat. Here a generic stress response was included that was assumed to be linked to the nutrient load, with higher rate of toxin accumulation when the nutrient level was low. In both of these investigations only one particular form for the growth rate function was used.

One of the goals of the current investigation is to extend these results to a more general form. In particular, modeling results are much more useful if they can be shown to be independent of much of teh biological details of the system. In this paper

we show that, although the process of persister formation is very complicated and is not completely understood, successful dosing strategies can be developed. The model and analysis is similar in spirit to a variety of models in the literature that seek to develop broad understanding of optimal control in the absence of complete biological understanding [1,7,6]

Our principal conclusions can be summarized as follows:

1. We generalize the model in [9] for persister formation by allowing more general growth rate functions, and it turns out that our qualitative conclusions do not depend on the particular form of these functions. In addition, while the model in [9] was a batch model, here we consider a chemostat setting. We also assume that persister cells are formed continuously, independently of whether or not the environment contains antibiotics. In [9], persister cells were assumed to be formed only when the environment contained antibiotics. Here we alter the model to include persister formation at a rate that depends only on the growth rate. In the absence of antibiotic challenge the persisters would not be evident except through slower growth rate; however, antibiotic challenge at various growth stage (i.e lag or exponential) would expose different concentrations of persister cells as demonstrated in [19].
2. Whereas the focus in [9] was on simulations, and the mathematical analysis on approximations of the model, here the focus is on the global mathematical analysis of the original model.
3. Our conclusions regarding the global behavior are modest, but they seem to answer the important question of whether or not the population is killed, or ultimately persists, by a given periodic treatment protocol. We do this by identifying a critical quantity, expressed in terms of the model parameters, whose value should fall below a threshold for treatment success. This quantity is the spectral radius of a certain matrix and plays a role which is very similar to that of the basic reproduction ratio in more classical epidemiological and population models [13]. Provided that the model parameters are known, the calculation of this quantity can precede an actual experiment or simulation run, and predict their outcome.
4. When treatment succeeds, the critical quantity is inversely proportional to the speed of convergence of solutions to the eradication steady state. We investigate numerically how the convergence speed depends on the duration of the antibiotic exposure of the bacterial population in a periodic dosing protocol. We find that this dependence is typically non-monotone and reaches a global maximum at a particular value of the duration. This may suggest to experimentalists how they should set up their periodic treatment experiment if the concern is to kill off the population as quickly as possible.

The manuscript is organized as follows: We begin by describing the model for the dynamics of the bacterial population in response to antibiotic challenge. We then develop the theory by analyzing two extreme cases (no dosing and constant dosing) and the intermediate case. This leads to a local sufficient condition for treatment success. Next we give a condition for global treatment failure, supported by numerical simulations of the model. We also show that the speed of eradication does

not necessarily depend monotonically on the duration of the administration of the antibiotic.

2 Model

Consider the following chemostat model:

$$\dot{B}_s = [(1 - k_d(t) - k_l)\, f(S) - D]\, B_s + k_g(t) B_p \tag{1}$$

$$\dot{B}_p = k_l f(S) B_s - [k_g(t) + D] B_p \tag{2}$$

$$\dot{S} = D(S^0 - S) - \frac{f(S) B_s}{Y} \tag{3}$$

where B_s is the concentration of the cells which are susceptible to antibiotics, B_p is the concentration of the persister cells and S is the concentration of the nutrient. This model deviates from the one in [9] because it is a chemostat model, which is reflected in the additional loss terms at rate D (called the dilution rate or washout rate), and the inflow (at the same rate D) of nutrient with an input concentration S^0. We have also incorporated an additional loss rate $k_l f(S) B_s$ for some positive k_l, of susceptible cells that become persister cells, regardless of whether or not antibiotics are administered. The per capita growth rate of the susceptible cells is denoted by $f(S)$, for which we assume the following throughout the rest of the paper:

$$f : \mathbb{R}_+ \to \mathbb{R}_+ \text{ is smooth and increasing and } f(0) = 0.$$

The persister cells do not consume nutrient, hence the lack of a corresponding growth term in the B_p-equation. The conversion of nutrient into new biomass occurs with a yield of $Y \in (0, 1)$.

The remaining functions $k_d(t)$ and $k_g(t)$ are non-negative, time-varying functions which describe the effect of antibiotics on the population. First, $k_d(t) f(S)$ is the killing rate of the susceptible population. Note in particular that the killing rate is proportional to the growth rate of the cells. It is positive when both antibiotic and nutrient are present, but zero when either one is missing. Secondly, $k_g(t)$ is the (per capita) rate at which persister cells revert to the susceptible state when antibiotic is absent (it is zero when antibiotic is present).

Since antibiotics are administered to the reactor vessel in a controlled (lab) environment, we will make the simplifying assumption that the functions $k_d(t)$ and $k_g(t)$ are τ-periodic (for some given $\tau > 0$), and of the bang-bang type with simultaneous switching instances: for some $p \in [0, 1]$, and for positive parameters k_d and k_g, there holds that

$$k_d(t) = \begin{cases} k_d & \text{for } \ t \in [0, p\tau) \\ 0 & \text{for } \ t \in [p\tau, \tau) \end{cases}, \quad \text{and} \quad k_g(t) = \begin{cases} 0 & \text{for } \ t \in [0, p\tau) \\ k_g & \text{for } \ t \in [p\tau, \tau) \end{cases}. \tag{4}$$

Thus, antibiotics are present during a fraction p of the period τ, and absent during the remaining fraction $1 - p$ of the period.

