




Phage–Antibiotic Synergy Inhibited by Temperate and Chronic Virus Competition

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Abstract

As antibiotic resistance grows more frequent for common bacterial infections, alternative treatment strategies such as phage therapy have become more widely studied in the medical field. While many studies have explored the efficacy of antibiotics, phage therapy, or synergistic combinations of phages and antibiotics, the impact of virus competition on the efficacy of antibiotic treatment has not yet been considered. Here, we model the synergy between antibiotics and two viral types, temperate and chronic, in controlling bacterial infections. We demonstrate that while combinations of antibiotic and temperate viruses exhibit synergy, competition between temperate and chronic viruses inhibits bacterial control with antibiotics. In fact, our model reveals that antibiotic treatment may counterintuitively increase the bacterial load when a large fraction of the bacteria are antibiotic resistant, and both chronic and temperate phages are present.

Keywords Bacteria · Bacteriophage · Phage · Latent · Lytic · Infection · Evasion · Recovery · Resistance · *Pseudomonas aeruginosa* · Cystic fibrosis · Population dynamics · Mathematical model

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1 Introduction

The rising number of antibiotic-resistant bacteria is causing an increase in the cost of treatment, higher mortality rates, and a longer period of sickness (Laxminarayan et al. 2013). For instance, infections by the multi-drug-resistant pathogen *Pseudomonas aeruginosa*, which is categorized as a serious threat by the United States Centers for Disease Control (C. for Disease Control and Prevention 2019), are frequently diagnosed among those with compromised immune systems to such an extent that it is the most common cause of death among patients with cystic fibrosis (Hancock and Speert 2000).

As antibiotic-resistant bacteria are becoming more prevalent, new strategies are needed to combat these bacterial infections. Recent experiments show that antibiotics and phages may synergistically target bacterial populations when used in tandem (Chaudhry et al. 2017; Easwaran et al. 2020). While some studies have revealed that phage cocktails in combination with antibiotics are effective against bacterial infections (Chaudhry et al. 2017), we demonstrate that certain combinations of viruses may inhibit bacterial infection control with antibiotics.

1.1 Biological Background

Bacteriophages employ a variety of strategies to infect bacteria and reproduce, including the lytic, temperate, and chronic lifestyles (Calendar 2006; Dimmock et al. 2016; Weinbauer 2004). Lytic viruses infect a bacterial host and use bacterial machinery to produce new viruses, which burst lethally from the cell. Temperate viruses have both a lytic cycle and a latent cycle, in which the viral genetic material is integrated into host genomes; latent proviruses remain dormant in the bacterial genome until induced to replicate (Weinbauer 2004). In chronic infections, productive host cells bud new viruses from the cell without killing the bacterium (Rakonjac 2012), and chronic infection is transmitted vertically to daughter cells (Lwoff 1953). Comparative genomics among closely related bacterial strains demonstrates that proviruses of all lifestyles are ubiquitous in bacterial genomes (Davies et al. 2016; Roux et al. 2015; Mathee et al. 2008; Mosquera-Rendón et al. 2016; Spencer et al. 2003; Kung et al. 2010).

When a bacterium is stressed, for instance, by radiation, excessive heat, or sub-lethal antibiotics, the cell may induce latent prophage and begin phage production (López et al. 2014; Martínez-García et al. 2015; Kaur et al. 2012). One mechanism for provirus induction is the SOS response, an evolved repair system found in many bacteria including *P. aeruginosa* (Fothergill et al. 2011; Rokney et al. 2008; Weinbauer 2004). Because induction of latent proviruses of the temperate lifestyle leads inevitably to cell death (Brives and Pourraz 2020), this phenomenon is proposed to be one of the mechanisms behind the synergistic effect of antibiotics and phage infection (Kaur et al. 2012; Kim et al. 2018).

1.2 Modeling Background

Since the synergistic role of bacteriophages and antibiotics in the fight against infections was first investigated with mathematical models, there has been a multitude of additional complexities that has attracted the attention of modelers (Sinha et al. 2018). These include the competition between the lytic and lysogenic strategy, bacteria–phage coevolution, phage and antibiotic resistance in bacteria, and spatial limitations.

The population dynamics of phage–bacterium interactions have been under investigation for at least the last half century (Levin et al. 1977; Payne and Jansen 2001, 2003). A few decades ago, antibiotic effects were first included in models (e.g., Levin and Bull 1996), which predict that phage therapy is more effective than antibiotic treatment; a caveat of the results is that the efficacy of phage therapy depends on adsorption rate, burst size, and initial phage dose, and the efficacy of antibiotic treatment depends on the initial antibiotic dose. These important, albeit early attempts, were later shown to be inconsistent with experimental observations (Weld et al. 2004).

As our biological understanding of virus lifestyles (chronic, temperate, etc.) and their strategies (lytic, lysogenic, productive, etc.) becomes more complete, so too do our mathematical models. More recently, the role of lysogeny has been studied in the absence of antibiotics to evaluate whether it provides a competitive advantage for either type of virus; it was determined that chronic viruses maximize steady state density by eliminating lysogeny, while temperate viruses benefit from a small, nonzero lysogeny frequency (Clifton et al. 2021).

