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A MODEL OF OPTIMAL DOSING OF ANTIBIOTIC TREATMENT IN BIOFILM

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Abstract. Biofilms are heterogeneous matrix enclosed micro-colonies of bacteria mostly found on moist surfaces. Biofilm formation is the primary cause of several persistent infections found in humans. We derive a mathematical model of biofilm and surrounding fluid dynamics to investigate the effect of a periodic dose of antibiotic on elimination of microbial population from biofilm. The growth rate of bacteria in biofilm is taken as Monod type for the limiting nutrient. The pharmacodynamics function is taken to be dependent both on limiting nutrient and antibiotic concentration. Assuming that flow rate of fluid compartment is large enough, we reduce the six dimensional model to a three dimensional model. Mathematically rigorous results are derived providing sufficient conditions for treatment success. Persistence theory is used to derive conditions under which the periodic solution for treatment failure is obtained. We also discuss the phenomenon of bi-stability where both infection-free state and infection state are locally stable when antibiotic dosing is marginal. In addition, we derive the optimal antibiotic application protocols for different scenarios using control theory and show that such treatments ensure bacteria elimination for a wide variety of cases. The results show that bacteria are successfully eliminated if the discrete treatment is given at an early stage in the infection or if the optimal protocol is adopted. Finally, we examine factors which if changed can result in treatment success of the previously treatment failure cases for the non-optimal technique.

1. **Introduction.** Bacteria are mostly found living in micro-colonies known as biofilms. The biofilm formation is the core reason for many microbial infections [5], [7], [18] and [13]. The foremost cause of failure of medical implants is bacterial infection, which is due to the formation of biofilms [2], [13] and [40] on the surfaces of indwelling medical devices and in some cases on the adjacent tissues as well. This phenomenon has been observed in various devices such as artificial joints, prosthetic heart valve, urinary catheters and contact lenses [7], [13], [14], [37] and [39]. Biofilms are heterogenous matrix-enclosed bacterial accumulations that

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may attach to living or non-living surfaces, surrounded by water channels [1] and [18]. Microbial colonies in biofilms are rooted in a glue-like matrix, which is mainly composed of exopolysaccharides; however minute amount of proteins and nucleic acid are also present[1]. The plaque that forms on the surface of teeth, causing tooth decay, is also a type of biofilm.

Biofilm formation is a growth cycle which initiates as the free-floating bacteria identify a surface and strongly adhere to it by excreting polymers that aid in attachment and matrix formation [1], [13], [18] and [37]. These attached cells multiply in number forming micro-colonies of bacteria, leading to maturation of the microbial cells. The resulting mature biofilm may form a mushroom-like structure, with open water channels acting as a circulatory system [1] and [18]. The biofilm bacteria show a change in phenotype with respect to the rate of growth and gene transcription [13] [37]; hence bacteria in biofilm have a genetic makeup different from the individual bacterial cells [37].

It is observed that at times, antibiotic treatment fails to cure the bacterial infections due to biofilm formation. The reason for this failure of antimicrobial therapy has been an active research area in the recent years [5],[7] and [14]. Several mathematical models explaining this have been developed, which have improved our understanding of this subject.

Several hypothesis explaining the reduced susceptibility of bacteria growing in biofilms to antimicrobial therapy have been devised. According to one hypothesis, the structure of biofilm prevents the antibiotic from penetrating inside the deeply embedded bacterial community [2], [5] [39]and [40]. Several mechanisms such as reaction with neutralizing agent, synthesis of antibiotic degrading enzymes and sorption of antibiotic by exo-polymeric substance leads to limited penetration of antimicrobial agents in biofilm [5], [6] and [2]. Phenotypic resistance is another actively researched mechanism that leads to decreased mortality of bacteria in biofilm [4], [42] and [44]. Genetically homogenous bacterial populations may be different phenotypically with respect to their tolerance to antimicrobial agents [6], [12] and[44]. Exposure of this type of phenotypically heterogenous population to antibiotics results in an increase in resistant bacterial population to antibiotic treatment, as the exposure time of bacteria to medication increases [5] and [44].

Another suggested preventive phenomena to antibiotic treatment in biofilms is the physiological resistance [5], [4], [6], [8] and [36]. According to this hypothesis, the slow growing and non-respiring bacteria are protected from antibiotic therapy because of their inactivity. The decreased growth rate and inadequate nutrient supply are common features of biofilm. It is suggested [4] and [36] that bacteria on the surface are killed at a faster rate than the those embedded deep in the biofilm [8]. It can then be expected that as the bacteria on surface are killed, the nutrient penetrates into the biofilms, hence rendering those bacteria susceptible to antibiotic treatment. By this reasoning, one may expect that this would lead to a complete eradication of bacteria; however this is not the case observed [4] [36]. Nevertheless, studies [5, 10, 36] have shown that nutrient limitation and decreased growth rate lead to reduced rate of killing of biofilm bacterial population and that these two factors are major causes of phenotypic tolerance as well [10]. This paper focuses on the conditions under which antibiotic treatment successfully eradicates the microbial population from biofilm and surrounding fluid compartment. In order to understand the working of antibiotic on bacteria, we take into consideration the pharmacology of the antimicrobial agent.

The pharmacology of antibiotics can be divided into two branches, pharmacokinetics and pharmacodynamics. Pharmacokinetics describes the movement of antibiotics into, through and out of the body whereas pharmacodynamics describes the relationship between the concentration of antibiotics, its effect on target bacteria (growth or decay)[11] and factors influencing this relationship. The elimination of the drug either by metabolism or excretion is very important in studying antibiotics, since it determines the frequency (both periodicity and concentration) of the antibiotic administered. If the metabolization of the antibiotic is very high, it must be given frequently as compared to that which is broken down slowly. One of the important objectives of pharmacokinetics is to decide the optimal frequency of an antibiotic for a successful treatment. On the other hand, pharmacodynamics describes in detail the relationship between concentration and its effects on the bacterial population in order to achieve the maximum removal of bacteria from the host.

Mathematical modelling of the effects of drug treatment has long been used side-by-side with experimental studies [5], [10], [22], [24], [30], [31], [36] and [42]. Most mathematical models of the effect of antimicrobial agent on bacterial population assume that bacteria grows at an exponential rate in the absence of the antimicrobial agent. The pharmacodynamics function, in our case, has to be determined only for the agent. So it is a mathematical expression for the decline in growth rate resulting from a given concentration of the antimicrobial agent.

As mentioned earlier, it is often observed that fast growing and rapidly reproducing bacteria are more prone to antibiotics and biocide treatments as compared to the bacteria that are reproducing less actively [4], [8], [10], [39], [42] and [43]. A slow growth rate and a restricted availability of nutrients could be the major contributors towards insensitivity of antibiotics to the kill rate of bacteria. This leads us to choose a pharmacodynamics function that depends on the concentration of antibiotic and limiting nutrients level. It has been noted that bacteria multiply more slowly in an experimental animal than *in vitro*, suggesting nutrient limitation *in vivo*[9] [10]. Therefore, it seems obvious that basic Monod model of microbial growth under nutrient limitation should be at the core of models that include the population dynamics of the pathogen [22].

Many other researchers have used the pharmacodynamics function that depends on the antimicrobial agent and limiting resource level. Corpet *et al.* [9] introduces pharmacodynamics function that depends on limiting both nutrient and antimicrobial agent. Cogan in [5] does so as well while considering persister cells. Cozen [10] notes that the restricted availability of iron and other nutrients appears to be typical of infection states. Robert and Stewart [36] construct a mathematical model to explore the possibility that the observed antibiotic tolerance of biofilms is due in part to nutrient limitation reducing bacterial growth and hence killing rates. Even if resource supply rates are relatively constant, one expects significant depletion in local resource levels as a bacterial infection progresses and we expect these changes to play a role in treatment by antimicrobial agent.

In this paper we will give a brief overview on how the antibiotic affects the growth of bacteria in biofilm and surrounding fluid. Flow of the nutrient, antimicrobial agent and bacterial population in the biofilm and fluid surrounding it are considered, taking into account the diffusive transport. The killing rate induced by antibiotic is built on the model proposed by [22]. The pharmacokinetics function is based on

[20], [22], [29] and [35]. Briefly described below are the key features of our work and where it differs from the previous work in this field:

1. We build a two compartment biofilm model based on the plasmid model of Imran and Smith [23] and include the antibiotic equation used by [3], [21] and [29]. This work is an extension of the work in [22], where antibiotic treatment was studied for one compartment and two types of bacteria - susceptible and resistant. We now consider the same kind of bacterial populations in a biofilm and the surrounding fluid. By making suitable assumptions, this system effectively reduces to a single compartment model.
2. A periodic antibiotic dosing protocol is initially investigated. We derive simple models to understand the effects of the killing effect of an antibiotic on bacterial population. Our model differs from other studied biofilm models by the consideration of limitation of microbial growth due to limited nutrient and the removal of antimicrobial agent by association with bacteria [23]. We use singular perturbation theory to characterize treatment failure, i.e., when the bacteria is not completely eliminated [22].
3. Overuse of antibiotics can lead to bacterial resistance to antibiotic action [34]. It is therefore imperative to apply them in measured doses while also eradicating bacteria [17]. We study the effects of dose tapering for our bactericidal model. In addition, we devise optimal dosing strategies that eliminate bacteria from our system while also minimizing antibiotic usage.