Clearly, this is a simplification of reality because the concentration of an antibiotic is not expected to be of the bang–bang type. In a more realistic model, the functions $k_d(t)$ and $k_d(t)$ would be replaced by functions depending on (at least) a new state variable for the concentration of the antibiotic, and the periodicity would arise through a periodic forcing term in the equation for this new variable. We leave the study of such a model to the future.

Throughout the rest of this paper we assume that the constant loss of susceptible cells to the persister compartment does not prevent a net positive growth of the susceptible class:

$$1 - k_l > 0, \tag{5}$$

but that the additional effect of the antibiotic is lethal to the susceptible cells:

$$1 - k_d - k_l < 0. \tag{6}$$

Note that this assumption is valid for the parameter values related to the experiments described in [9].

The main purpose of this paper is to investigate how the behavior of system (1)–(3) with (4) changes both qualitatively and quantitatively in terms of p.

3 Preliminary results

In this section we collect a couple of basic results concerning the dynamical behavior of (1)–(3) with (4). For a real-valued function $x(t)$, we denote the extended real numbers $\liminf_{t\to\infty} x(t)$ and $\limsup_{t\to\infty} x(t)$ by x_∞ and x^∞, respectively.

Lemma 1 *System (1)–(3) with (4) has $\mathbb{R}^3_+$ as a forward invariant set, and its solutions are ultimately bounded by a uniform bound.*

Proof The first assertion is obvious. The second follows from consideration of the dynamics of

$$M = B_s + B_p + YS,$$

given by

$$\dot{M} = D(YS^0 - M) - k_d(t) f(S) B_s \le D(YS^0 - M),$$

and hence

$$M^\infty \le YS^0.$$

Not only are all state components of every solution ultimately bounded from above by some constant which does not depend on initial conditions, we also notice in the following Lemma, that $S(t)$ is ultimately bounded from below by some positive constant which is independent of initial conditions as well.

Lemma 2 *There is a constant $\theta > 0$ such that for all solutions of (1)–(3) with (4), there holds that $S_\infty \geq \theta$.*

Proof Consider the function $g(x) := f(x)S^0 - D(S^0 - x)$. Then g is increasing with $g(0) < 0$ and $g(S^0) > 0$, hence by the intermediate value theorem, there is a unique $\theta \in (0, S^0)$ such that $g(\theta) = 0$. We will show that $S_\infty \geq \theta$. If not, then since $B_s^\infty \leq Y S^0$ by the proof of Lemma 1, it follows from Corollary 2.4 in [30]—a consequence of the famous Fluctuation Lemma—applied to (2) that

$$\begin{aligned} 0 &\geq \liminf_{t\to\infty} \left[D(S^0 - S_\infty) - \frac{f(S_\infty)B_s(t)}{Y} \right] \\ &\geq D(S^0 - S_\infty) - f(S_\infty)S^0 \\ &> D(S^0 - \theta) - f(\theta)S^0, \end{aligned}$$

which contradicts that $g(\theta) = 0$.

4 Analysis of the extreme cases $p = 0$ and $p = 1$

First we study the cases where antibiotic is either present or absent for all times. Our conclusions are the expected ones: When antibiotic is present continuously, all susceptible cells are either killed or washed out (note that none of the persister cells revert to the susceptible state). Consequently, since their rate of formation $k_l f(S)B_s$ tends to zero, all persisters are ultimately washed out.

When the population is never exposed to antibiotics, then ultimately the population will consist of a mixture of susceptible and persister cells, provided the dilution rate is not too high, as made precise below in condition (7) (Notice that the left-hand side in (7) is an increasing function in D by (5) which is 0 when $D = 0$.). Moreover, the system is uniformly persistent.

Theorem 1 1. *Case $p = 1$* (**continuous antibiotic dosing**). *The eradication steady state $(B_s, B_p, S) = (0, 0, S^0)$ of system (1)–(3) with (4) is globally asymptotically stable.*

2. *Case $p = 0$* (**no antibiotic dosing**). *Assume that*

$$D + k_l \frac{D^2}{k_g + D(1 - k_l)} < f(S^0). \tag{7}$$

The eradication steady state $(B_s, B_p, S) = (0, 0, S^0)$ is unstable, and there exists a unique positive steady state $(B_s, B_p, S) = (B_s^*, B_p^*, S^*)$ which is locally asymptotically stable. System (1)–(3) with (4) is uniformly persistent. More precisely, there is some $\epsilon > 0$ (independent of initial conditions), such that all solutions with $B_s(0) + B_p(0) > 0$, have the property that:

$$B_s(t) > \epsilon \quad \text{and} \quad B_p(t) > \epsilon, \quad \text{for all sufficiently large } t.$$

Proof 1. If $p = 1$, then $k_d(t) \equiv k_d$ and $k_g(t) \equiv 0$ for all t. By (6) it follows that for all solutions, $\dot{B}_s \leq -DB_s$, and hence $B_s(t) \to 0$ as $t \to \infty$. This suggests that we should study the linear limiting system

$$\begin{aligned} \dot{B}_p &= -DB_p \\ \dot{S} &= D(S^0 - S) \end{aligned}$$

whose solutions clearly converge to $(B_p, S) = (0, S^0)$. The conclusion now follows immediately by applying Theorem F.1 in [27].

