A MODEL 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 a periodic dose of antibiotic on elimination of microbial population from biofilm. The bacteriocidal and bacteriostatic effects of antibiotic are considered individually. In this model the two main branches of pharmacology of antibiotic, pharmacodynamics and pharmacokinetics, are considered in parallel. 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 condition for treatment success, elimination of bacteria from the biofilm. 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 and suggest why a continuous dosing strategy is better than a discrete one. 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 discrete 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 [7, 9, 18]. The foremost cause of failure of medical implants is the bacterial infection, which is due to the formation of biofilm [2, 18, 44] on the surface of indwelling medical devices and in some cases on the adjacent tissues as well. This phenomena has been observed in various devices for example, artificial joints,

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prosthetic heart valve, urinary catheters and contact lenses [9, 18, 19, 39, 43]. Biofilms are heterogenous matrix-enclosed bacterial accumulation that may attach to living or non-living surfaces, surrounded by water channels [4, 21]. 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[4]. The plague that forms on the surface of the teeth, causing tooth decay is also a type of biofilm.

The biofilm formation is a growth cycle which initiates as the free-floating bacteria identifies a surface, strongly adheres to it by excreting polymers that aids in attachment and matrix formation [4, 21, 39, 18, 13]. These attached cell 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 circulatory system [4, 21]. The biofilm bacteria shows a change in phenotype with respect to the rate of growth and gene transcription [18, 39], hence bacteria in biofilm have a genetic makeup different from the individual bacterial cells [39].

It is observed that at times, antibiotic treatment may fail 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 [7, 9, 19]. Several mathematical models explaining this have been developed, which has improved the 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, 6, 44]. Several mechanisms such as reaction with neutralizing agent, synthesis of antibiotic degrading enzymes and sorption of antibiotic by exo-polymeric substance leads to this penetration limitation of antimicrobial agents in biofilm [2, 6]. Another actively researched mechanism, that leads to decreased mortality of bacteria in biofilm is phenotypic resistance [6, 48, 46]. Genetically homogenous bacterial population may be different phenotypically with respect to its tolerance to antimicrobial agent [48]. 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 [6, 48].

Another preventive phenomena to antibiotic treatment in biofilms, suggested is the physiological resistance [7, 8, 11, 38]. 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, [8, 38] that bacteria on the surface are killed at a faster rate than the one embedded deep in biofilm[11]. So it can be expected that as the bacteria on surface are killed, the nutrient penetrate 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 [8, 38]. Nevertheless, studies [6, 15, 38] have shown that nutrient limitation and decreased growth rate lead to reduced rate of killing of biofilm bacterial population and these two factors are major causes of phenotypic tolerance as well [15]. 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 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)\[16\] and factors influencing this relationship. The elimination of 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 \[3\]. 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 modeling of the effects of drug treatment has long been used side-by-side with experimental studies\[6, 15, 32, 31, 35, 38, 45, 46\]. Though, 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 this 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 biocides treatment as compared to the bacteria that are less actively reproducing \[6, 11, 43, 46, 47, 15, 35\]. 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 \textit{in vitro}, suggesting nutrient limitation \textit{in vivo}\[12, 15\]. 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\[25, 27\].

Many other researchers have used the pharmacodynamics function that depends on the antimicrobial agent and limiting resource level. Corpet \textit{et al.} \[12\] introduces pharmacodynamics function that depends on limiting both nutrient and antimicrobial agent. Cogan \[6\] does as well in his considerations of persister cells. Cozen \[15\] note that the restricted availability of iron and other nutrients appears to be typical of infection states. Robert and Stewart \[38\] 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 is considered, taking into account the diffusive transport. The killing rate induced by antibiotic is built on the model proposed by Cogan \[6\]. The pharmacokinetics function is based on \[3, 23, 28, 37\]. The only difference is this that we have used a periodic supply of an antibiotic with the term showing the loss of antibiotic due to its action on bacteria. In order to examine the effect of antibiotic on two
populations - one in the fluid and the other in the biofilm - we build a model based on the plasmid model of Imran and Smith [26], include the antibiotic equation used by [3, 24, 28].

We have developed mathematical models that take into account both the pharmacokinetics of the antibiotic and pharmacodynamics of its effect on a bacterial population in order to study antibiotic treatment. We consider both the effects, cidal and static, of an antibiotic on bacterial population and drive simple models of the effects of periodically-administered discrete dosing or constant antimicrobial dosing strategies on a bacterial population whose growth is checked by nutrient-limitation and possibly by host defenses if not by the antimicrobial agent itself. Distinguishing features of our model are the inclusion of nutrient limitation of microbial growth and accounting for the removal of antimicrobial agent by association with bacteria [26]. Successful treatment requires eradication of both fluid and biofilm bacterial populations. The mathematical theory of persistence plays a major role here as it is used to characterize treatment failure[27].

In the next section, we formulate and analyze models of antibiotic treatment of a bacterial population in fluid and biofilm compartment.

2 Model of bactericidal antibiotic treatment in biofilm

We consider the effect of antibiotic treatment in a two compartment model, the fluid environment and the wall growth or biofilm environment. First taking the effect of antibiotic to be bacteriocidal, that is killing the bacteria, assuming that the kill rate of bacteria is lower in biofilm as compared to fluid, due to the inactivity of bacteria in biofilm as explained in the Introduction. In a later section the model that takes the effect of antibiotic as bacteriostatic, inhibiting the growth of bacteria, is considered. Supposing that the content of fluid compartment have a high mixing rate with the biofilm compartment, we begin with the basic model of antibiotic treatment [27]. 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 concentration of limiting nutrient and antibiotic agent in fluid and $s$ and $a$ denote concentration 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

\[ U' = \text{growth} - \text{washout} - \text{disinfection} - \text{diffuse to biofilm} \]
\[ u' = \text{growth} - \text{disinfection} - \text{transfered from fluid}. \]

The disinfection term is directly linked to the growth as well as type of the antimicrobial agent used. It is shown that the slow growing bacteria are more resistant to antibiotic agent as compared to the fast growing. Our model assumes that the rate of the killing by the antibiotic is directly proportional to the growth rate. Moreover, some antibiotic 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 [5]. Slower growth rate weakens the effect of bactericidal antibiotic. We use the two different pharmacodynamics function for fluid and biofilm compartment

\[ f_1(S, A) \]
and
\[ f_{1u}(s, a) \]
respectively.