One limitation of our model is that we have treated the biofilm as a single layered structure. The nutrient and antibiotic are both taken to be present in sufficient quantities so as to penetrate the biofilm entirely. This allows us to treat all the bacteria in the biofilm uniformly.

In the next section, we formulate and analyze models of antibiotic treatment of bacterial populations in fluid and biofilm compartments.

2. **Model of bactericidal antibiotic treatment.**

We consider the effect of antibiotic treatment in a two compartment model, the fluid environment and the wall growth or biofilm environment. We take the effect of the antibiotic to be bactericidal, that is killing the bacteria. We have assumed that the killing rate of bacteria is lower in the biofilm as compared to that in the fluid due to the inactivity of bacteria in biofilm, as explained in the Introduction. We begin with the basic model of antibiotic treatment proposed in [22] and suppose that the contents of the fluid compartment have a high mixing rate with the biofilm compartment. The term $U(t)$ denotes the bacterial population in fluid and $u(t)$ denotes the population of the bacteria in biofilm. Let S and A denote the concentrations of limiting nutrient and antibiotic agent in fluid respectively and let s and a denote the concentrations of limiting nutrient and antibiotic in biofilm respectively.

The population of the bacteria changes due to growth, death due to antibiotic action, loss due to wash out and loss because of diffusion from one compartment to the other. Thus, the equations governing the dynamics of the bacteria in fluid and biofilm are based on

$$\begin{aligned} U' &= \text{growth} - \text{washout} - \text{antibiotic-killing} - \text{biofilm attachment} \\ &\quad + \text{biofilm sloughing} \\ u' &= \text{growth} - \text{antibiotic-killing} + \text{biofilm attachment} - \text{biofilm sloughing}. \end{aligned}$$

The disinfection term is directly linked to the growth as well as to the type of antimicrobial agent used. It is shown that the slow growing bacteria are more resistant to antibiotic agent as compared to the fast growing species. Our model assumes that the rate of the killing by the antibiotic is directly proportional to the growth rate. Moreover, some antibiotics are effective for growing and some are effective for non-growing bacteria. The bactericidal antibiotics are mostly effective against cells that are growing and dividing [33]. Slower growth rate weakens the effect of bactericidal antibiotic. We use $f_1(S, A)$ and $f_{1u}(s, a)$ respectively as the pharmacodynamics functions for the fluid and biofilm compartments.

2.1. **The model and preliminary results.** We take the model based on the *in vivo* model of antibiotic treatment. The equations are:

$$\begin{aligned} VS' &= F(S^0 - S) - \gamma^{-1}VUf(S) - r_s(S - s) \\ VA' &= F(A_0(t) - A) - VUg(A) - r_a(A - a) \\ VU' &= (f(S) - F/V - f_1(S, A))\,VU - r_u(U - u) \\ vs' &= r_s(S - s) - \gamma^{-1}vuf_u(s) \\ va' &= r_a(A - a) - vug_u(a) \\ vu' &= (f_u(s) - f_{1u}(s, a))vu + r_u(U - u) \end{aligned} \tag{1}$$

where V denotes the volume of fluid compartment, v the volume of biofilm compartment and F the mixing rate of the contents of the fluid compartment to the biofilm compartment. The terms r_s,r_u and r_a are the flow rates of nutrient,bacteria and antibiotic respectively from one compartment to the other. We define $\epsilon = \frac{V}{F}$; assuming that F has a large value, implying that the fluid compartment has a very high mixing rate with the biofilm compartment, we infer that ϵ is small. Such a situation arises where the contents of the biofilm environment are stagnant while the surrounding fluid is moving very fast.

Since *flow rate/volume* of the compartment gives the dilution rate, we manipulate the above equations and replace the terms r_i/V and r_i/v by D_i and d_i respectively, for $i = s, a, u$, to obtain the following set of equations:

$$\begin{aligned} \epsilon S' &= (S^0 - S) - \epsilon\left[\gamma^{-1}Uf(S) + D_s(S - s)\right] \\ \epsilon A' &= (A_0(t) - A) - \epsilon\left[Ug(A) + D_a(A - a)\right] \\ \epsilon U' &= -U + \epsilon\left[(f(S) - f_1(S, A))\,U - D_u(U - u)\right] \\ s' &= d_s(S - s) - \gamma^{-1}uf_u(s) \\ a' &= d_a(A - a) - ug_u(a) \\ u' &= (f_u(s) - f_{1u}(s, a))\,u + d_u(U - u) \end{aligned} \tag{2}$$

where D_s, D_a and D_u give the dilution rates of nutrient, antibiotic and bacteria respectively in the fluid compartment and d_s, d_a and d_u are the dilution rates of nutrient, antibiotic and bacteria respectively in the biofilm compartment.

We take the fresh nutrient at constant concentration S^0 as input and antibiotic concentration at time t, $A_0(t)$, as the input. The yield constant γ gives the conversion of nutrients to organism. The functions $f(S)$ and $f_u(s)$ are the growth rate of bacteria at nutrient concentration S and s in fluid and biofilm compartment respectively. Classically, we take f to be Monod type but our results hold more generally. The only requirement for the growth function is to be monotonically increasing in

S:

$$f(0) = 0, \qquad f'(S) \geq 0$$

and the same holds for $f_u(s)$.

Most of the models of antibiotic treatment [3] are based on the assumption that bacteria has no effect on antibiotic concentration; its concentration at the infection site is dependent only on the input to the model. However, here we take antimicrobial concentration at the site of infection as a dynamic variable with periodic dosing as input to the model. In this case of oscillatory antibiotic concentration, the critical parameter is the "invasion eigenvalue" which establishes whether pathogens can infect an environment in which the antimicrobial level have reached their asymptotic periodic pharmacodynamics regime $a^*(t)$: classically, a recurring cycle of exponential decay and rise following a discrete dose, as shown in Figure 1.

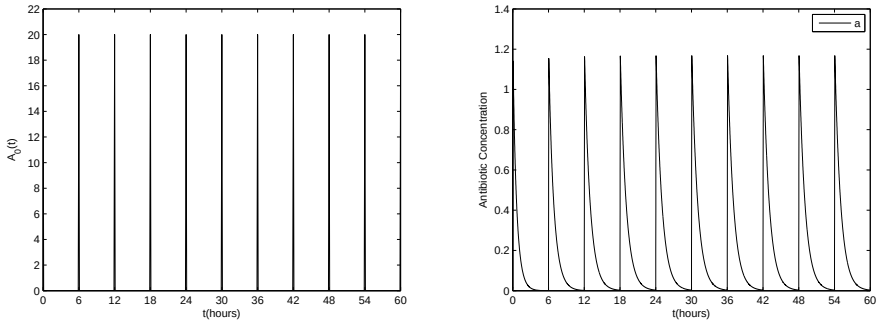

FIGURE 1. *Left* figure shows periodic discrete dosing of an antibiotic. *Right* figure shows resulting pharmacokinetics $a^*(t)$.

Characteristically, antibiotics are administered either as a constant dose $A_0(t) = A_0 =$ or periodically $A_0(t) = A_0(t+T) \geq 0$ with T as the dosing period. Although our model allows a general non-negative periodic dosing function $A_0(t)$, in reality it is typically a sequence of discrete doses which might be approximated by:

$$A_0(t) = \sum_i d\delta(t - iT).$$

Parameter d measures dose and δ is the Dirac impulse function [22]. The simulations in Figure (1) take the dosing period as $T = 6hrs$.

The function $f_1 = f_1(S, A)$ is the pharmacodynamics function for the fluid compartment and $f_{1u} = f_{1u}(s, a)$ is the pharmacodynamics function for the biofilm compartment, which describes the kill rate induced by the antimicrobial agent per unit of bacteria. In general, the killing rate depends on the bacteria and the antibiotic used as well as the nutrient levels.

Qualitative assumptions are made regarding the pharmacodynamic functions $f_1(S, A)$ and $f_{1u}(s, a)$. They should vanish if there is no antibiotic and increase

as the antibiotic concentration is increasing:

$$f_1(S,0)=0, \qquad \frac{\partial f_1}{\partial A}\geq 0$$
$$f_{1u}(s,0)=0, \qquad \frac{\partial f_{1u}}{\partial a}\geq 0.$$

Moreover, adding nutrient should not decrease the net bacterial growth rate:

$$f(S)-f_1(S,A) \text{ is non decreasing for } 0\leq S\leq S^0.$$

The same holds for $f_{1u}(s)$.