2. If $p = 0$, then $k_d(t) \equiv 0$ and $k_g(t) = k_g$ for all t. By linearizing, we find that the Jacobian matrix at the eradication steady state $(0, 0, S^0)$ is

$$\begin{pmatrix} (1-k_l)f(S^0) - D & k_g & 0 \\ k_l f(S^0) & -(k_g + D) & 0 \\ -\frac{f(S^0)}{Y} & 0 & -D \end{pmatrix},$$

which has a negative eigenvalue $-D$. The two other eigenvalues are those of the matrix

$$\begin{pmatrix} (1-k_l)f(S^0) - D & k_g \\ k_l f(S^0) & -(k_g + D) \end{pmatrix},$$

whose determinant is negative because of (7). Hence, the Jacobian matrix at $(0, 0, S^0)$ has one positive and two negative eigenvalues, and thus it is unstable.
Let us next establish the existence of the unique positive steady state (B_s^*, B_p^*, S^*). If such a steady state exists, it must satisfy the following equations:

$$A(S)B = 0 \tag{8}$$

$$f(S)B_s = YD(S^0 - S), \tag{9}$$

where we used the following notation:

$$A(S) = \begin{pmatrix} (1-k_l)f(S) - D & k_g \\ k_l f(S) & -(k_g + D) \end{pmatrix}, \quad B = \begin{pmatrix} B_s \\ B_p \end{pmatrix}.$$

Before attempting to solve (8)–(9), we note that $A(S)$ is quasi-monotone (i.e., its off-diagonal entries are all non-negative) and irreducible (we can exclude $S = 0$ from the analysis). Then by the Perron–Frobenius theorem [4] it has a real dominant simple eigenvalue with a corresponding (entry-wise) positive eigenvector. The second eigenvalue is real as well (but distinct from the dominant one) since $A(S)$ is two-dimensional.
We start by calculating

$$\det(A(S)) = -(k_g + D(1-k_l))f(S) + D(k_g + D), \tag{10}$$

which is decreasing in S because $f(S)$ is increasing and because of (5). Since $f(0)=0$, it follows that $\det(A(0))>0$, and thus both eigenvalues of $A(0)$ are negative because the trace of $A(0)$ is negative. From the previous study of the linearization at the eradication steady state $(0,0,S^0)$, we already know that $\det(A(S^0))<0$, and thus that $A(S^0)$ has one positive and one negative eigenvalue. By the intermediate value theorem, it follows that there is a unique S^* such that $\det(A(S^*))=0$. For all $S<S^*$, we have that $\det(A(S))>0$, and thus $A(S)$ has two negative distinct eigenvalues. By continuity of the eigenvalues of $A(S)$, the matrix $A(S^*)$ has one negative eigenvalue, and its dominant eigenvalue is necessarily 0. The Perron–Frobenius theorem implies that the eigenvector of $A(S^*)$ corresponding to the dominant eigenvalue 0 is a positive vector B^*:

$$A(S^*)B^*=0.$$

But eigenvectors are only unique up to multiplication by a scalar, and so it may appear as if there are infinitely many possible positive choices for B^*. Equation (9) determines B^* uniquely however, since it fixes the value of B_s^* to $DY(S^0-S^*)/f(S^*)$, and this in turn will determine the value of B_p^* since $(B_s^*,B_p^*)^T$ must be an eigenvector of $A(S^*)$ associated to the dominant eigenvalue 0. In summary, we have established that there is a unique positive steady state $(B_s,B_p,S)^T=(B_s^*,B_p^*,S^*)^T$.

To show that it is locally stable we determine the characteristic polynomial of the Jacobian matrix evaluated at $(B_s^*,B_p^*,S^*)^T$. The calculation is lengthy but straightforward and therefore omitted, and yields the following polynomial:

$$\lambda^3+a_2\lambda^2+a_1\lambda+a_0,$$

where we have used the fact that $\det(A(S^*))=0$. The a_i are as follows:

$$\begin{aligned}
a_2 &= \big(D-(1-k_l)f(S^*)+k_g+D\big)+\left(D+\frac{f'(S^*)B_s^*}{Y}\right)\\
a_1 &= \big(D-(1-k_l)f(S^*)+k_g+D\big)\left(D+\frac{f'(S^*)B_s^*}{Y}\right)\\
&\quad +(1-k_l)f(S^*)\frac{f'(S^*)B_s^*}{Y}\\
&= \big(D+k_g+D\big)\left(D+\frac{f'(S^*)B_s^*}{Y}\right)-(1-k_l)f(S^*)D\\
a_0 &= \big(D(k_g+D)\big)\left(\frac{f'(S^*)B_s^*}{Y}\right)=:(\alpha)\,(\beta)
\end{aligned}$$

Since $\mathrm{tr}(A(S^*))<0$, it follows that a_2, a_1 and a_0 are all positive. It suffices by the Routh–Hurwitz criterion to show that

$$a_1a_2-a_0>0.$$

But since $a_2 > \beta$, it suffices to show that

$$a_1 > \alpha,$$

or equivalently, that

$$D\left(D + \frac{f'(S^*)B_s^*}{Y}\right) + (k_g + D)\frac{f'(S^*)B_s^*}{Y} - (1 - k_l)f(S^*)D > 0.$$