### 2.1 The model and preliminary results

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

\[
\begin{align*}
VS' &= F(S^0 - S) - \gamma^{-1} VU f(S) - r_s(S - s) \\
VA' &= F(A_0(t) - A) - VU g(A) - r_a(A - a) \\
VU' &= (f(S) - F/V - f_1(S, A)) VU - r_u(U - u) \\
vs' &= r_s(S - s) - \gamma^{-1} vuf_u(s) \\
va' &= r_a(A - a) - vug_u(a) \\
vu' &= (f_u(s) - f_{1u}(s, a)) vu + r_u(U - u).
\end{align*}
\]  

where \( V \) denotes the volume of fluid compartment, \( v \) denotes the volume of biofilm compartment and \( F \) is the mixing rate (flow rate) of the contents of fluid compartment to biofilm compartment. The term \( r_s \) is the flow rate of nutrient from one compartment to another. Similarly \( r_u \) and \( r_a \) is the flow rate of bacteria and antibiotic from one compartment to another. Assuming that the fluid compartment has a very high mixing rate with the biofilm compartment, implying that \( F \) is a large value, we define \( \epsilon = \frac{V}{F} \) a very small parameter. We model a situation, where the contents of the biofilm environment are stagnant while its surrounding fluid is moving very fast. In this case the above model becomes:

\[
\begin{align*}
\epsilon S' &= (S^0 - S) - \frac{\epsilon}{V} \left[ r_s f(S) + \gamma^{-1} U f(S) \right] \\
\epsilon A' &= (A_0(t) - A) - \frac{\epsilon}{V} \left[ U g(A) + r_a (A-a) \right] \\
\epsilon U' &= -U + \frac{\epsilon}{V} \left[ (f(S) - f_1(S, A)) U - r_u(U - u) \right] \\
s' &= \frac{r_s}{v}(S - s) - \gamma^{-1} u f_u(s) \\
a' &= \frac{r_a}{v}(A - a) - u g_u(a) \\
u' &= (f_u(s) - f_{1u}(s, a)) u + \frac{r_u}{v}(U - u).
\end{align*}
\]  

Since flow rate/volume of the compartment gives the dilution rate, replacing the terms \( r/V \) as \( D \), respectively in the above equations, gives the following set of equations:
\[ \epsilon S' = (S^0 - S) - \epsilon \left[ \gamma^{-1} U f(S) + D_s(S - s) \right] \]

\[ \epsilon A' = (A_0(t) - A) - \epsilon \left[ U g(A) + D_a(A - a) \right] \]

\[ \epsilon U' = -U + \epsilon \left[ \left( f(S) - f_1(S, A) \right) U - D_u(U - u) \right] \]

\[ s' = d_s(S - s) - \gamma^{-1} u f_u(s) \]

\[ a' = d_a(A - a) - u g_u(a) \]

\[ u' = (f_u(s) - f_{1u}(s, a)) u + d_u(U - u). \]

Where \( D_s \) is the dilution rate of nutrients in the fluid compartment, \( D_a \) and \( D_u \) give the dilution rate of antibiotic and bacteria in the fluid compartment. \( d_s, d_a \) and \( d_u \) are the dilution rate of nutrient, antibiotic and bacteria respectively in 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. Parameter \( \gamma \) is the yield constant, which gives the conversion of nutrients to organism and is measured as below [40]

\[
\frac{\text{mass of organism formed}}{\text{mass of substrate used}}
\]

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 monotonic increasing in \( S \):

\[ f(0) = 0, \quad f'(S) \geq 0 \]

The same holds for \( f_u(s) \).

Mostly 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 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.

Characteristically, antibiotics are administered either as a constant dose \( A_0(t) = A_0 = 0 \) 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 \( \delta \) is the Dirac impulse function [27]. The simulations in figure (1) take the dosing period as \( T = 6 \text{hrs} \).
Figure 1: The left figure shows periodic discrete dosing of an antibiotic. The right figure shows resulting pharmacokinetics $a^*(t)$

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 function $f_1(S, A)$ and $f_{1u}(s, a)$. It should vanish if there is no antibiotic and increase as the antibiotic concentration is increasing:

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

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

$$S \rightarrow f(S) - f_1(S, A)$$

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. This function $g$ can be taken in accordance with the Michaelis-Menten kinetics or simply take it as $g(A) = cA$. Mostly pharmacodynamics do not include this kind of term. In some cases, it is reasonable to assume that $g(A) = 0$, if the antibiotic removal rate is relatively independent of $U$. This case is of special interest mathematically, since it decouples the pharmacokinetics from the rest of the model. We assume that $g$ vanishes with $A$ and is nondecreasing in $A$:

$$g(0) = 0, \quad g'(A) \geq 0$$

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

Let us define

$$y = \begin{pmatrix} S \\ A \\ U \end{pmatrix}$$
and
\[ x = \begin{pmatrix} s \\ a \\ u \end{pmatrix}. \]

Taking the right hand side of the first three equations, denoting the fluid compartment dynamics as \( G(t, x, y, \epsilon) \) and the last three equations, denoting the biofilm compartment dynamics as \( F(t, x, y, \epsilon) \), the model (3) can be written as:

\[
\begin{align*}
\epsilon y' &= G(t, x, y, \epsilon) \\
x' &= F(t, x, y, \epsilon).
\end{align*}
\]

(4)

At \( \epsilon = 0 \), implying that the flow rate of fluid compartment \( F \) is very large, we have the reduced problem

\[
x' = F(t, x, y, 0), \quad 0 = G(t, x, y, 0)
\]

(5)

Hence this simplifies the problem of the two compartment, to essentially a single compartment model, the biofilm environment.

### 3 Periodic Solutions and their Stability

In this section we will discuss the existence of periodic solutions and their stability properties. It is clear from the first three equations that the reduced system

\[ 0 = G(t, x, y, 0) \]

has a periodic solution

\[ E_0(t) = (S^0, A_0(t), 0) \]

Also, from the theorem 2.1 ([27]), we can see that

\[ x' = F(t, x, y, 0) \]

has a unique periodic solution \( \tilde{E}_0(t) = (S^0, a^*(t), 0) \) with \( u = 0 \) and \( u(0) = 0 \). Thus the reduced model (5) has a bounded “outer” solution which we call the trivial solution

\[ E_0(t) = (S^0, A_0(t), 0, S^0, a^*(t), 0) \]

so that \( (y, x) = (S^0, A_0(t), 0, S^0, a^*(t), 0) \) satisfies (5). This is the “sterile state” or “infection-free state”, which has no bacteria present and the nutrient level match 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 as the asymptotic pharmacokinetics since every solution of the above differential equation is asymptotic to it as $t$ becomes large. It has the following properties

$$[a^*]_m = [A_0]_m, \quad \min_t A_0 \leq \min_t a^* \leq \max_t a^* \leq \max_t A_0$$

The infection-free state can be taken as the desired target state, so that the successful treatment 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 $\bar{U}(t) \geq 0$, $\bar{u}(t) > 0$ and all other components are positive periodic functions. Such states correspond to the treatment failure. It is interesting to note that there is no solution for the model such that $U(t) > 0$ and $u(t) = 0$. So this implies that presence of bacteria in fluid guarantee that bacteria are present in biofilm but the converse is not true so it maybe that bacteria are not present in fluid but are there in the biofilm.