Finally, equation (1) includes a removal rate of antibiotic due to its association with bacteria, modeled by the term $-g(A)U$ for the fluid compartment and $-g_u(a)u$ for the biofilm compartment [21], [29] and [32]. This function g can be taken in accordance with the Michaelis-Menten kinetics or simply as $g(A)=cA$. Many authors neglect the factor g(A) which results in the decoupling of the pharmacokinetics from the rest of the model. We assume that g vanishes with A and is nondecreasing in A:

$$g(0)=0, \qquad g'(A)\geq 0.$$

The same assumptions hold for $g_u(a)$.

A list of appropriate pharmacodynamic functions appears in [22]. In the work that follows, we have used the following forms for these functions:

$$f_u(s)=\frac{ms}{b+s}, \quad g_u(a)=\frac{m_1 a}{L_1+a} \quad \textit{and} \quad f_{1u}(s,a)=k_u\frac{s}{b+s}\frac{a}{L+a}$$

where m, b, b_1, k_u, L and L_1 are constants.

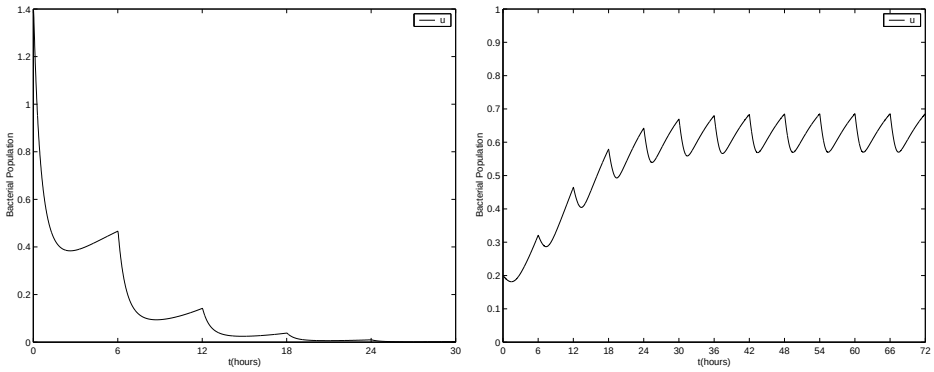

FIGURE 2. *Left* figure shows treatment success when $\lambda = -0.1826 < 0$. *Right* figure shows the treatment failure when $\lambda = 0.1662 > 0$.

Table 1: Descriptions and values of the parameters used

Description	Symbol	Value
Substrate feed concentration in fluid	S^0	0.2
Maximum antibiotic dosage concentration	A_m	8
Substrate dilution rate bifilm (fluid)	$d_s(D_S)$	0.23
Antibiotic dilution rate bifilm (fluid)	$d_a(D_A)$	0.23
Bacteria dilution Rate bifilm (fluid)	$d_u(D_U)$	0.23
Yield constant	γ	0.8
Maximum growth rate for bacteria in biofilm	m	0.417
Maximum antibiotic uptake in biofilm	m_1	0.345
Maximum killing rate for bacteria in biofilm	k_u	0.96
Half saturation constant for bacteria growth	b	0.1
Half saturation constant for bacteria removal	L	0.1
Half Saturation constant for antibiotic uptake	L_1	0.1
Antibiotic cost sensitivity	W	0.001
Susceptible bacteria cost sensitivity	W_u	1
Period of dosing regimen	T	6

3. **Bacteria-free states, infection states, and their stability.** In this section we will discuss the existence of periodic solutions and their stability properties.

For $\epsilon = 0$, (2) reduces to $S = S^0$, $A = A_0(t)$, $U = 0$ and

$$\begin{aligned} s' &= d_s(S^0 - s) - \gamma^{-1} u f_u(s) \\ a' &= d_a(A_0(t) - a) - u g_u(a) \\ u' &= (f_u(s) - f_{1u}(s, a) - d_u)\, u. \end{aligned} \tag{3}$$

where $d_s S_0$ is a flux of nutrient into the infected region, supplied by surrounding tissues or the blood, and d_s the removal rate of nutrient which might also include uptake by host cells. $d_a A_0$ is now interpreted as flux of antibiotic into the infected region and d_a its removal rate.

System (3) has a periodic bounded bacteria-free solution

$$E_0(t) = (S^0, A_0(t), 0, S^0, a^*(t), 0).$$

The existence and uniqueness of the latter solution follows from Theorem 2.1 of [22]. This is the "sterile state" or "disease-free state", which has no bacteria present and the nutrient level matches the feed level. The term $a^*(t)$ is the unique periodic solution of

$$a' = d_a(A_0(t) - a)$$

where $a^*(t)$ can be called the asymptotic pharmacokinetics since every solution of the above differential equation is asymptotic to it as t becomes large.

The infection-free state can be taken as the desired target state, so that successful treatments must drive the system state to it.

Furthermore, there may or may not be one or more "disease states" or "infection states" of the form

$$E_u(t) = (\bar{S}(t), \bar{A}(t), \bar{U}(t), \bar{s}(t), \bar{a}(t), \bar{u}(t))$$

where $\bar{U}(t) > 0$, $\bar{u}(t) > 0$ and all other components are positive periodic functions. Such states correspond to treatment failure.

The local stability of the infection-free state can be determined using the Floquet exponent of the variational equation about $E_0(t)$. It turns out that two of these are negative: the third, the 'invasion exponent' for the fluid and biofilm model respectively, is given by:

$$\lambda = f_u(S^0) - [f_{1u}(S^0, a^*)]_m - d_u$$

where $[.]_m$ gives the time-averaged value of a time-dependent function. Thus, in the above case, λ depends on the net time-averaged bacterial kill rate in the asymptotic pharmacokinetic state, involving the pharmacodynamics function, the nutrient level and the bacterial growth rate.

Theorem 3.1. *1. There exist ϵ_0,ρ_0 and ρ_1 with $0 \leq \rho_1 \leq \rho_0$ such that for each ϵ satisfying $0 \leq \epsilon \leq \epsilon_0$, (2) has a unique disease-free periodic solution $E_0(t,\epsilon) = (E_0^\star(t,\epsilon), E_0^\circ(t,\epsilon))$ where*

$$\begin{aligned} E_0^\star(t,\epsilon) &= \bar{E}_0(t) + \epsilon O(M(\epsilon)) \\ E_0^\circ(t,\epsilon) &= \tilde{E}_0(t) + O(M(\epsilon)), \ \text{as } \epsilon \to 0 \end{aligned}$$

satisfying $\| O(M(\epsilon)) \| \leq \rho_1$, $\| \epsilon O(M(\epsilon)) \| \leq \rho_1$ and where $M(\epsilon)$ is a nondecreasing function with $\lim_{\epsilon \to 0} M(\epsilon) = 0$. This solution is continuous in ϵ uniformly in $t \in R$.

2. The T periodic solution $E_0(t,\epsilon)$ of (2) is asymptotically stable if $f_u(S^0) - [f_{1u}(S^0, a^*(t))]_m - d_u < 0$ and is unstable if $f_u(S^0) - [f_{1u}(S^0, a^*(t))]_m - d_u > 0$.

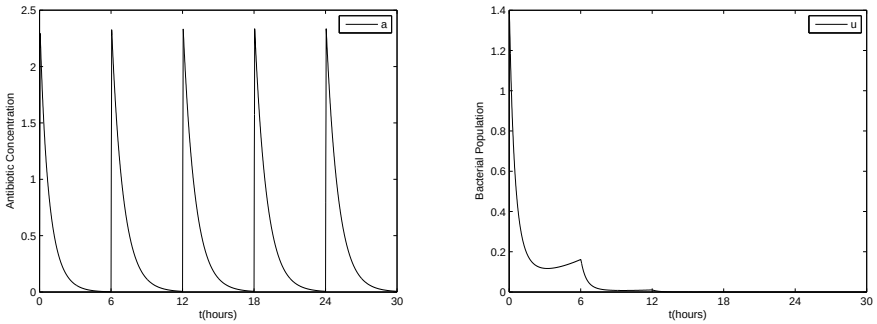

FIGURE 3. *Left* figure shows the effect of increasing the Antibiotic dose by a factor of 1.5, on bacterial population for treatment success. *Right* Bacteria eliminated at a higher rate as compared to Figure 2.

The above theorem asserts that the sign of the invasion exponent λ is critical in determining stability of sterile state and non-sterile state. This result is illustrated in Figure 2. For the purpose of our simulations, we use the growth rate, pharmacodynamics and antibiotic uptake functions defined at the end of Section 2.