Since $f' \geq 0$, this is satisfied if

$$D^2 - (1 - k_l)f(S^*)D > 0,$$

or if

$$f(S^*) < \frac{D}{1 - k_l},$$

where we recall that $1 - k_l > 0$ by (5). Now, from $\det(A(S^*)) = 0$, it follows that

$$f(S^*) = \frac{D(k_g + D)}{k_g + (1 - k_l)D}.$$

It is easily verified that

$$\frac{D(k_g + D)}{k_g + (1 - k_l)D} < \frac{D}{1 - k_l}, \tag{11}$$

is satisfied which shows that $(B_s^*, B_p^*, S^*)^T$ is locally asymptotically stable. Finally, uniform persistence of (1)–(3) with (4) can be established by an application of Theorem 4.6 in [30]. Using the notation of that reference, we have here that

$$X_1 = \mathbb{R}^3_+, \quad X_2 = \partial\mathbb{R}^3_+, \quad Y_2 = \{(B_s, B_p, S)^T | B_s = B_p = 0, \ S \geq 0\},$$
$$\Omega_2 = \{(0, 0, S^0)^T\},$$

and Ω_2 has an acyclic isolated covering $\{(0, 0, S^0)^T\}$. Moreover, $\{(0, 0, S^0)^T\}$ is a weak repellor for X_1, because $(0, 0, S^0)^T$ is an unstable hyperbolic steady state with a stable manifold that does not intersect X_1, although we do not show that here. The steps of the proof are the same as those in the proof of uniform persistence of a within-host virus dynamics model in [15], and are therefore omitted.

5 The case of periodic dosing: $p \in (0, 1)$

In this section we deal with the τ-periodic model (1)–(3) with (4), assuming that $p \in (0, 1)$. We will also assume that the dilution rate is not too large, as in condition (7) of Theorem 1.

Notice that system (1)–(3) with (4) has a steady state $E_0 := (B_s, B_p, S) = (0, 0, S_0)$, regardless of the value of p. To determine its stability properties, we define $z = (B_s, B_p, S - S^0)$ and calculate the τ-periodic variational equation at E_0:

$$\dot{z} = \begin{pmatrix} (1 - k_d(t) - k_l) f(S^0) - D & k_g(t) & 0 \\ f(S^0) k_l & -(k_g(t) + D) & 0 \\ -\frac{f(S^0)}{Y} & 0 & -D \end{pmatrix} z.$$

One of the Floquet multipliers of this system is $\mathrm{e}^{-D\tau}$ (which is of course inside the unit circle of the complex plane). The other two Floquet multipliers are those of the following system:

$$\dot{x} = \begin{pmatrix} (1 - k_d(t) - k_l) f(S^0) - D & k_g(t) \\ f(S^0) k_l & -(k_g(t) + D) \end{pmatrix} x$$

Using (4), these Floquet multipliers are the eigenvalues of the following matrix:

$$\Phi := \mathrm{e}^{(1-p)\tau A_2}\, \mathrm{e}^{p\tau A_1}, \tag{12}$$

where

$$A_1 := \begin{pmatrix} (1 - k_d - k_l) f(S^0) - D & 0 \\ k_l f(S^0) & -D \end{pmatrix}, \quad \text{and}$$
$$A_2 := \begin{pmatrix} (1 - k_l) f(S^0) - D & k_g \\ k_l f(S^0) & -(k_g + D) \end{pmatrix} \tag{13}$$

are quasimonotone matrices. Notice also that all eigenvalues of A_1 are in the open half-plane by (6), and that A_2 has one negative and one positive eigenvalue, as shown in the proof of Theorem 1.

Since A_1 and A_2 are quasi-monotone, it follows that their matrix exponentials are (entry-wise) non-negative matrices and then their product Φ is a (entry-wise) positive matrix whose spectral radius $\rho(\Phi)$ is an eigenvalue by the Perron–Frobenius theorem [4]. Consequently, to determine stability of E_0, we need to establish whether or not $\rho(\Phi)$ is inside the unit circle: If $\rho(\Phi) < 1$, then E_0 is locally asymptotically stable. If $\rho(\Phi) > 1$, then E_0 is unstable. Summarizing, we have established

Theorem 2 *Let $p \in (0, 1)$, and assume that (7) holds. Then the steady state $E_0 = (0, 0, S^0)$ is locally stable for (1)–(3) with (4) if $\rho(\Phi) < 1$, but unstable if $\rho(\Phi) > 1$.*

Our main concern is knowing how $\rho(\Phi)$ varies as a continuous function of p (this variation is continuous since eigenvalues of a matrix are continuous functions of its entries, and clearly the entries of Φ are continuous in p). For $p = 0$ (never using antibiotic), and hence also for p near 0 by continuity of $\rho(\Phi)$, we have that $\rho(\Phi) = \rho(\mathrm{e}^{\tau A_2}) > 1$ because A_2 has a positive eigenvalue. This is in accordance with

Theorem 1 where it was shown that the microbial population persists uniformly. For $p = 1$ (using antibiotic continuously) we have by (6) that $\rho(\Phi) = \rho(\mathrm{e}^{\tau A_1}) = \mathrm{e}^{-D\tau} < 1$. The inequality also holds for p near 1. This is in accordance with Theorem 1 as well because it was shown there that all solutions converge to E_0 in this case.

6 Conditions for global treatment failure

In this section we show that the spectral radius $\rho(\Phi)$ also plays a key role in the global behavior of system (1)–(3) with (4) and $p \in (0, 1)$. We will show that if $\rho(\Phi) > 1$, then not only is E_0 unstable as we have shown in Theorem 2, but treatment fails globally, because both cell populations persist uniformly. In addition we will show that there are positive periodic solutions.