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:

$$\lambda = f_u(s) - [f_{iu}(s, a^*)]_m - d_u$$

In the above case, $\lambda$ depends on the net, time-averaged bacterial growth rate in the asymptotic pharmacokinetic state, involving the growth dynamics, the pharmacodynamics function and the bacterial growth rate.

Let us define

$$y_1 = y - \bar{E}_0(t)$$
$$x_1 = x - \bar{E}_0(t),$$

then expanding (3) about the trivial solution and renaming $x_1$ and $y_1$ to $x$ and $y$ gives

$$\epsilon y' = C(t, \epsilon)x + D(t, \epsilon)y + h_2(t, x, y, \epsilon)$$
$$x' = A(t, \epsilon)x + B(t, \epsilon)y + h_1(t, x, y, \epsilon)$$

where

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

In the above case, $\lambda$ depends on the net, time-averaged bacterial growth rate in the asymptotic pharmacokinetic state, involving the growth dynamics, the pharmacodynamics function and the bacterial growth rate.
\[ A(t, \epsilon) := \begin{pmatrix} -d_s & 0 & -\gamma^{-1} f_u(S^0) \\ 0 & -d_a & -g_u(a^*(t)) \\ 0 & 0 & f_u(S^0) - f_{1u}(S^0, a^*(t)) - d_u \end{pmatrix}, \]

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 (6).

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

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

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

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

For system (6) we have \( C(t, \epsilon) = \epsilon C(t, \epsilon), |C(t, \epsilon)| \leq M \) and \( h_2(t, x, y, \epsilon) = \epsilon \bar{h}_2(t, x, y, \epsilon) \) where both \( \bar{h}_2(t, x, y, \epsilon) \) and \( h_1(t, x, y, \epsilon) \) satisfy the estimates of (H4).

**Theorem 3.1** (a) Assume (H1)-(H4) hold. Then there exists \( \epsilon_0, \rho_1 \) with \( 0 \leq \rho_1 \leq \rho_0 \) such that for each \( \epsilon \) satisfying \( 0 \leq \epsilon \leq \epsilon_0 \) (3) has a unique periodic solution \( E_0(t, \epsilon) = (E_0^s(t, \epsilon), E_0^w(t, \epsilon)) \) where

\[
E_0^s(t, \epsilon) = \bar{E}_0(t) + \epsilon O(M(\epsilon))
\]

\[
E_0^w(t, \epsilon) = \bar{E}_0(t) + O(M(\epsilon)), \text{ as } \epsilon \to 0
\]

satisfying \( \|x\| \leq \rho_1, \|y\| \leq \rho_1 \) and this solution is continuous in \( \epsilon \) uniformly in \( t \in R \).

(b) The period solution \( E_0(t, \epsilon) \) of (3) is asymptotically stable if \( f_u(S^0) - [f_{1u}(S^0, a^*(t))]_m - d_u < 0 \) and is unstable if \( f_u(S^0) - [f_{1u}(S^0, a^*(t))]_m - d_u > 0 \).

The above theorems ascertains that the invasion exponent \( \lambda \) sign is critical in determining stability of sterile state and non-sterile state. This result is shown in figure 2. For the purpose
Figure 2: The left figure shows treatment success when $\lambda = -0.1826 < 0$. The right figure shows the treatment failure when $\lambda = 0.1662 > 0$.

Figure 3: The figure shows the effect of increasing the Antibiotic dose by a factor of 1.5, on bacterial population for treatment success. Bacteria eliminated at a higher rate as compared to figure 2.
of our simulations we take the growth rate and pharmacodynamics function based on the Michaelis-Menten kinetics

$$f_u(s) = \frac{ms}{b + s}, \quad g_u(a) = \frac{b_1a}{L_1 + a} \quad \text{and} \quad f_{iu} = k_u\frac{s}{b + s}L + a.$$  

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 parameters values are same in both figures except the killing rate $k_u$. This is chosen such that $\lambda < 0$ in left figure and $\lambda > 0$ in right figure. Output has been scaled by $s/b$, $u/(b\gamma)$, $a/L$. Time $t$ is scaled by $1/d_s$, $S_0$ is scaled by $1/(b)$, $A_0$ is scaled by $1/L$ and $d_u, d_a, m$ and $k$ are all scaled by $1/d_s$. Parameter values are chosen as in Refs [27, 38]. Particularly, the yield constant $\gamma = 0.8$, maximum specific growth rate $m = 0.417$, $v = 0.28$; removal rate $d_s = 0.23$; half saturation constants $b = 0.1$, $L = 1$ and $L_1 = 1$; maximum disinfection rate $k = 0.529$ for the left figure and $k = 0.057$ for the right; concentration of the substrate feed $S^0 = 0.2$. Figure 3 shows the effect of increasing the antibiotic concentration on the treatment success case. For a higher dose of antibiotic the bacteria are eliminated at a greater rate.

The figure 4 below 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 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.

Figure 5(a) 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 is given for a small initial population ($u(0) = 0.45$) and large initial population ($u(0) = 0.76$) at the beginning of the treatment. The solution for small population shows treatment success whereas solution for large initial populations shows unsuccessful treatment. Both $E_0(t)$ and $E_u(t)$ are simultaneously locally stable. Except for $S^0 = 0.5$, the values of all parameters are the same as for previous figures, with $k_u = 0.529$. This
implies that early antibiotic treatment, before bacterial population becomes large, will be most effective.

However, the case of treatment failure for figure 5(a) can be converted to treatment success by changing the values of certain parameters. In the simulations shown in figure 5(b) antibiotic dose is increased (as shown in figure 3), this eliminates the bacteria for the large initial population of bacteria. Similarly, simulations in figure 5(c) shows that if the killing rate is increased the complete eradication of bacteria can be achieved even for a higher initial microbial population. Moreover figure 5(d) demonstrates the effect of reducing the interval between antibiotic dose. As the time period of dosing is reduced from $T = 6\text{hrs}$ (as in figure 5(a)) to $T = 4\text{hrs}$ (figure 5(d)), 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 (a). Changing certain parameter values can result in eradication of bacteria for the higher initial population (b), (c) and (d)
The following theorem gives the conditions for existence of a treatment failure periodic solution by using persistence theory.