The left side of Figure 2 shows that treatment is successful when $\lambda < 0$ and treatment failure results when $\lambda > 0$, since in this case bacteria can grow when rare. All the functions and parameter values are the same in both figures except the killing rate k_u. This is chosen such that $\lambda < 0$ in the left figure and $\lambda > 0$ in the right figure. Output is scaled by s/b, $u/(b\gamma)$, a/L. Time t is scaled by

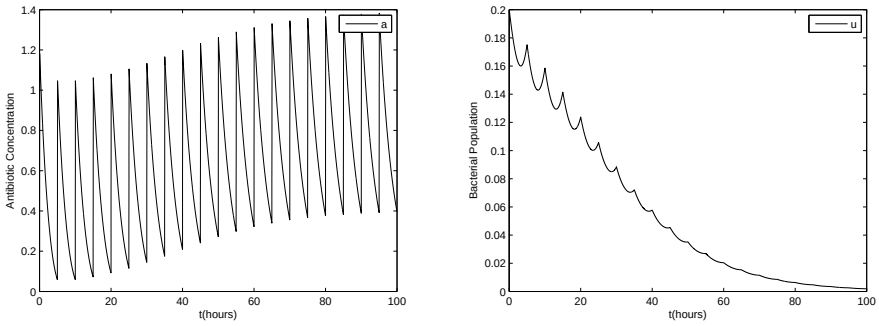

FIGURE 4. *Left* New dose given without the complete washout of the previous dose. *Right* The treatment failure case is converted into treatment success for the right side of Figure 2.

$1/d_s$, S_0 is scaled by $1/(b)$, A_0 is scaled by $1/L$ and d_a, d_u, m and k are all scaled by $1/d_s$. Parameter values are chosen as in [22] and [36]. Particularly, the yield constant $\gamma = 0.8$, maximum specific growth rate $m = 0.417$, $m_1 = 0.28$; removal rate $d_s = 0.23$; half saturation constants $b = 0.1$, $L = .1$ and $L_1 = .1$; maximum disinfection rate $k_u = 0.529$ for the figure on the left and $k_u = 0.25$ for that on the right; concentration of the substrate feed has been taken as $S^0 = 0.2$. Figure 3 shows the effect of increasing the antibiotic concentration on the treatment success case. For a stronger dose of antibiotic the bacteria are eliminated at a greater rate.

Figure 4 shows the effect of giving a new dose of antibiotic without the complete washout of the previous dose. This shows a treatment success for the previously treatment failure case Figure 2. Note that the dose is administered in a periodic fashion, but the new dose is given before the complete time period T, hence the quantity of antibiotic is increasing with time.

Figure 5 (upper left) demonstrates that $\lambda < 0$ leads to treatment success when the initial population of bacteria is small except for some special cases. Plots of bacterial population versus time are given for a small initial population ($u(0) = 0.45$) and a large initial population ($u(0) = 0.76$). The solution for the former shows treatment success whereas solution for the latter shows unsuccessful treatment. Both $E_0(t)$ and $E_u(t)$ are simultaneously locally stable. Except for $S^0 = 0.5$ and $k_u = 0.529$, the values of all parameters are the same as for previous figures. This implies that early antibiotic treatment, before bacterial population becomes large, is more effective.

However, the case of treatment failure for Figure 5 (upper left) can be converted to treatment success by changing the values of certain parameters. In the simulations shown in Figure 5 (upper right) by increasing the antibiotic dose(as shown in Figure 3), the bacteria for the large initial population is eliminated. Similarly, simulations in Figure 5 (lower left) shows that if the killing rate is increased, the complete eradication of bacteria can be achieved even for a higher initial microbial populations. Moreover Figure 5 (lower right) demonstrates the effect of reducing the interval between antibiotic dose. As the time period of dosing is reduced from $T = 6hrs$ (as in Figure 5 upper left) to $T = 4hrs$ (Figure 5 lower right), the bacteria are successfully eliminated for the treatment failure case.

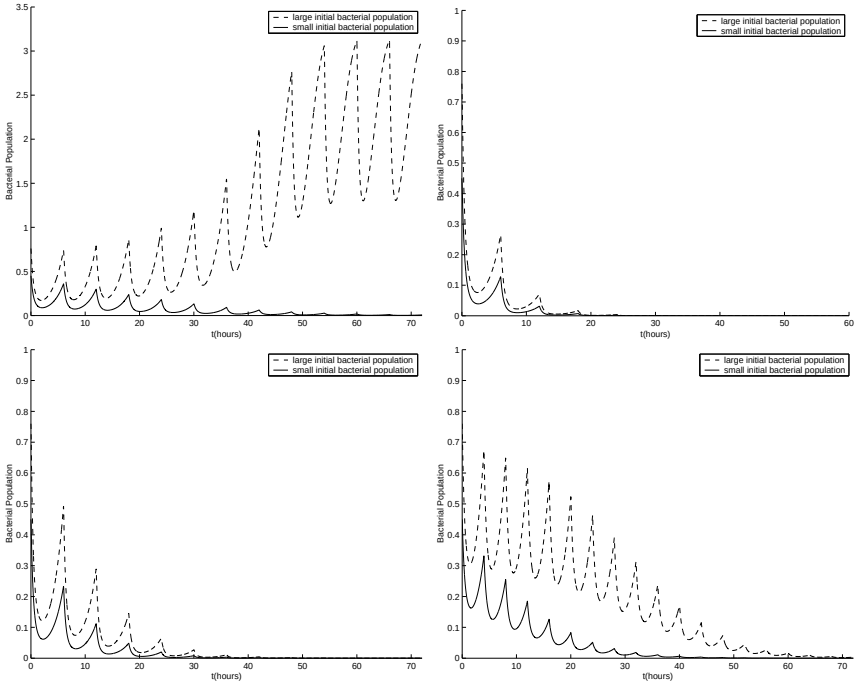

FIGURE 5. Initial Conditions can generate different outcomes when $\lambda < 0$: treatment success and failure results from the same system but different initial values upper left. Changing certain parameter values can result in eradication of bacteria for the higher initial population other three figures.

The following theorem gives the conditions for existence of a treatment failure periodic solution by using persistence theory. The proof is contained in Appendix A.

Theorem 3.2. *1. If $f_u(S^0) - [f_{1u}(S^0, a^*(t))]_m - d_u > 0$ then bacterial population of the biofilm compartment uniformly strongly persists. More precisely there exists $\epsilon > 0$, independent of initial data, such that for all solutions of the above model satisfying $u(0) > 0$, we have*

$$u(nT) > \epsilon$$

for all sufficiently large n. In this case, there exists a T periodic "disease" solution

$$E_u(t) = (E_u^*(t), E_u^\circ(t))$$

of (3), where $E_u^*(t)$ is the same as $\bar{E}_0(t)$ and $E_u^\circ = (s, a, u)$ caters to the biofilm part.

2. For each T periodic solution $E_u(t)$ of (3), there exists ϵ_0 such that for $0 < \epsilon < \epsilon_0$, there exists a T periodic "disease" solution

$$E_u(t, \epsilon) = (E_u^*(t, \epsilon), E_u^\circ(t, \epsilon))$$

of system (2) with

$$\begin{aligned} E_u^*(t,\epsilon) &= E_u^*(t) + \epsilon O(M(\epsilon)) \\ E_u^\circ(t,\epsilon) &= E_u^\circ(t) + O(M(\epsilon)) \end{aligned}$$

where $M(\epsilon)$ *is a nondecreasing function with* $\lim_{\epsilon\to 0} M(\epsilon) = 0$.

3. *There is* ϵ_0 *such that for* $0 < \epsilon < \epsilon_0$ *the* T *periodic solution* $E_u(t,\epsilon)$ *is asymptotically stable if and only if the linear system* $z_1' = A(t,0)z_1$ *is asymptotically stable where*

$$A(t,0) := \begin{pmatrix} -d_s - \gamma^{-1}\bar{w}f_u'(\bar{s}) & 0 & -\gamma^{-1}f_u(\bar{s}) \\ 0 & -d_a - \bar{u}g_u'(\bar{a}) & -g_u(\bar{a}) \\ -(f_u(\bar{s}) - f_{1u}'(\bar{s},\bar{a}))\bar{u} & -f_{1u}(\bar{s},\bar{a}) & f_u(\bar{s}) - f_{1u}(\bar{s},\bar{a}) - d_u \end{pmatrix}.$$

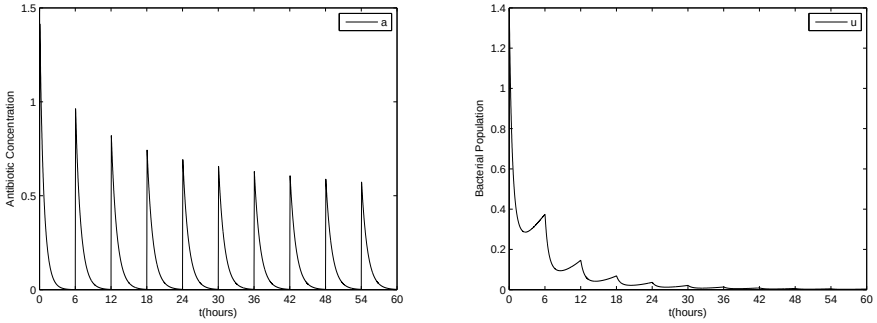

FIGURE 6. Dose decreasing with time, the quantity of dose administered is higher, such that bacteria decreases but decreases slowly as compared to treatment success case.