Theorem 3 *Let $p \in (0, 1)$, and assume that (7) holds. If $\rho(\Phi) > 1$, then treatment fails and the population is uniformly persistent. More precisely, there is some $\epsilon^* > 0$ (independent of initial conditions), such that all solutions of (1)–(3) with (4) and $B_s(0) > 0$, have the property that:*

$$B_s(t) > \epsilon^*, \quad \textit{and} \quad B_p(t) > \epsilon^*, \quad \textit{for all sufficiently large } t.$$

Moreover, there are τ-periodic solutions $(B_s(t), B_p(t), S(t))$ with $B_s(t), B_p(t) > 0$ for all t.

Proof Define the following matrix:

$$\tilde{\Phi}(\epsilon) = \mathrm{e}^{(1-p)\tau \tilde{A}_2(\epsilon)}\, \mathrm{e}^{p\tau \tilde{A}_1(\epsilon)},$$

where

$$\tilde{A}_1(\epsilon) := \begin{pmatrix} f(S^0 - \epsilon) - (k_d + k_l) f(S^0 + \epsilon) - D & 0 \\ k_l f(S^0 - \epsilon) & -D \end{pmatrix}, \quad \text{and}$$

$$\tilde{A}_2(\epsilon) := \begin{pmatrix} f(S^0 - \epsilon) - k_l f(S^0 + \epsilon) - D & k_g \\ k_l f(S^0 - \epsilon) & -(k_g + D) \end{pmatrix}$$

Notice that $\tilde{\Phi}(0) = \Phi$, and thus since $\rho(\Phi) > 1$, it follows that

$$\rho(\tilde{\Phi}(\epsilon)) > 1, \quad \text{for all sufficiently small } \epsilon > 0, \tag{14}$$

as well, because the spectral radius of any matrix is continuous with respect to its entries. We fix some $\epsilon > 0$ such that (14) holds.

We will first show that B_s is uniformly weakly persistent, i.e., that there is some $\epsilon' > 0$ such that if $B_s(0) > 0$, then $B_s^\infty \geq \epsilon'$. By contradiction, if B_s is not uniformly

weakly persistent, then there is some solution $(B_s(t), B_p(t), S(t))$ with $B_s(0) > 0$ such that

$$B_s^\infty \leq \frac{YD}{2f(S^0)}\epsilon. \tag{15}$$

By Corollary 2.4 in [30] applied to (3), and since $S^\infty \leq S^0$ by (3), we have that

$$\begin{aligned} 0 &\geq \liminf_{t\to\infty}\left[D(S^0 - S_\infty) - \frac{f(S_\infty)B_s(t)}{Y}\right] \\ &\geq D(S^0 - S_\infty) - \frac{f(S^0)B_s^\infty}{Y}, \end{aligned}$$

and hence by (15) that

$$S_\infty \geq S^0 - \frac{\epsilon}{2}.$$

Thus, for some $T^* > 0$, there holds that $S^0 - \epsilon \leq S(t) \leq S^0 + \epsilon$ for all $t \geq T^*$ (the latter inequality follows from the proof of Lemma 1). It follows from (1)–(2), that for all $t \geq T^*$:

$$\begin{pmatrix}\dot{B}_s \\ \dot{B}_p\end{pmatrix} \geq \begin{pmatrix} f(S^0-\epsilon) - (k_d(t)+k_l)f(S^0+\epsilon) - D) & k_g(t) \\ k_l f(S^0-\epsilon) & -(k_g(t)+D)\end{pmatrix}\begin{pmatrix}B_s \\ B_p\end{pmatrix} \tag{16}$$

where the vector inequalities should be interpreted componentwise. Notice that the vector field on the right-hand side of (16) is that of a τ-periodic, cooperative linear system whose principal fundamental matrix solution evaluated over one period τ equals $\tilde{\Phi}(\epsilon)$. By Kamke's comparison Theorem (see e.g., Theorem B.1 in Appendix B of [27]) it follows that for all $t \geq T^*$, the vector $(B_s(t), B_p(t))^T$ is not smaller (component-wise) than the solution starting in $(B_s(T^*), B_p(T^*))^T$ of the τ-periodic, cooperative linear system with vector field given in the right-hand side of (16). But all non-zero, non-negative solutions of the linear system diverge because $\rho(\tilde{\Phi}(\epsilon)) > 1$. Then so does $(B_s(t), B_p(t))$, and this contradicts (15). We have thus shown that B_s is uniformly weakly persistent.

Next we establish that B_s is in fact uniformly strongly persistent. This follows from Theorem 1.3.3 in [32], applied to the map P which maps $(B_s(0), B_p(0), S(0))^T \in X$ to $(B_s(\tau), B_p(\tau), S(\tau))^T$, where $X := \{(B_s, B_p, S)^T \in \mathbb{R}^3_+ | B_s + B_p + YS \leq YS^0\}$, $X_0 := \{(B_s, B_p, S)^T \in X \mid B_s \neq 0\}$ and $\partial X_0 := \{(B_s, B_p, S)^T \in X \mid B_s = 0\}$. The map P is continuous and maps X_0 into itself, and it has a global attractor because it is compact and dissipative. It follows that there is some $\epsilon_1^* > 0$, independent of initial conditions, such that if $B_s(0) > 0$, then $\liminf_{n\to\infty} B_s(n\tau) > \epsilon_1^*$, and also that $\liminf_{t\to\infty} B_s(t) > \epsilon_1^*$ by Theorem 3.1.1 in [32].