**Theorem 3.2**  
(a) If \( f_u(S^0) - [f_1u(S^0, a^*(t))]_m - d_u > 0 \) then bacterial population for \( x' = F(t, x, y, 0) \) 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 solution of \( x' = F(t, x, y, 0) \) of the form \( E^*_2 = (s, a, u) \) such that

\[
E^* = (E^*_1(t), E^*_2(t))
\]

is a solution of (5).

(b) For each \( T \) periodic solution \( E^*(t) \) of (5) there is \( \epsilon_0 \) such that for \( 0 < \epsilon < \epsilon_0 \) there exists a \( T \) periodic solution

\[
E(t, \epsilon) = (E_1(t, \epsilon), E_2(t, \epsilon))
\]

of system (3) with

\[
E_1(t, \epsilon) = E^*_1(t) + \epsilon O(M(\epsilon))
\]

\[
E_2(t, \epsilon) = E^*_2(t, \epsilon) + O(M(\epsilon))
\]

(c) There is \( \epsilon_0 \) such that for \( 0 < \epsilon < \epsilon_0 \) the \( T \) periodic solution \( E(t, \epsilon) \) is asymptotically stable if and only if the linear system \( z'_1 = A(t, 0)z_1 \) is asymptotically stable where

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

Treatment failure is guaranteed when \( \lambda > 0 \) in all cases since bacteria can grow when rare. Furthermore, by using persistence theory, we showed that there exist at least one (periodic) infection state \( E_u(t) \), generalizing it, this solution may be non-unique and we do not know its stability properties.

Dose tapering technique has been effective in antibiotic treatment [36]. 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. So it has been suggested that antibiotic dose should be reduced with time. However one should be careful with this since if the dose is reduced below a certain level, then the bacteria might start to increase. We have used our model with the bacteriocidal effect of antibiotic to show the simulations for this effect.

We take \( A_0(t) \) a function 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. So it is clear from figure 7, that as the dose is reduced as compared to figure 6, the bacterial population starts increasing after 54 hours. So dosing should be appropriate such that the bacteria is completely eliminated.
4 Optimal Antibiotic Treatments

In this section, we address the problem of finding a course of treatment which minimizes active bacteria at the end while also balancing the total antibiotic applied. Several studies have indicated the counter-productive effects of over-deployment of antibiotics. Indeed, this may even increase the susceptibility to infection [36]. It is therefore essential to manage the antibiotic load to ensure that its harmful effects are minimized.

At the same time, the dilution rate, low relative to the maximum growth rate, is unable to flush out the bacteria on its own. Antibiotic application therefore becomes imperative. 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.

In this regard, our work mirrors that of Cogan. The results, however, are strikingly different. While Cogan’s model requires a cycling strategy between antibiotic withdrawal and application, we obtain a continuous dosing program. We also show how the qualitative differences between the two models lie at the basis of these vastly different results.

Based on this, we derive optimal strategies for different initial conditions, sensitivity parameters and dilution rates. We observe that such a strategy precludes the bistability that appeared in the periodic antibiotic application scenario, i.e., the zero bacteria steady state is globally stable. Under our model thus, employing such a course of treatment ensures bacteria eradication and by implication disease treatment. Finally, we show that finding an optimal discrete dosage strategy leads us back to the previously found continuous protocol.

4.1 Finding the Optimal Control Function

To achieve our objective of finding an optimal strategy, we rely on theory of constrained optimization. To minimize the antibiotic applied while also reducing bacteria population at the end, we want to reduce the respective ”costs” both these factors incur. We want to find
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.

the form of the function $A_0(t)$ such that it minimize a functional of the form

$$C(A_0(t), u(t_f)) = \frac{1}{2} \int_{t_0}^{t_f} W A_0(t)^2 dt + W_u u(t_f)$$

(7)

The functional $C(A_0(t), u(t_f))$ is the mathematical form of the intuitive idea of the costs associated with a protocol. The integral term depends on how much antibiotic is applied while the second term gives the bacteria population left at time $t_f$, the time at the end of the treatment. The parameters $W$ and $W_u$ determine the respective contributions to the sum. Together, this functional gives us a method of evaluating a particular strategy’s cost; the problem is reduced to minimizing 7 by finding the optimal $A_0(t)$.

As in [11], we define a Lagrangian

$$\mathcal{L} = \frac{1}{2} W A_0(t)^2 + \lambda_1 s' + \lambda_2 a' + \lambda_3 u'$$

(8)

where the $\lambda_i$ are adjoint functions. By Pontryagin’s principle, these functions obey

$$\frac{d\lambda_1}{dt} = - \frac{\partial \mathcal{L}}{\partial s}$$
$$\frac{d\lambda_2}{dt} = - \frac{\partial \mathcal{L}}{\partial a}$$
$$\frac{d\lambda_3}{dt} = - \frac{\partial \mathcal{L}}{\partial u}$$

(9)

with $\lambda_1(t_f) = 0$, $\lambda_2(t_f) = 0$ and $\lambda_3(t_f) = W_u$. Furthermore, at optimum, $\frac{\partial \mathcal{L}}{\partial A_0} = 0$. Using (5) and (8), this translates to

$$A_0(t) = - \frac{\lambda_2 a}{W}$$

(10)
We have now ended up with a system of six ODEs, namely the system (5) and equations (9), along with their boundary values. The solution of this system can be used to yield the form of the control function by (10).

Several techniques exist for numerically solving this system. The method we employ is the forward-backward sweep method implemented in MATLAB, as detailed in [29]. First making an initial guess for adjoint functions $\lambda_1, \lambda_2$ and $\lambda_3$, we use the Runge-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 adjoints. These iterations are continued until convergence occurs; (10) is then used to give the form of the optimal treatment function.

4.2 Results and Variations

The recommended strategy and the resulting changes in antibiotic and bacteria levels are illustrated in Figure 8. The values of initial conditions and parameters are the same as in Figure 2(a), which allows a comparison to be made. The sensitivity parameters are set to $W = 0.0001$ and $W_u = 100$. The expectedly jerky nature of bacteria elimination in the discrete application case aside, observe that both treatments are similarly efficacious in eliminating bacteria.

Figure 2(b) illustrates a treatment failure case under the discrete protocol. The new treatment, however, eliminates the disease for the same initial conditions and parameter values; this is shown in Figure 9. This indicates one advantage of the newly developed strategy over the discrete protocol.