Treatment failure is thus guaranteed when $\lambda > 0$, since bacteria can grow when rare. Furthermore, by using persistence theory, we showed that there exists at least one (periodic) infection state $E_u(t)$ when $\lambda > 0$. Generalizing it, this solution may be non-unique and we do not know its global stability properties or its Floquet exponent for local stability.

In the next sections we will discuss about the optimizing the amount of dose administered. First we will talk about dose tapering and then optimal control strategy.

4. **Dose tapering.** Dose tapering technique has been effective in antibiotic treatments [34]. Prolonged treatment by using high doses of antibiotic might result in harmful side effects and stopping the treatment at an early stage might result in re-emergence of the disease. It has therefore been suggested that antibiotic doses should be reduced with time. However one should be careful with this since if the dose is reduced below a certain level, the bacteria might start to increase. We have used our model to show the simulations of this effect.

We take $A_0(t)$ as a function whose peaks are decreasing with time. Dose tapering can result in successful elimination of the disease and but it might lead to re-emergence of the disease in case of reducing dose below a certain level. The simulations for this are given in Figure 6 and 7. It is evident from Figure 7 that as the dose is reduced, compared to Figure 6, the bacterial population starts increasing

after 54 hours. Dosing should therefore be appropriately decreased such that the bacteria is completely eliminated. The following section considers this problem in more depth.

5. **Optimal antibiotic treatments.** In this section, we address the problem of finding a course of antibiotic treatment which kills the active bacteria while also minimizing the amount of total antibiotic applied. Several studies have indicated the counter-productive effects of over-deployment of antibiotics [46]. Indeed, it has also been suggested that this may even increase the susceptibility to infection by increasing the effective resistance [34]. The high costs of antibiotics are another factor in our motivation to minimize the quantity of antibiotic applied over the course of the treatment.

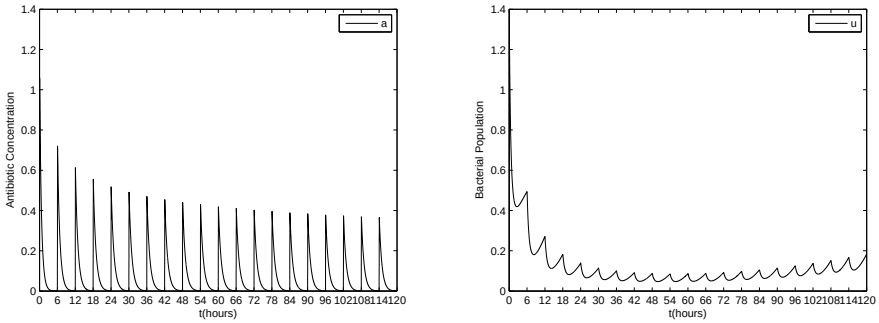

FIGURE 7. Results with dose tapering. Reducing dose with time, results in first decrease in bacteria and then it shows an increase. This signifies that if the complete dose is not given then the infection could prolong.

At the same time, application of antibiotics is imperative because the dilution rate is not sufficiently high to flush out the bacteria on its own. However, the paucity of development of newer and better antibiotics to combat microbial strains implies that we have to work with the available antibiotics more effectively [17]. Our work focuses on coming up with an optimal strategy of antibiotic application that eliminates bacteria while at the same time ensuring that antibiotic deployment is at a minimum.

As in [8], we use optimal control theory to suggest the best strategy for our model. The results suggest that a regimen of cycling between application and withdrawal of antibiotic is the optimal treatment course, with a gradual reduction in the strength of applied dosages.

Based on this, we derive optimal dosing strategies for different initial conditions and parameter values. We observe that these strategies preclude the bi-stability that appeared in the non-optimal antibiotic application scenario, i.e., bacteria free state is stable for a wide range of initial bacterial concentrations. Under our model thus, employing such a course of treatment ensures bacteria eradication and by implication disease treatment.

5.1. **Finding the optimal control function.** We now come to the problem of finding dosing strategies that minimize the total antibiotic deployment while also eliminating bacteria of both types. We rely on theory of constrained optimization to achieve our objective of finding an optimal strategy. We assign costs to antibiotic usage and to surviving active bacteria. To minimize the amount of antibiotic applied while also eliminating bacteria population, we look to reduce the sum of these costs.

Let $A_0(t) = A_m\delta(t)$, where A_m gives the maximum concentration of antibiotic that can be applied and $\delta(t)$ is a function that controls the precise timing of the dosing protocol by determining the amount of antibiotic being let through at time t, $\delta(t) \in [0, 1]\ \forall\ t \geq 0$. This can be expressed mathematically by assigning costs to all dosing protocols depending on their killing efficacy and antibiotic usage. In particular, we assign costs to bacterial concentration. For instance, greater antibiotic deployment would lead to a higher antibiotic cost while a higher density of surviving bacteria at the end of the treatment would give a higher bacterial cost. The problem now is to find the form of $\delta(t)$ that minimizes the functional

$$C(\delta(t), u(t_f)) = \frac{1}{2}\int_0^{t_f} W\delta(t)^2 dt + W_u u(t_f) \tag{4}$$

where W and W_u are suitable cost sensitivity parameters and t_f is the time at the end of the treatment. This cost functional is defined for each dosing strategy $\delta(t)$ and the surviving bacterial populations at time t_f.

The integral term in (4) is a measure of the total antibiotic applied over the course of the treatment. The other term is due to the bacterial concentration present at time t_f . This functional $C(\delta(t), u(t_f))$ sums the two factors that determine the penalty incurred by employing a particular regimen and hence its total cost. Depending on the sensitivity parameters, the relative penalty of antibiotic usage and bacterial survival may be adjusted. The problem is thus reduced to minimizing (4) by finding the optimal $\delta^*(t)$. Similar functionals appear extensively in literature and have been used to great effect in [8], [15], [25], [26] and [45].

We first present the following result. The proof is contained in Appendix B.

Theorem 5.1. *There exists a unique control function $\delta^*(t)$ that minimizes the cost functional defined in (4).*

The above result thus guarantees the existence of a unique course of treatment that minimizes (4). An instance of what this optimal treatment may look like is shown in Figure 8. Appendix B contains the details of the procedure used to find it. In the following section, we present the results that we obtained for different scenarios.

5.2. **Results and variations.** The recommended strategy and the resulting changes in antibiotic and bacteria levels are illustrated in Figure 8. The sensitivity parameters are set to $W = 0.1$ and $W_u = 100$ and the maximum antibiotic dosage concentration is $A_m = 8$. The values of initial conditions are set at $s(0) = 2.9$, $a(0) = A_m$ and $u(0) = 2.5$. The optimal treatment is applied over cycles of 6 hours. Observe the gradual decrease in dosage strength and the corresponding steady decline in bacterial and antibiotic concentration levels.

Figure 9 illustrates the strategy and bacterial decay when the values of initial conditions and parameters are the same as in Figure 2 (left); this allows a comparison to be made. Observe that in this case, the optimal treatment is just as efficacious in combating bacteria as the non-optimal treatment discussed earlier. In

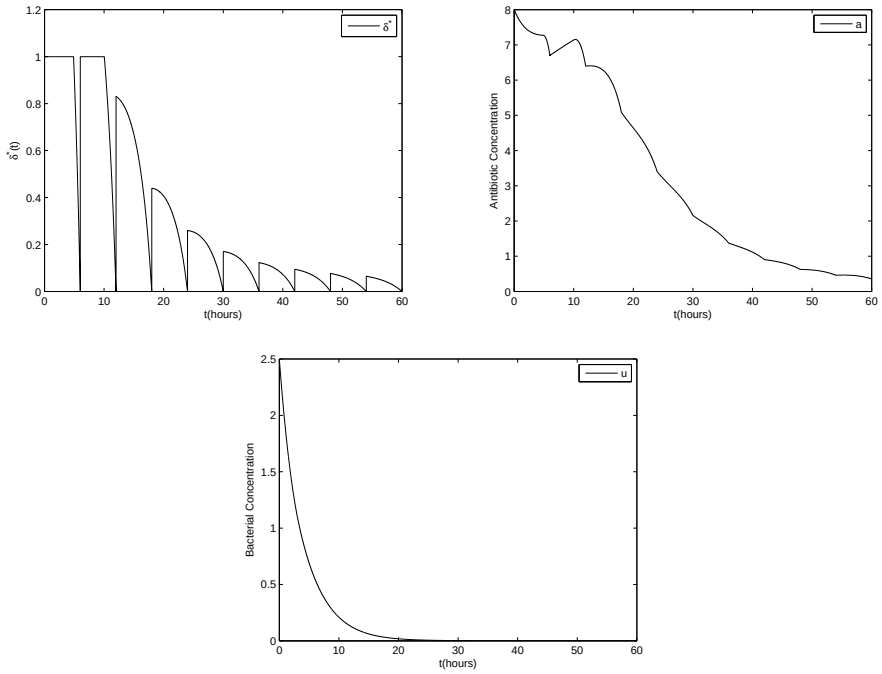

FIGURE 8. The optimal strategy along with the resulting changes in antibiotic and bacterial concentrations, for $s(0) = 2.9$, $a(0) = A_m$ and $u(0) = 2.5$.

fact, both treatments take the bacterial concentration to zero in almost the same time.