Next we show that uniform strong persistence of B_s, implies uniform strong persistence of B_p. Consider equation (2) and notice that for all sufficiently large t:

$$\dot{B}_p \geq k_l f(\theta) B_s - (k_g(t) + D) B_p \geq k_l f(\theta) \frac{\epsilon_1^*}{2} - (k_g(t) + D) B_p,$$

where θ is the positive constant from Lemma 2. It is not hard to show that the linear equation

$$\dot{z} = k_l f(\theta) \frac{\epsilon_1^*}{2} - (k_g(t) + D) z,$$

has a positive τ-periodic solution $p(t)$ and that all non-negative solutions converge to it. Therefore, it follows that for all sufficiently large t,

$$B_p(t) \geq \frac{p_\infty}{2},$$

establishing uniform strong persistence for B_p, since p_∞ is independent of initial conditions. We conclude the proof of uniform strong persistence of B_s and B_p by setting $\epsilon^* = \min\{\epsilon_1^*, \frac{p_\infty}{2}\}$.

Finally, to show that there are τ-periodic solutions with $B_s(t), B_p(t) > 0$, we apply Theorem 1.3.6 from [32] applied to the continuous map P defined above. We have already remarked that this map is continuous, maps X_0 into itself, is dissipative and compact, and we have just proved that it is uniformly strongly persistent with respect to $(X_0, \partial X_0)$. Observe also that X_0 is relatively open in X, and that X_0 is convex. Then by Theorem 1.3.6 from [32], the map P has a fixed point in X_0, and this in turn implies the existence of τ-periodic solutions with $B_s(t) > 0$ for (1)–(3). The same argument as the one used above to establish uniform strong persistence of $B_p(t)$, shows that these τ-periodic solutions are such that $B_p(t) > 0$ as well.

7 Numerical example

In the following, we describe results of numerical simulations of the model equations. These numerical experiments illustrate the intermediate cases of periodic dosing. In particular, we see that the spectral radius provides a simple test for the success or failure of a dosing strategy. The period of application is by far the simplest lab control that is available, we vary the dose duration while keeping the other parameters fixed. We use the following numerical values: The per capita growth rate is of Michaelis-Menten type:

$$f(S) = \frac{\mu S}{k_s + S}, \quad \text{where } \mu = 0.417 \text{ hs}^{-1} \quad \text{and} \quad k_s = 0.2 \text{ mgl}^{-1},$$

which are typical values [25]. Parameters describing the loss/gain and period are

$$k_d = 3, \quad k_l = 0.1, \quad k_g = 0.5 \text{ h}^{-1}, \quad k_l = 0.001 \text{ h}^{-1} \quad \text{and} \quad \tau = 10 \text{ h}.$$

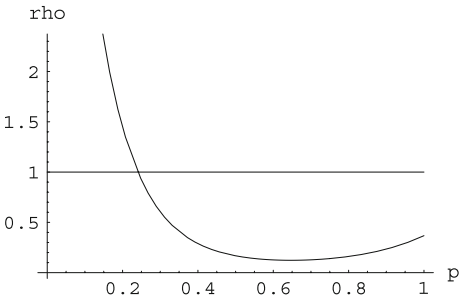

Fig. 1 Spectral radius of Φ for $p \in [0, 1]$ ($k_d = 3$, other parameters in text)

These are consistent with values in [9], that were determined by fitting the experiments using *E. coli* [19].

The chemostat setting requires that we specify two additional parameters:

$$D = 0.1 \text{ h}^{-1} \quad \text{and} \quad S^0 = 1 \text{ mgl}^{-1}.$$

It can be easily verified that these choices satisfy the conditions (6) and (7). Note that there is no need to specify the yield coefficient Y in order to calculate $\rho(\Phi)$. Indeed, the matrix Φ in (12) does not depend on Y.

We note that the bulk of the results shown in this section agree with experimental expectations [5,22]; however, we are unaware of any direct experimental results available for comparison. These results are consistent with mathematical results that have been described before [9,10] with additional insight that there are only mild requirement on the transition rate from susceptible to persister in order for periodic dosing to be successful. This suggests that a simple experiment done in a chemostat could verify that there is an optimal strategy for eliminating a population of bacteria. Moreover, our analysis indicates that, although we have fixed the kinetics in the simulations, the results should generalize to a variety of bacteria and nutrients.

The graph of the spectral radius $\rho(\Phi)$ in terms of p, determined using formula (12), is given in Fig. 1. We see that $\rho(\Phi) = 1$ when p is approximately equal to 0.241. Clearly, $\rho(\Phi)$ is not monotone, and it has a global minimum of approximately 0.123 which is achieved at $p \approx 0.647$ (determined numerically using Mathematica). Thus, the optimal strategy in a dosing experiment with a period of $\tau = 10$ h occurs for a dosing duration of $p\tau \approx 6.5$ h. Here, optimality means that eradication happens as quickly as possible.

Let us also illustrate what happens if p equals approximately 0.205. Then $\rho(\Phi)$ equals approximately 1.38, implying that treatment fails. It appears that the solution of (1)–(3) with $Y = 1$ (and all other parameters as above) starting from the initial condition $(B_s, B_p, S) = (0.3, 0, 0.4)$ converges to a τ-periodic solution, see Figs. 2 and 3. These observations are in accordance with Theorem 3.

We also see that if $p = \frac{1}{3}$, then $\rho(\Phi)$ equals approximately 0.464. Then it follows from Theorem 2 that E_0 is locally stable. Figures 4 and 5 illustrate this by indicating that for the solution with the same initial condition $(B_s, B_p, S) = (0.3, 0, 0.4)$ as above, treatment is successful.