Moreover, applying the optimal treatment removes the bi-stability that is exhibited in Figure 5(a), for $S_0 = 0.5$. It can be seen that while bacteria is severely reduced in several of these cases, it is not completely eliminated. This is a trade-off for minimizing the antibiotic load, whose effect has to be tolerated. 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. This will then cause an even greater diminishing of bacteria population in the biofilm. Figure 10 indicates that the low-bacteria state is globally stable for any initial bacteria level. The optimal strategy hence ensures the eventual near-eradication of bacteria over time which was not guaranteed as a result discrete applications.

This leads us to investigating the effect of varying the sensitivity parameters. With only two of those, the point of interest is only their relative values. We thus hold $W_u$ constant at a value of 4 and vary $W$ substantially. The suggested strategies and the resultant bactericide is shown in Figure 11. As would be expected, increasing the cost associated with antibiotic application tends to reduce it. For $W=1$ and 100, the quantity of antibiotic applied is substantially lower than that for $W = 0.001$. The corresponding reduction in bacterial concentration is then negatively correlated with the value of $W$.

We make two relevant observations at this point. Firstly, notice that the almost coincident curves for $W = 1$ and $W = 100$ in both diagrams suggest that however high the antibiotic cost, it is always prudent to utilize them to prevent a bacteria outbreak. Secondly, for very low antibiotic cost, i.e., $W = 0.001$, the bacteria population is almost wiped out. This is the point we made earlier while discussing the stability of the low-bacteria state.
Figure 8: The optimal strategy and the resulting change in antibiotic and bacterial concentration, for $s(0)=1.9$, $a(0)=0.01$ and $u(0)=1.4$, using the parameter values of Figure 2(a). The bacterial decay for the latter is also shown.
Figure 9: The treatment failure case from Figure 2(b) can be treated successfully under the optimal strategy.

Figure 10: 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(a) to illustrate bi-stability.
Figure 11: Varying the values of $W$ yields these treatments and the corresponding bacteria decays.
4.3 Discretizing the Dosages

As mentioned before, Cogan in [11] has obtained periodic optimal strategies for eliminating bacteria, contrary to our findings. However, it must be kept in mind that Cogan incorporates susceptible and persistent type bacteria into his model. The latter are not harmed by antibiotic at all. Indeed, it is assumed that a higher antibiotic concentration prevents them from reverting back to susceptible-type bacteria. Thus, the optimal strategy must involve periodic cycling of antibiotic dosages so as to allow the persistent bacteria to transition to susceptible and then get rid of them.

As opposed to this, the bacteria in our model is assumed to possess no such immune system. Hence, there is no reason to withdraw antibiotics other than when we can afford to dispense with it at the cost of a higher final bacterial level. A continuous protocol is obtained as a consequence.

Even if we attempt to change our control function so that the antibiotic is applied in discrete chunks periodically, we end up with the continuous protocol. Suppose we let

\[ A_0(t) = \delta(t) \sin^4 \left( \frac{\pi t}{p} \right) \]

where \( \delta(t) \) is the control function and \( p \) is the time period between dosages. Altering the system and solving as before, \( \delta(t) \) becomes very large as \( t \) approaches \( hp, h \in \mathcal{N} \). Consequently, we get finite limits as \( t \) approaches \( hp \) so the form of \( A_0(t) \) returned is the same as above. Thus, attempting to artificially discretize the optimal strategy fails.

5 Bacteriostatic Effect of Antibiotic

In the model (3) we have taken the effect of antibiotic as bacteriocidal in nature, that is killing the bacteria. In this section we consider the bacteriostatic effect of antibiotic, which means that antibiotic will inhibit the growth of bacteria and restrict the uptake of nutrient by bacteria. The model (3) is now modified as below

\[
\begin{align*}
\epsilon S' &= (S^0 - S) - \epsilon \left[ \gamma^{-1} U f_2(S, A) + D_s(S - s) \right] \\
\epsilon A' &= (A_0(t) - A) - \epsilon \left[ U g(A) + D_a(A - a) \right] \\
\epsilon U' &= -U + \epsilon \left[ f_2(S, A)U - D_u(U - u) \right] \\
s' &= d_s(S - s) - \gamma^{-1} u f_{2u}(s, a) \\
a' &= d_a(A - a) - u g_a(a) \\
u' &= f_{2u}(s, a)u + d_u(U - u).
\end{align*}
\]

The pharmacodynamic functions \( f_2(S, A) \) and \( f_{2u}(s, a) \) for the fluid and biofilm respectively are assumed to be monotonically increasing in \( S \) and monotonically decreasing in \( A \). All other parameters are the same as described in section (2.1). Since the flow rate \( F \), is taken to be very large, so as described earlier we say that \( \epsilon = \frac{V}{F} \) is equal to zero, hence the model can be reduced to

\[ x' = F_1(t, x, y, 0), \quad 0 = G_1(t, x, y, 0) \]
Where \( x \) and \( y \) are same as defined in section (2.1). \( F_1 \) is the right hand side of last three equations and \( G_2 \) is the right hand side of first three equations of the model (11). The above model will have the periodic solutions corresponding to the sterile state and infectious state which are given by:

\[
E_s^0(t) = (S^0, A_0(t), 0, S^0, a^*(t), 0)
\]

and

\[
E_s^*(t) = (\bar{S}(t), \bar{A}(t), \bar{U}(t), \bar{S}(t), \bar{a}(t), \bar{u}(t))
\]

respectively.

To check the local stability of the sterile state, we use the floquent exponent of the variational equation about \( E_s^0(t) \). Two of these floquent exponent come out to be negative, and the third floquent exponent, which we call as the invasion exponent is given as

\[
\lambda_1 = [f_{2u}(s, a^*)]_m - d_u
\]

In this case again, \( \lambda \) depends on the net, time-averaged bacterial growth rate in the asymptotic pharmacokinetic state, involving the growth dynamics, the pharmacodynamics function and the bacterial growth rate, as for the case of bacteriocidal effect.

As in section (3) we define

\[
y_2 = y - \bar{E}_s^0(t) \quad x_2 = x - \bar{E}_s^0(t),
\]

then expanding (11) about the trivial solution and renaming \( x_2 \) and \( y_2 \) to \( x \) and \( y \) gives

\[
\begin{align*}
ey'(t) &= C_1(t, \epsilon)x + D_1(t, \epsilon)y + h_{2s}(t, x, y, \epsilon) \\
x'(t) &= A_1(t, \epsilon)x + B_1(t, \epsilon)y + h_{1s}(t, x, y, \epsilon)
\end{align*}
\]

(13)

Where the forms of the matrix \( A_1, B_1, C_1 \) and \( D_1 \) are same as the corresponding matrix forms given in section (3).

The hypothesis H1-H4 in section (3) hold for the above model as well. The following theorems give the main result for the model (11).