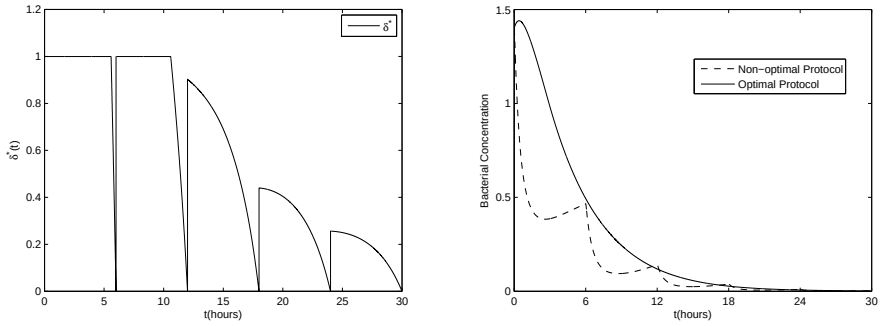

FIGURE 9. The optimal strategy and the resulting change in bacterial concentration, for $s(0)$=1.9, $a(0)$=0.01 and $u(0)$=1.4. The parameter values are the same as those for the case in Figure 2(a), which has also been shown.

Figure 2 (right) illustrates a treatment failure case under the discrete protocol. The new optimal treatment, however, eliminates the disease for the same initial

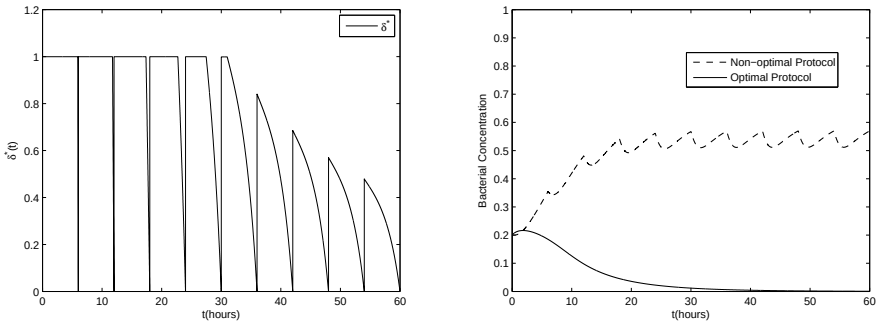

FIGURE 10. The treatment failure case from Figure 2(b) can be treated successfully under the optimal strategy.

conditions and parameter values; this is shown in Figure 10, along with the bacteria curve of the original failed treatment. This indicates one advantage of the newly developed strategy over the discrete protocol. Thus, the optimal strategy has been shown to work successfully in cases where the discrete treatment may or may not succeed.

Moreover, applying the optimal treatment removes the bi-stability that is exhibited in Figure 5 (upper left), for $S^0 = 0.5$. Figure 11 indicates that the bacteria free state is locally stable for several initial bacteria concentrations. In all cases, the optimal treatment is shown to reduce bacteria concentration substantially. The optimal strategy hence ensures the eventual near-eradication of bacteria over time which was not guaranteed as a result of the previously discussed strategy.

It can be seen on close observation that while bacteria is severely minimized in all of these cases, it may not be completely eliminated for higher values of antibiotic sensitivity parameter W. This is a trade-off of minimizing the antibiotic load during the treatment and has to be borne. Due to the higher antibiotic cost, the system reduces the emphasis on bactericide in favour of reduced antibiotic application. If such a bacteria level is still harmful, we can generate the optimal strategy which takes into account higher costs associated with end-of-treatment bacteria, i.e., increase W_u. This will then cause an even greater diminishing of bacteria population in the biofilm as the penalty associated with bacteria survival would then be higher.

This leads us to investigating the effect of varying the sensitivity parameters. With only two of those, our interest lies only in their relative values. We hold W_u constant at a value of 100 and vary W over a wide range of values. The suggested strategies and the resultant bactericide are shown in Figure 12. As would be expected, increasing the cost associated with antibiotic application tends to reduce its deployment. For W=100 and 1000, the quantity of antibiotic applied is substantially lower than that for $W = 0.00001$. The corresponding reduction in bacterial concentration is then negatively correlated with the value of W.

We make some relevant observations at this point. Firstly, the bacterial concentrations for for $W = 0.1$ and $W = 0.00001$ clearly go to zero while those for $W = 100$ and $W = 1000$ approach non-zero values. This is the point we made earlier while discussing the stability of the low-bacteria state. Secondly, all optimal treatments advocate a gradual decrease in antibiotic application over successive cycles. This is a contrast to the non-optimal treatments previously used, which applied the same

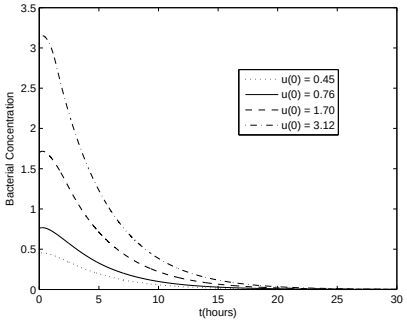

FIGURE 11. Different initial levels of bacteria are treated successfully by the optimal treatment for each case with $S^0 = 0.5$. Note in particular the bacteria reduction for $u(0) = 0.76$ that was used in Figure 5(upper left) to illustrate bi-stability.

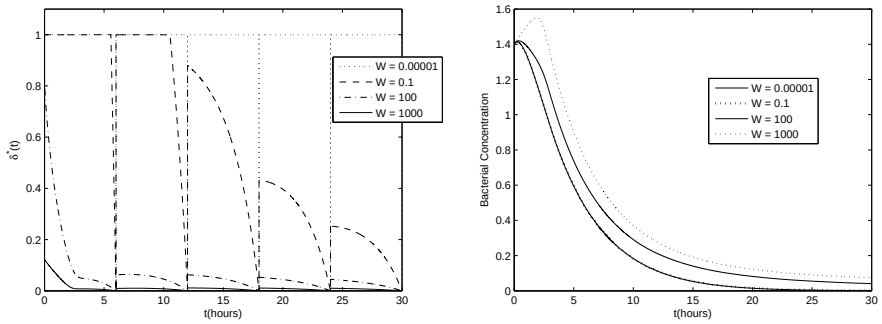

FIGURE 12. Varying the values of W yields these suggested treatments and the corresponding bacteria decays.

quantity of antibiotic in each cycle, and is in agreement with the dose tapering technique.

6. **Discussion.** Microbial biofilms have several detrimental effects on the host surface on which they are formed. A few of these are the contamination of food products, dental plaque, cystic fibrosis and failure of medical implants [5], [4], [7], [13], [14], [37], [39] and [40]. In this paper we have presented a mathematical model of antibiotic treatment for microbial population in biofilm and fluid compartment.

In the model described here, we used the theory of singularly perturbed non-autonomous systems given in [38] to analyze the model. Assuming that the antibiotic is bactericidal in nature, the biological results we obtain in this paper are based on the supposition that the flow rate F, of fluid compartment is very high. Hence, we reduce the six dimensional system to a three dimensional system, i.e., only the biofilm system. We have used the results proved in [22] to investigate the dynamics of this reduced model.

We first investigate a periodic dosing regimen. Under such a strategy, we show that periodic solutions exist for our model and that there are solutions corresponding

to sterile and infection states. Theorem 3.1 establishes that the sign of invasion exponent λ characterizes the local stability of the sterile state or treatment success. It provides a concise criterion for the stability of the periodic solutions and hence for treatment success or treatment failure. Simulations in Figure 2 show that the bacteria population is eliminated in the case of $\lambda < 0$ but bacteria persists in the case of $\lambda > 0$.

We have further used the model to illustrate the phenomenon of bi-stability. For different initial bacterial populations with all the other parameter values same and $\lambda < 0$, Figure 5 (upper left) shows that we can get vastly different outcomes. The simulation results show that a higher population results in treatment failure whereas a lower initial microbial population results in treatment success. The former case can, however, be converted to a success by various methods. Figure 5 (upper right) shows the effect of increasing the antibiotic dose, Figure 5 (lower left) gives the effect of increased killing rate for bacterial biofilm and Figure 5 (lower right) illustrates the impact of reducing the dosing period. In all of these cases, the bacterial population is eliminated for the higher initial population. These results suggest that if the treatment is started late, appropriate adjustments should be made to the dosing strategy, such as increasing the dosing strength or reducing the dosing period.