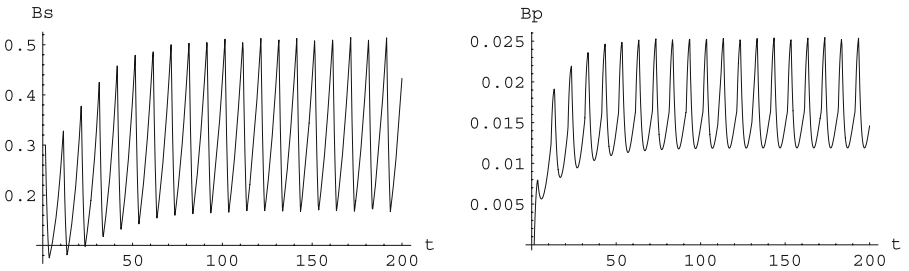

Fig. 2 Times series for B_s and B_p. ($p \approx 0.205$, $\rho(\Phi) \approx 1.38 > 1$, so treatment fails)

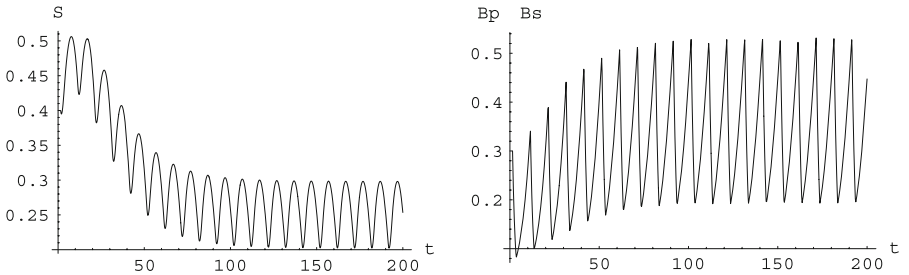

Fig. 3 Times series for S and $B_s + B_p$. ($p \approx 0.205$, $\rho(\Phi) \approx 1.38 > 1$, so treatment fails)

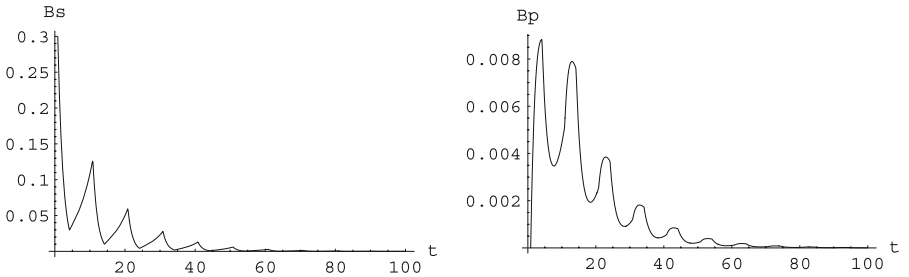

Fig. 4 Times series for B_s and B_p. ($p = \frac{1}{3}$, $\rho(\Phi) \approx 0.464 < 1$, so treatment succeeds)

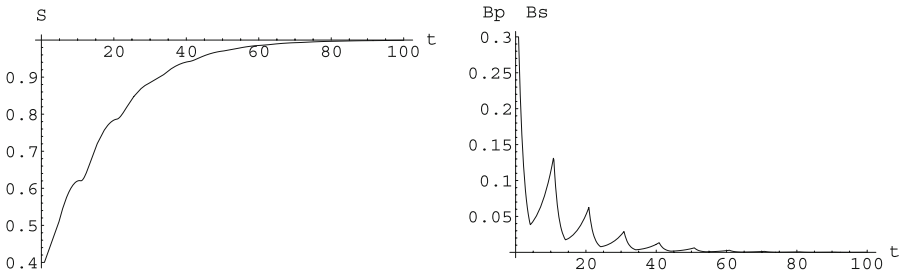

Fig. 5 Times series for S and $B_s + B_p$. ($p = \frac{1}{3}$, $\rho(\Phi) \approx 0.464 < 1$, so treatment succeeds)

Finally, we note that the spectral radius of Φ may be monotone: If we change the value of k_d from 3 to 1, and leave all other parameters unchanged, then $\rho(\Phi)$ is a decreasing function of $p \in [0, 1]$, and achieves its minimum at $p = 1$. This

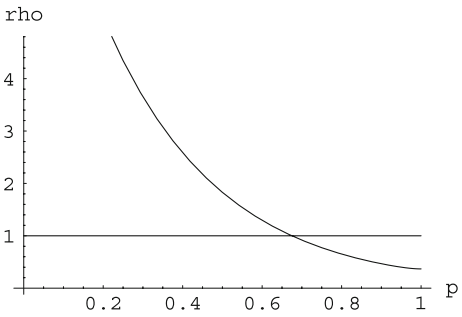

Fig. 6 Spectral radius of Φ for $p \in [0, 1]$ ($k_d = 1$, other parameters in text)

indicates that for this case, the optimal strategy is to use antibiotics continuously, see Fig. 6.

8 Conclusions

Bacterial infections are a source of problems in a wide variety of situations including industrial, environmental and clinical settings. Growing understanding of the inability of antibiotics and biocides to treat these infections has driven investigations into the cause of the failure of treatments. It is becoming increasingly evident that persister cells must play an important role in protecting populations of bacteria. It has been observed that other protective mechanisms including physiological and physical processes are not sufficient to explain the observed failures [8,11]. Moreover, because it is very difficult to investigate and classify persister cells experimentally, mathematical modeling can play an important role in supporting hypotheses as well as generating useful predictions.