**Theorem 5.1**

(a) Assume (H1)-(H4) hold. Then there exists \( \epsilon_0, \rho_1 \) with \( 0 \leq \rho_1 \leq \rho_0 \) such that for each \( \epsilon \) satisfying \( 0 \leq \epsilon \leq \epsilon_0 \) (11) has a unique periodic solution \( E_s^0(t, \epsilon) = (E_{s0}^s(t, \epsilon), E_{s0}^s(t, \epsilon)) \) where

\[
\begin{align*}
E_{s0}^s(t, \epsilon) &= \bar{E}_s^0(t) + \epsilon O(M(\epsilon)) \\
E_{s0}^s(t, \epsilon) &= \bar{E}_s^0(t) + O(M(\epsilon)), \ as \ \epsilon \to 0
\end{align*}
\]

satisfying \( \| x \| \leq \rho_1, \| y \| \leq \rho_1 \) and this solution is continuous in \( \epsilon \) uniformly in \( t \in R \).

(b) The \( T \) periodic solution \( E_s^0(t, \epsilon) \) of (11) is asymptotically stable if \( f_{2u}(s, a) - d_u < 0 \) and is unstable if \( f_{2u}(s, a) - d_u > 0 \).
The above theorems establishes that the sign of the Floquet exponent is important in determining the stability of the sterile state solution. This is shown by the simulations given in figure 12.

The following theorem give the conditions for existence of a periodic solution for treatment failure case using persistence theory.

**Theorem 5.2**

(a) If \( f_{2u}(s,a) - d_u > 0 \) then bacterial population for \( x' = F_1(t,x,y,0) \) 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 solution of \( x' = F_1(t,x,y,0) \) of the form \( E_{2s}^{ss} = (s,a,u) \) such that

\[
E^{ss} = (E_{1s}^{ss}(t), E_{2s}^{ss}(t))
\]

is a solution of (12).

(b) For each \( T \) periodic solution \( E^{ss}(t) \) of (12) there is \( \epsilon_0 \) such that for \( 0 < \epsilon < \epsilon_0 \) there exists a \( T \) periodic solution

\[
E^{s}(t,\epsilon) = (E_{1s}(t,\epsilon), E_{2s}(t,\epsilon))
\]

of system (11) with

\[
E_{1s}(t,\epsilon) = E_{1s}^{ss}(t) + \epsilon O(M(\epsilon))
\]

\[
E_{2s}(t,\epsilon) = E_{2s}^{ss}(t,\epsilon) + O(M(\epsilon))
\]

(c) There is \( \epsilon_0 \) such that for \( 0 < \epsilon < \epsilon_0 \) the \( T \) periodic solution \( E^{s}(t,\epsilon) \) is asymptotically stable if and only if the linear system \( z'_1 = A_1(t,0) z_1 \) is asymptotically stable where

\[
A_1(t,0) := \begin{pmatrix}
-d_s - \gamma^{-1} \bar{w} f'_{2u}(\bar{s},\bar{a}) & 0 & -\gamma^{-1} f_{2u}(\bar{s},\bar{a}) \\
0 & -d_a - \bar{u} g'_u(\bar{a}) & -g_u(\bar{a}) \\
f'_{2u}(\bar{s},\bar{a}) \bar{u} & f_{2u}(\bar{s},\bar{a}) & f_{2u}(\bar{s},\bar{a}) - d_u 
\end{pmatrix}.
\]

Whenever \( \lambda > 0 \) treatment failure is assured since bacteria can grow when rare. Besides this, persistence theory facilitates us in showing that there exist at least one periodic infection state \( E_{2s}^{ss}(t) \), generalizing this, the solution may be non-unique and we do not know its stability properties.

The simulations are done for this model by assuming the pharmacodynamics function of the form

\[
f_{2u} = \frac{ms}{a + s} \exp(-bA)
\]

All other parameters are taken to be the same as for the simulations of the bacteriocidal case, shown in figure 2.

It is observed by the figure (12) that bacteriostatic effect of antibiotic eradicates the bacteria at a slower rate. By looking at the treatment failure case, it can be seen that the bacteria grows but grows very slowly, since the antibiotic has an inhibiting effect on the growth rate of bacteria.
6 Discussion

Microbial biofilms have several detrimental effects on the host surface on which it is formed. A few of which are contamination of food products, dental plaque, cystic fibrosis and failure of medical implants [7, 8, 9, 18, 19, 39, 43, 44]. 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 theory of singularly perturbed nonautonomous systems given in [42] to analyze the model. Assuming that antibiotic is bacteriocidal 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 dynamics of six dimensional system to a three dimensional system, only the biofilm system. We have used the results proved in [27] to investigate the dynamics of this reduced model.

This paper considers two different model of antibiotic treatment, the bacteriocidal effect, killing the bacteria and bacteriostatic, inhibiting the bacterial growth. The pharmacodynamics function used in both the cases are different. Although the form of pharmacodynamics function for both fluid and biofilm environment is taken similar, the values of the parameters are different in both environment. The killing rate $k_w$ in biofilm is much smaller than killing rate $k$ in fluid compartment. A possible explanation for this could be the inability of the antibiotic to fully penetrate the biofilm region since the bacteria in the biofilm are not respiring and are inactive. The antibiotics tend to be more effective on active microbial population[6].

We show that for the model periodic solution exist and there are solutions corresponding to sterile state and infection state. The sign of invasion exponent $\lambda$ is crucial in determining the treatment success or treatment failure of the microbial population and hence the stability of the periodic solutions. Simulation in figure 2 are for the bacteriocidal effect of antibiotic, showing that the bacteria are eliminated in case of $\lambda < 0$ and bacteria persists in the case of $\lambda > 0$. The theorem 3.1 establishes that the invasion exponent $\lambda$, whose sign characterizes
the local stability of the sterile state or treatment success, can provide useful information concerning the global dynamics of the model in certain cases.

The infectious state solution has the property $U(t) \geq 0$ and $u(t) > 0$. Since we reduced the six dimensional model to three dimensional model. So this condition implies that for the treatment failure, bacterial population for biofilm should be greater than zero. Hence elimination of bacteria just from the fluid compartment is not sufficient to attain treatment success. Conversely, if bacteria are eliminated from biofilm, $u(t) = 0$, then this would imply that $U(t) = 0$ which then guarantees successful treatment, both biofilm and fluid are in infection free state.