Managing antibiotic load is essential due to the development of bacterial resistance and the high financial cost incurred by their application. Control theory has been used to come up with optimal antibiotic strategies that minimize antibiotic deployment while at the same time eliminate bacteria in section 5. We devise the best treatments while keeping in mind the relative costs of both these factors. The results show that optimal treatments are vastly more successful than the previously used discrete ones. Under the optimal protocol, several treatment failure cases are converted to successful treatment ones. It is also shown that the bacteria-free state is stable for a wide range of starting bacterial concentrations, implying that the bistability observed previously now disappears. We note that the optimal treatments advocate periodic application and withdrawal of antibiotic with a gradual decline in dosage strength, similar to that recommended in [8] for the persisting bacteria case.

In conclusion to our study, we have described a model that takes into account the antibiotic treatment in biofilm and surrounding fluid environment. Successful treatment requires that bacteria is eradicated in both the biofilm and fluid; this is given as the "sterile state" solution. The pharmacodynamics function is formulated such that it takes into consideration the slow growth rate and limited supply of nutrients, which are the major attributes of biofilm bacterial population. There are several mechanisms that result in antibiotic treatment failure in biofilms. We have presented conditions under which antibiotic therapy may result in successful elimination of microbial population from the biofilm environment. Finally, we have formulated the optimal antibiotic application strategies that take into account bacterial elimination as well as the amount of antibiotic applied.

Appendix A. Proof of Theorem 3.1 and Theorem 3.2. Proofs of Theorem 3.1 and Theorem 3.2 are given in this appendix.

Let us define

$$y = \begin{pmatrix} S & A & U \end{pmatrix}^T$$

and

$$x = \begin{pmatrix} s & a & u \end{pmatrix}^T$$

Let

$$\begin{aligned} y_1 &= y - \bar{E}_0(t) \\ x_1 &= x - \tilde{E}_0(t), \end{aligned}$$

then expanding (2) about the trivial solution and renaming x_1 and y_1 as x and y gives

$$\begin{aligned} \epsilon y' &= C(t,\epsilon)x + D(t,\epsilon)y + h_2(t,x,y,\epsilon) \\ x' &= A(t,\epsilon)x + B(t,\epsilon)y + h_1(t,x,y,\epsilon) \end{aligned} \tag{5}$$

where

$$C(t,\epsilon) := \begin{pmatrix} \epsilon D_s & 0 & 0 \\ 0 & \epsilon D_a & 0 \\ 0 & 0 & \epsilon D_u \end{pmatrix},$$

$$D(t,\epsilon) := \begin{pmatrix} -1-\epsilon D_s & 0 & -\epsilon\gamma^{-1} f(S^0) \\ 0 & -1-\epsilon D_u & -\epsilon g(A_0(t)) \\ 0 & 0 & -1+\epsilon f(S^0) - \epsilon f_1(S^0, A_0(t)) - \epsilon D_u \end{pmatrix},$$

$$A(t,\epsilon) := \begin{pmatrix} -d_s & 0 & -\gamma^{-1} f_u(S^0) \\ 0 & -d_a & -g_u(a^*(t)) \\ 0 & 0 & f_u(S^0) - f_{1u}(S^0, a^*(t)) - d_u \end{pmatrix},$$

and

$$B(t,\epsilon) := \begin{pmatrix} d_s & 0 & 0 \\ 0 & d_a & 0 \\ 0 & 0 & d_u \end{pmatrix}.$$

The following hypotheses hold for the above system (5).

(H1) $A(t,\epsilon)$, $B(t,\epsilon)$, $C(t,\epsilon)$, $D(t,\epsilon)$ are continuous and bounded matrix functions defined on $R \times [0,\epsilon_0]$. Moreover, they are continuous in ϵ, uniformly in $t \in R$. We let $\bar{M}$ denote a common bound for the norm of each of these matrices for $(t,\epsilon) \in R \times [0,\epsilon_0]$.

(H2) $D(t,0) = D_0$ a constant matrix having no eigenvalues on the imaginary axis; $C(t,0) \equiv 0$.

(H3) The system $z' = A(t,0)z$ is noncritical.

(H4) h_1, h_2 are continuous functions of all four arguments (t,x,y,ϵ) such that $t \in R$, $|x|$, $|y| \le \rho_0$, $0 \le \epsilon \le \epsilon_0$ and both functions are continuous in (x,y,ϵ) uniformly in $t \in R$. Furthermore, there exists nondecreasing functions $M(\epsilon)$ and $\eta(\rho,\epsilon)$, $0 \le \epsilon \le \epsilon_0$, $0 \le \rho \le \rho_0$ satisfying $\lim_{\epsilon\to 0} M(\epsilon) = 0$, $\lim_{(\rho,\epsilon)\to(0,0)} \eta(\rho,\epsilon) = 0$, such that $|h_2(t,0,0,\epsilon)| \le M(\epsilon)$, $|h_1(t,0,0,\epsilon)| \le M(\epsilon)$, $t \in R$, $0 \le \epsilon \le \epsilon_0$, and

$$|h_2(t,x,y,\epsilon) - h_2(t,\bar{x},\bar{y},\epsilon)| \le \eta(\rho,\epsilon)[|x-\bar{x}| + |y-\bar{y}|]$$

$$|h_1(t,x,y,\epsilon) - h_1(t,\bar{x},\bar{y},\epsilon)| \le \eta(\rho,\epsilon)[|x-\bar{x}| + |y-\bar{y}|]$$

holds for all $t \in R$, $|x|$, $|\bar{x}|$, $|y|$, $|\bar{y}| \le \rho$, $0 \le \epsilon \le \epsilon_0$, $0 \le \rho \le \rho_0$.

For system (5) we have $C(t,\epsilon) = \epsilon\overline{C}(t,\epsilon)$, $|\overline{C}(t,\epsilon)| \le \bar{M}$ and $h_2(t,x,y,\epsilon) = \epsilon\overline{h}_2(t,x,y,\epsilon)$ where both $\overline{h}_2(t,x,y,\epsilon)$ and $h_1(t,x,y,\epsilon)$ satisfy the estimates of (H4).

Proof. Proof for Theorem 3.1 is given first.

(1) For the proof of the this part of the theorem see [38].

(2) It follows from [38] that the T periodic solution $(x^*(t,\epsilon), y^*(t,\epsilon))$ of (5) is asymptotically stable provided

$$z_1' = A(t,0)z_1 \tag{6}$$

and

$$z_2' = D_0 z_2 \tag{7}$$

are asymptotically stable where

$$D_0 := \begin{pmatrix} -1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & -1 \end{pmatrix}$$

and

$$A(t,0) := \begin{pmatrix} -d_s & 0 & -\gamma^{-1} f_u(S^0) \\ 0 & -d_a & -g_u(a^*(t)) \\ 0 & 0 & f_u(S^0) - f_{1u}(S^0, a^*(t)) - d_u \end{pmatrix}.$$

A computation yields the fundamental matrix $\phi_1(t)$ of (7):

$$\phi_1(t) := \begin{pmatrix} e^{-t} & 0 & 0 \\ 0 & e^{-t} & 0 \\ 0 & 0 & e^{-t} \end{pmatrix}$$

evaluating $\phi_1(t)$ at $t = T$ we obtained the Floquet exponents -1, -1 and -1. This shows that (7) is asymptotically stable. Also the fundamental matrix $\phi_2(t)$ of (6):

$$\phi_2(t) := \begin{pmatrix} e^{-d_s t} & 0 & \phi_{13} \\ 0 & e^{-d_a t} & \phi_{23} \\ 0 & 0 & e^{\int_0^t \left(f_u(S^0) - f_{1u}(S^0, a^*(s)) - d_u\right) ds} \end{pmatrix}.$$

Evaluating this fundamental matrix $\phi_2(t)$ at $t = T$ we obtained the Floquet exponents $-d_s$, $-d_a$ and $f_u(S^0) - [f_{1u}(S^0, a^*(t))]_m - d_u$. It follows at once that (6) is asymptotically stable if $f_u(S^0) - [f_{1u}(S^0, a^*(t))]_m - d_u < 0$. Thus the T periodic solution $E_0(t,\epsilon)$ is asymptotically stable if and only if $f_u(S^0) - [f_{1u}(S^0, a^*(t))]_m - d_u < 0$. □

Proof. Proof for Theorem 3.2.