We have described and analyzed a general model for the dynamics of persister formation in response to antibiotic challenge, and have provided a condition for the success/failure of antibiotic challenge in a chemostat. It relies on the value of a certain quantity -the spectral radius of a particular matrix- that can be determined analytically and numerically in terms of the model parameters. If these are known it can be a useful tool to predict the outcome of a dosing experiment or model simulation alike. It can also be used to guide the experimentalist in setting up a periodic dosing protocol which eliminates the bacteria as quickly as possible. These results indicate that periodic dosing is an effective treatment protocol for a variety of bacteria, substantially strengthening the results in [9]. The theoretical results were also confirmed by direct numerical simulations.

Acknowledgments Supported by NSF grant DMS-0614651.

References

1. Agur Z, Hassin R, Levi S (2006) Optimizing chemotherapy scheduling using local search heuristics. Oper Res 54(5):829846
2. Ayati BP, Klapper I (2007) A multiscale model of biofilm as a senescence-structured fluid. SIAM Multi Model Sim 6:347–365

3. Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S (2005) Bacterial persistence as a phenotypic switch. Science 305:1622–1625
4. Berman A, Plemmons R (1994) Nonnegative matrices in the mathematical sciences. SIAM, Philadelphia
5. Bigger J (1944) Treatment of stapphylococcal infections with penicillin by intermittent sterilisation. Lancet 244(6320):497–500
6. Burden T, Ernstberger J, Fister R (2004) Optimal control applied to immunotherapy. Discrete Continuous Dyn Syst Ser B 4(1):135146
7. Cappuccio A, Elishmereni M, Agur Z (2007) Optimization of interleukin-21 immunotherapeutic strategies. J Theor Biol 248(2):259–266
8. Chambliss JD, Hunt SM, Stewart PS (2006) A three-dimensional computer model of four hypothetical mechanisms protecting biofilms from antimicrobials. Appl Environ Microbiol 72:2005–2013
9. Cogan NG (2006) Effects of persister formation on bacterial response dosing. J Theor Biol 238: 694–703
10. Cogan NG (2007) Incorporating toxin hypothesis into a mathematical model of persister formation and dynamics. J Theor Biol 248:340–349
11. Cogan NG, Cortez R, Fauci LJ (2005) Modeling physiological resistence in bacterial biofilms. Bull Math Biol 67(4):831–853
12. Costerton J (2001) Cystic fibrosis pathogenesis and the role of biofilms in persistent infection. Trends Microbiol 9:50–52
13. Cushing J (1998) An introduction to structured population dynamics. SIAM, Philadelphia
14. Davies D (2003) Understanding biofilm resistance to antibacterial agents. Nat Rev Drug Discovery 2:114–122
15. De Leenheer P, Smith HL (2003) Virus dynamics: a global analysis. SIAM J Appl Math 63: 1313–1327
16. Desai M, Buhler T, Weller P, Brown M (1998) Increasing resistance of planktonic and biofilm cultures of *Burkholderia cepecia* × to ciproflaxacin and ceftazidime during exponential growth. J Antimicrob Chemother 42:153–160
17. File TM Jr (1999) Overview of resistance in the 1990s. Chest 115:3–8
18. Imran M, Smith HL (2006) The pharmacodynamics of antibiotic treatment. J Comput Math Methods Med 7:229–263
19. Keren I, Kaldalu N, Spoering A, Wang Y, Lewis K (2004) Persister cells and tolerance to antimicrobials. FEMS Microbiol Lett 230:13–18
20. Klapper I, Gilbert P, Ayati BP, Dockery J, Stewart PS (2007) Senescence can explain microbial persistence. Microbiology 153:3623–3630
21. Lappin-Scott HM, Costerton JW (eds) (1995) Microbial biofilms. In: Mechanisms of the protection of bacterial biofilms from antimicrobial agents. Cambridge University Press, Cambridge, pp 118–130
22. Lewis K (2001) Riddle of biofilm resistance. Antimicrob Agents Chemother 45:999–1007
23. Lewis K (2005) Persister cells and the riddle of biofilm survival. Biochem Moscow 70:267–285
24. Neu HC (1992) The Crisis in antibiotic resistance. Science 257(5073)
25. Roberts ME, Stewart PS (2004) Modeling antibiotic tolerance in biofilms by accounting for nutrient limitation. Antimicrobial Agents Chemother 48:4852
26. Stewart EJ, Madden R, Paul G, Taddei F (2005) Aging and death in an organism that reproduces by morphologically symmetric division. PLoS Biol 3:295–300
27. Smith HL, Waltman P (1995) The theory of the chemostat. Cambridge University Press, Cambridge
28. Spoering A, Lewis K (2001) Biofilms and planktonic cells of pseudomonas aeruginosa have similar resistance to killing by antimicrobials. J Bacteriol 183(23):6746–6751
29. Szomoloy B, Klapper I, Dockery J, Stewart P (2005) Adaptive responses to antimicrobial agents in biofilms. Environ Microbiol 7(8):1186–1191
30. Thieme HR (1993) Persistence under relaxed point-dissipativity (with applications to an endemic model). SIAM J Math Anal 24:407–435
31. Wiuff C, Anderson D (2007) Antibiotic treatment in vitro of phenotypically tolerant bacterial populations. J Antimicrob Chemother 59(2):254–263
32. Zhao X-Q (2003) Dynamical systems in population biology. Springer, New York