The effect of giving a new antibiotic dose without the complete washout of the previous dose, has been checked using this model. The simulations are shown in figure (4). The first figure on the left shows that the dose is administered periodically, but the new dose is given before the complete time period required for the washout of previous dose. Results for treatment success case (not shown) are obvious, bacteria are eliminated at a higher rate. However, for the treatment failure case (shown in figure 2 (right)), the bacteria are now successfully eliminated (figure 4). Hence this suggest that if the antibiotic dose is given with the previous dose still present in the body, this would facilitate in eradication of bacteria.

We have further used the model to show that different initial bacterial population, with all other parameter values same and $\lambda < 0$, can result in different outcomes (figure 5(a)). The simulation results show that higher population results in treatment failure whereas lower initial microbial population results in treatment success. Also the treatment failure for higher initial population can be converted into treatment success by changing some of the parameters values. Figure 5(b) shows the effect of increasing the antibiotic dose. This is increased by a factor of 1.5, and the treatment failure turns into treatment success. Similarly figure 5(c) shows that if the killing rate for bacterial biofilm is increased from $k_u = 0.51$ to $k_u = 0.62$ then again the treatment failure results in treatment success. Figure 5(d) shows that as the dosing period is reduced from $T = 6\text{hrs}$ to $T = 4\text{hrs}$ the bacterial population is eliminated for higher initial population. These results suggest that if the treatment is started late, the bacterial population is higher at the start of treatment the dosing should be increased or the dosing period should be reduced. The bi-stability shown in figure 5(a) suggests that it is best to have an early treatment, antibiotic therapy should be done before bacterial population increases. In case the bacterial population has increased to the limit where treatment failure is observed than the antibiotic should be administered in the way that successful treatment is achieved.

The results and the simulations for the effect of bacteriostatic antibiotic is given in section 5. The results are same as described for the bacteriocidal case, the existence of periodic solution for both the infection and sterile state is proved as for the bacteriocidal case. However the simulations show that the bacteria are eliminated at a slower rate as compared to bacteriocidal case. Since the effect of bacteriostatic antibiotic is to reduce the growth of bacteria rather than killing it, the simulation in figure (12) shows that the bacteria are completely eradicated in case of $\lambda < 0$ and it takes about 19 days ($\approx 450\text{hrs}$) to eliminate the bacteria, whereas it takes 1.25 days to eliminate the bacteria in case of bacteriocidal antibiotic.

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. Also there are several other mechanism 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.

References


Proofs of our result are given in this appendix.

Proof for Theorem 3.1 is given below:

(a) For the proof of the this part of the theorem see [42].

(b) It follows from [42] that the T periodic solution \((x^*(t, \epsilon), y^*(t, \epsilon))\) of (6) is asymptotically stable provided

\[
\dot{z}_1' = A(t,0)z_1 \tag{14}
\]

and

\[
\dot{z}_2' = D_0 z_2 \tag{15}
\]

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 (15):

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

evaluating \( \phi_1(t) \) at \( t = T \) we obtained the Floquet exponents \(-1, -1\) and \(-1\). This shows that (15) is asymptotically stable. Also the fundamental matrix \( \phi_2(t) \) of (14):

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

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 (14) 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 for Theorem 3.2 is given below:

Proof: (a) We apply the theorem (4.1) of [22]. Using the notation, we set \( X = \{(s, u, a) \in \mathbb{R}_+^3 : \gamma S + u \leq \gamma S^0, A \leq M_1, \ \text{where} \ M_1 = \max_{t \in \mathbb{R}_+} A_0(t)\}, \ X_1 = \{(s, u, a) \in \mathbb{R}_+^3 : u \neq 0\}, \ \text{and} \ X_2 = \{(s, u, a) \in \mathbb{R}_+^3 : u = 0\}. \) 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

\[ \lim_{n \to \infty} d(h^n(X), X_2) > \epsilon. \]

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 \( W^s(M) = \{x \in X : h^n(x) \to M \ \text{as} \ n \to \infty_+\} \)
In order to show that $M$ is isolated in $X$ we will apply the Theorem 2.3 of ([34]).

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 $\tilde{K} \subseteq W^s(E_1)$.

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 $\tilde{K} \subseteq W^u(E_1)$.

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

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

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

(b) Suppose that the coefficients in the system (5) 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

$$y = y - E_1^*(t)$$

$$x = x - E_2^*(t)$$

then expanding (3) about this outer solution gives

$$\epsilon y' = C(t, \epsilon) x + D(t, \epsilon) y + h_2(t, x, y, \epsilon)$$

$$x' = A(t, \epsilon) x + B(t, \epsilon) y + h_1(t, x, y, \epsilon)$$  \hspace{1cm} (16)$$

where

$$C(t, \epsilon) := \left( \begin{array}{ccc} \epsilon D_s & 0 & 0 \\ 0 & \epsilon D_a & 0 \\ 0 & 0 & \epsilon D_u \end{array} \right),$$

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

$$A(t, \epsilon) := \left( \begin{array}{ccc} -d_s - \gamma^{-1} u f'_u(\bar{s}) & 0 & -\gamma^{-1} f_u(\bar{s}) \\ 0 & -d_a - \bar{w} g'_w(\bar{a}) & -g_u(\bar{a}) \\ -(f_u(\bar{s}) - k u f'_1 u(\bar{s}, \bar{a})) \bar{u} & -k u f_1 u(\bar{s}) - k u f'_1 u(\bar{s}, \bar{a}) - d_u & f_u(\bar{s}) - k u f'_1 u(\bar{s}, \bar{a}) - d_u \end{array} \right),$$

and

$$B(t, \epsilon) := \left( \begin{array}{ccc} d_s & 0 & 0 \\ 0 & d_a & 0 \\ 0 & 0 & d_u \end{array} \right).$$
Since \((H1) - (H4)\) hold for the model (3). So by theorem (1.4) of [42], the system (3) has a \(T\) periodic solution \(E(t, \epsilon) = (E_1(t, \epsilon), E_2(t, \epsilon))\) with
\[
E_1(t, \epsilon) = E_1^*(t) + \epsilon O(M(\epsilon)) \\
E_2(t, \epsilon) = E_2^*(t) + \epsilon O(M(\epsilon))
\]