(1) We apply the theorem (4.1) of [19]. Using the notation, we set $X = \{(s,u,a) \in \mathbb{R}^3_+ : \gamma S + u \leq \gamma S^0,\ A \leq M_1,$ where $M_1 = \max_{t \in R^+} A_0(t)\}$, $X_1 = \{(s,u,a) \in \mathbb{R}^3_+ : u \neq 0\}$, and $X_2 = \{(s,u,a) \in \mathbb{R}^3_+ : u = 0\}$. Define a map h such as $h(s(0), a(0), u(0)) = (s(T), a(T), u(T))$. We want to show that there exists $\epsilon > 0$ such that

$$\liminf_{n \to \infty} d(h^n(X), X_2) > \epsilon.$$

Given that

(i) X is compact metric space.
(ii) $h : X \to X$ is continuous map.
(iii) $h(X_1) \subset X_1$
(iv) M is maximal compact invariant set in X_2.

In our case $M = E_0(0)$, since the omega limit set of solutions starting in X_2 is, by our hypotheses, $E_0(0)$ where $E_0(0) = (S^0, a^*(0), 0)$. We want to show that

(i) M is isolated in X, that is, there exists a closed neighborhood U of M such that M is the largest invariant set in U, and
(ii) $W^s(M) \subset X_2$, where $W^s(M) = \{x \in X : h^n(x) \to M$ as $n \to \infty_+\}$

In order to show that M is isolated in X we will apply the Theorem 2.3 of ([27]). We will show that M is isolated in X and $W^s(M) \subset X_2$.

Let V be the neighborhood given by the theorem. Assume that there exists an invariant set $\tilde{K}$ such that

$$M \subset \tilde{K} \subseteq V \cap X.$$

Since $\tilde{K}$ is positively invariant, all solutions that begin in $\tilde{K}$ stay in $\tilde{K}$ and so in V for positive time. Thus $\tilde{K} \subset W^s(E_1)$.

Since $\tilde{K}$ is negatively invariant, all solutions that begin in $\tilde{K}$ stay in $\tilde{K}$ and so in V for negative time. Thus $\tilde{K} \subset W^u(E_1)$.

$$W^s(E_1) \cap W^u(E_1) = E_1$$

thus $\tilde{K} = M = E_1$. Therefore K is an isolated compact invariant set in X.

It is clear that in this case $W^s(M) = X_2$.

(2) Suppose that the coefficients in system 3 are such that it has a positive periodic solution. Denote this periodic solution by $(\bar{s}, \bar{a}, \bar{u})$. Let us denote this outer solution by $E^*(t) = (S^0, A_0(t), 0, \bar{s}(t), \bar{a}(t), \bar{u}(t))$. We define

$$y_1 = y - E_u^*(t)$$
$$x_1 = x - E_u^\circ(t)$$

then expanding (2) about this outer solution and renaming x_1 and y_1 as x and y respectively gives

$$\begin{aligned} \epsilon y' &= C(t,\epsilon)x + D(t,\epsilon)y + h_2(t,x,y,\epsilon) \\ x' &= A(t,\epsilon)x + B(t,\epsilon)y + h_1(t,x,y,\epsilon) \end{aligned} \tag{8}$$

where

$$C(t,\epsilon) := \begin{pmatrix} \epsilon D_s & 0 & 0 \\ 0 & \epsilon D_a & 0 \\ 0 & 0 & \epsilon D_u \end{pmatrix},$$

$$D(t,\epsilon) := \begin{pmatrix} -1-\epsilon D_s & 0 & -\epsilon\gamma^{-1} f(S^0) \\ 0 & -1-\epsilon D_a & -\epsilon g(A_0(t)) \\ 0 & 0 & -1+\epsilon f(S^0) - \epsilon k f_1(S^0, A_0(t)) - \epsilon D_u \end{pmatrix},$$

$$A(t,\epsilon) := \begin{pmatrix} -d_s - \gamma^{-1}\bar{u} f_u'(\bar{s}) & 0 & -\gamma^{-1} f_u(\bar{s}) \\ 0 & -d_a - \bar{w} g_w'(\bar{a}) & -g_u(\bar{a}) \\ -(f_u(\bar{s}) - k_u f_{1u}'(\bar{s},\bar{a}))\bar{u} & -k_u f_{1u}(\bar{s}) & f_u(\bar{s}) - k_u f_{1u}(\bar{s},\bar{a}) - d_u \end{pmatrix},$$

and

$$B(t,\epsilon) := \begin{pmatrix} d_s & 0 & 0 \\ 0 & d_a & 0 \\ 0 & 0 & d_u \end{pmatrix}.$$

Since $(H1) - (H4)$ hold for the model (2). So by theorem (1.4) of [38], the system (2) has a T periodic solution

$$E_u(t,\epsilon) = (E_u^*(t,\epsilon), E_u^\circ(t,\epsilon))$$

with

$$\begin{aligned} E_u^*(t,\epsilon) &= E_u^*(t) + \epsilon O(M(\epsilon)) \\ E_u^\circ(t,\epsilon) &= E_u^\circ(t) + O(M(\epsilon)) \end{aligned}$$

(3) It follows from [38] that the T periodic solution $(x^*(t,\epsilon), y^*(t,\epsilon))$ of (5) is asymptotically stable provided

$$z_1' = A(t,0)z_1 \tag{9}$$

and

$$z_2' = D_0 z_2 \tag{10}$$

are asymptotically stable where

$$D_0 := \begin{pmatrix} -1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & -1 \end{pmatrix}$$

and

$$A(t,0) := \begin{pmatrix} -d_s - \gamma^{-1}\bar{w}f_u'(\bar{s}) & 0 & -\gamma^{-1}f_u(\bar{s}) \\ 0 & -d_a - \bar{u}g_u'(\bar{a}) & -g_u(\bar{a}) \\ -(f_u(\bar{s}) - f_{1u}'(\bar{s},\bar{a}))\bar{u} & -f_{1u}(\bar{s}) & f_u(\bar{s}) - f_{1u}(\bar{s},\bar{a}) - d_u \end{pmatrix}.$$

Computation yields the fundamental matrix $\phi_1(t)$ of (10):

$$\phi_1(t) := \begin{pmatrix} e^{-t} & 0 & 0 \\ 0 & e^{-t} & 0 \\ 0 & 0 & e^{-t} \end{pmatrix}$$

evaluating $\phi_1(t)$ at $t = T$ we obtained the Floquet exponents -1, -1 and -1. This shows that (10) is asymptotically stable. Thus, $E_u(t,\epsilon)$ is locally asymptotically stable if 9 is locally asymptotically stable. □

Appendix B. Proof of Theorem 5.1.

Proof. Let $\vec{r}(t,x,u)$ be the right hand side of (3), where x is as defined in Appendix A. We make the following observations regarding our problem and the control function δ.

(i) $\vec{r}(t,x,u)$ possesses the Lipschitz property with respect to the state variables contained in x.
(ii) As a constant control is permitted, the set of all allowable control functions is non-empty.
(iii) $\vec{r}(t,x,u)$ is linear in the control function,i.e., $\vec{r}(t,x,u) = \vec{p}(t,x) + \vec{q}(t,x)u$.
(iv) The range of the control function, $[0,1]$, is closed, convex and compact.
(v) The integrand in (4) is convex with respect to the control function δ.

By Corollary 4.1 of [16], as our system has properties (i)-(v), we conclude that a unique optimal control exists for our problem that minimizes the functional in (4). □

To find the optimal control function, we begin by defining a Lagrangian

$$\mathcal{L} = \frac{1}{2}W\delta(t)^2 + \lambda_1 s' + \lambda_2 a' + \lambda_3 u' \tag{11}$$

where the λ_i are adjoint functions and s',a' and u' refer to (3). By Pontryagin's principle, the adjoint functions obey

$$\begin{aligned} \frac{d\lambda_1}{dt} &= -\frac{\partial \mathcal{L}}{\partial s} \\ \frac{d\lambda_2}{dt} &= -\frac{\partial \mathcal{L}}{\partial a} \\ \frac{d\lambda_3}{dt} &= -\frac{\partial \mathcal{L}}{\partial u} \end{aligned} \tag{12}$$

with $\lambda_1(t_f) = 0$, $\lambda_2(t_f) = 0$ and $\lambda_3(t_f) = W_u$. Furthermore, at optimum, $\frac{\partial \mathcal{L}}{\partial \delta} = 0$. Using (3) and (11), this translates to

$$\delta^*(t) = -\frac{\lambda_2 d_a A_m}{W} \tag{13}$$

We have now ended up with a system of six ODEs, namely the systems (3) and (12), along with their boundary values. The solution of this combined system can be used to yield the form of the control function by (13).

Finding the analytic solution of this system is a difficult problem due to the nonlinearity of the system. However, several techniques exist for solving it numerically. The method we employ is the forward-backward sweep method, as detailed in [28]. First making an initial guess for adjoint functions λ_1, λ_2 and λ_3, we use the Runga-Kutta algorithm to solve for s, a and u forward in time - in a sense, sweeping forward. These solutions are then used to sweep backward in time for the solutions of the adjoint functions. These iterations are continued until convergence occurs; (13) is then used to give the form of the optimal treatment function.

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