\((e)\) It follows from [42] that the \(T\) periodic solution \((x^*(t, \epsilon), y^*(t, \epsilon))\) of (6) is asymptotically stable provided
\[
z_1' = A(t, 0)z_1 \tag{17}
\]
and
\[
z_2' = D_0z_2 \tag{18}
\]
are asymptotically stable where
\[
D_0 := \begin{pmatrix}
-1 & 0 & 0 \\
0 & -1 & 0 \\
0 & 0 & -1
\end{pmatrix}
\]
and
\[
A(t, 0) := \begin{pmatrix}
-d_s - \gamma^{-1}\bar{w}f_u'(\bar{s}) & 0 & -\gamma^{-1}f_u(\bar{s}) \\
0 & -d_a - \bar{u}g_a'(\bar{a}) & -g_a(\bar{a}) \\
-(f_u(\bar{s}) - f_{1u}'(\bar{s}, \bar{a}))\bar{u} & -f_{1u}(\bar{s}) & f_u(\bar{s}) - f_{1u}(\bar{s}, \bar{a}) - d_u
\end{pmatrix}.
\]
Computation yields the fundamental matrix \(\phi_1(t)\) of (18):
\[
\phi_1(t) := \begin{pmatrix}
e^{-t} & 0 & 0 \\
0 & e^{-t} & 0 \\
0 & 0 & e^{-t}
\end{pmatrix}
\]
evaluating \(\phi_1(t)\) at \(t = T\) we obtained the Floquet exponents \(-1, -1\) and \(-1\). This shows that (18) is asymptotically stable. It follows that (17) 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 for Theorem 5.1 is given below:

**Proof:**  
(a) For the proof of the this part of the theorem see [42].  
(b) It follows from [42] that the \(T\) periodic solution \((x^*(t, \epsilon), y^*(t, \epsilon))\) of (13) is asymptotically stable provided
\[
z_1' = A(t, 0)z_1 \tag{19}
\]
and
\[
z_2' = D_0z_2 \tag{20}
\]
are asymptotically stable where

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

and

\[ A(t, 0) := \begin{pmatrix} -d_s & 0 & -\gamma^{-1}f_{2u}(S^0, a^*(t)) \\ 0 & -d_a & -g_u(a^*(t)) \\ 0 & 0 & f_{2u}(S^0, a^*(t)) - d_u \end{pmatrix}. \]

A computation yields the fundamental matrix \( \phi_1(t) \) of (20):

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

evaluating \( \phi_1(t) \) at \( t = T \) we obtained the Floquet exponents \(-1, -1, -1\). This shows that (20) is asymptotically stable. Also the fundamental matrix \( \phi_2(t) \) of (19):

\[ \phi_2(t) := \begin{pmatrix} e^{-d_s t} & 0 & \phi_{13} \\ 0 & e^{-d_a t} & \phi_{23} \\ 0 & 0 & e^{\int_0^t (f_{2u}(S^0, a^*(t)) - d_u) ds} \end{pmatrix}. \]

Evaluating this fundamental matrix \( \phi_2(t) \) at \( t = T \) we obtained the Floquet exponents \(-d_s, -d_a\) and \( f_{2u}(S^0, a^*(t)) - d_u \). It follows at once that (19) is asymptotically stable if \( f_{2u}(S^0, a^*(t)) - d_u < 0 \). Thus the \( T \) periodic solution \( E_0(t, \epsilon) \) is asymptotically stable if and only if \( f_{2u}(S^0, a^*(t)) - d_u < 0 \).

**Proof for Theorem 5.2** is given below

**Proof:** (a) Proof for this part of the is similar to proof for theorem 3.2, part (a).

(b) Proof for this is on the similar lines as for the theorem 3.2, part (b). Suppose that the coefficients in the system (12) 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 \( E_{s}^*(t) = (S^0, A_0(t), 0, \bar{s}(t), \bar{a}(t), \bar{u}(t)) \). We define

\[ y = y - E_{s}^*(t) \]
\[ x = x - E_{s}^*(t) \]

then expanding (11) about this outer solution gives

\[ \epsilon y' = C_1(t, \epsilon)x + D_1(t, \epsilon)y + h_2(t, x, y, \epsilon) \]

\[ x' = A_1(t, \epsilon)x + B_1(t, \epsilon)y + h_1(t, x, y, \epsilon) \]

where
\[ C_1(t, \epsilon) := \begin{pmatrix} \epsilon D_s & 0 & 0 \\ 0 & \epsilon D_a & 0 \\ 0 & 0 & \epsilon D_u \end{pmatrix}, \]

\[ D(t, \epsilon) := \begin{pmatrix} -1 - \epsilon D_u & 0 & -\epsilon \gamma^{-1} f_2(S^0, A_0(t)) \\ 0 & -1 - \epsilon D_a & -\epsilon g(A_0(t)) \\ 0 & 0 & -1 + \epsilon f_2(S^0, A_0(t)) - \epsilon D_u \end{pmatrix}, \]

\[ A(t, \epsilon) := \begin{pmatrix} -d_s - \gamma^{-1} \bar{u} f'_2(s, \bar{a}) & 0 & -\gamma^{-1} f_2(s, \bar{a}) \\ 0 & -d_a - \bar{u} g'_a(\bar{a}) & -g_a(\bar{a}) \\ -(f'_2 u)(\bar{s}, \bar{a}) \bar{u} & f_2 u(s, \bar{a}) & f_2 u(s, \bar{a}) - d_u \end{pmatrix}, \]

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 (11). So by theorem (1.4) of [42], the system (11) has a \(T\) periodic solution \(E(t, \epsilon) = (E_1(t, \epsilon), E_2(t, \epsilon))\) with

\[ E_1(t, \epsilon) = E_1^*(t) + \epsilon O(M(\epsilon)) \]

\[ E_2(t, \epsilon) = E_2^*(t) + \epsilon O(M(\epsilon)) \]

(c) It follows from [42] that the \(T\) periodic solution \((x^*(t, \epsilon), y^*(t, \epsilon))\) of (6) is asymptotically stable provided

\[ z'_1 = A(t, 0) z_1 \] (22)

and

\[ z'_2 = D_0 z_2 \] (23)

are asymptotically stable where

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

and

\[ A_1(t, 0) := \begin{pmatrix} -d_s - \gamma^{-1} \bar{u} f'_2(s, \bar{a}) & 0 & -\gamma^{-1} f_2(s, \bar{a}) \\ 0 & -d_a - \bar{u} g'_a(\bar{a}) & -g_a(\bar{a}) \\ f'_2 u(s, \bar{a}) \bar{u} & f_2 u(s, \bar{a}) & f_2 u(s, \bar{a}) - d_u \end{pmatrix}. \]

Computation yields the fundamental matrix \(\phi_1(t)\) of (23):

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

evaluating \(\phi_1(t)\) at \(t = T\) we obtained the Floquet exponents \(-1, -1, -1\). This shows that (23) is asymptotically stable. It follows that (22) is asymptotically stable if \(f_2 u(S^0, a^*(t)) - d_u < 0\). Thus the \(T\) periodic solution \(E_0(t, \epsilon)\) is asymptotically stable if and only if \(f_2 u(S^0, a^*(t)) - d_u < 0\).