When the effect of antibiotics is included in models, interesting features of the synergy between antibiotics and phages are revealed (Clifton et al. 2019). For instance, it was found that temperate phage induction may act in synergy with antibiotics, even if the bacteria are antibiotic-resistant. In another study that validated a stochastic mathematical model with *Escherichia coli* experiments, two strains of lytic phages and antibiotics were combined with bacteria that were either phage-sensitive or phage-resistant (Tazyman and Hall 2015). The study found that the prevalence of antibiotic-resistant bacteria depends on the relative rates of bacterial adaptation to phage strains and antibiotics. A more recent study (Pourtois et al. 2021) investigated the effect of antibiotics on *P. aeruginosa* infection control, when the bacteria do and do not harbor chronic (filamentous) phages. The modeling study, corroborated with in vitro experiments, found that frequent administration of intermediate doses of antibiotics is optimal for maintaining a bacterial population that is infected with phage, leading to slower bacterial growth.

One of the challenges of phage therapy is its efficacy when phage-resistant bacteria dominate. Hence, the merit in studying the administration of phage cocktails was emphasized in Li et al. (2020). Using optimal control theory to determine the most effective protocol, the study found that cocktails of phage targeting phage-resistant bacteria and phage acting on phage-sensitive bacteria provide the most effective phage therapy. In addition, the study found that the optimal timing of phage dosing is at the beginning of the treatment, echoing the earlier findings of Levin and Bull (1996).

Another modeling challenge is including the role of the immune system. In a study of combination therapy (phages–antibiotics–immune response) in the context of

P. aeruginosa infections (Rodriguez-Gonzalez et al. 2020), it was found that combination therapy is more effective than phage therapy or antibiotic treatment alone. Moreover, when innate immune responses do not exist or are compromised, then phage–antibiotic synergy fails to completely eliminate the infection in most of the models considered.

1.3 Research Questions

This paper investigates the following questions regarding phage–antibiotic synergy in the treatment of human pathogens, such as *P. aeruginosa* and *E. coli*:

1. Previous studies suggest that phage induction in the presence of antibiotic stress results in phage–antibiotic synergy even if bacteria are resistant to antibiotics, assuming that cross-infection by different viral strains is inevitable (Clifton et al. 2019). Given that a bacterium infected by one virus may exclude other viruses (De Smet et al. 2017), we explore if antibiotics and phage still act in synergy if cross-infection does not occur.
2. Previous studies show that a compromised immune system or phage resistance may prevent phage–antibiotic synergy (Rodriguez-Gonzalez et al. 2020; Tazzyman and Hall 2015). We investigate other conditions under which phage–antibiotic synergy may fail when an immune response or phage resistance is not included.
3. Previous studies indicate that the timing and concentration of antibiotic and/or phage dosing influences the efficacy of treatment (Levin and Bull 1996; Li et al. 2020; Clifton et al. 2019). Given that phages are naturally present in bacterial populations (Davies et al. 2016; Roux et al. 2015; Mathee et al. 2008; Mosquera-Rendón et al. 2016; Spencer et al. 2003; Kung et al. 2010) and that antibiotics are typically administered in standard doses at periodic intervals (e.g., once or twice daily) (Geller et al. 2011; Hodson et al. 1987), we study the role of antibiotic dosing frequency on bacterial infection control in the absence of deliberate phage therapy.

This paper uses a simple mathematical model to investigate the role of two competing virus lifestyles, temperate and chronic, on the effectiveness of various antibiotic dosing frequencies. In most cases, we find that as the frequency of antibiotic dosages increases, the bacterial population decreases even if the bacteria are antibiotic-resistant. However, our model reveals that antibiotic treatment counterintuitively *increases* the bacterial load when a large fraction of the bacteria are antibiotic-resistant and both temperate and chronic phages are present in the system.

2 Model

We begin with a model of temperate and chronic virus competition (Clifton et al. 2021), which assumes that two virus types, temperate viruses (V_T) with lytic and latent lytic stages and chronic viruses (V_C) with productive and latent chronic stages, may infect a single strain of bacteria that is initially susceptible (S) to both viral types. See Fig. 1 for a model overview.

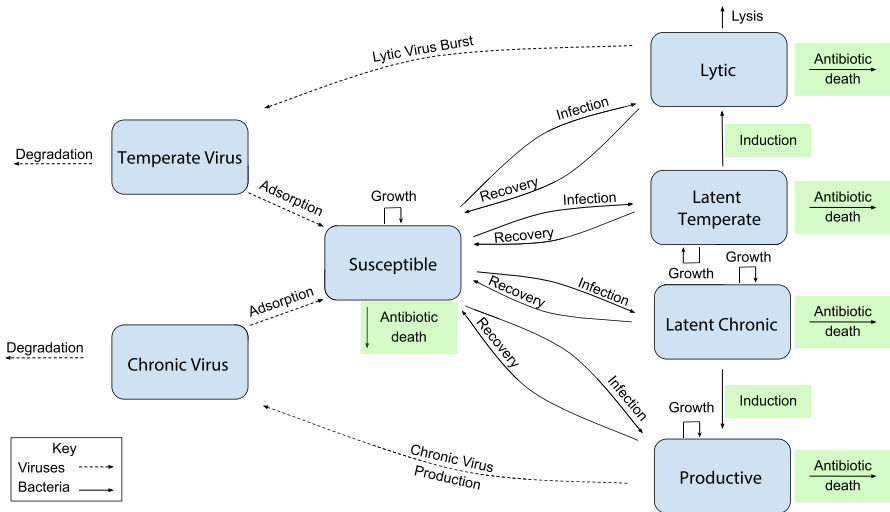


Fig. 1 Compartmental model of bacteria–phage system with temperate and chronic phages. Solid arrows represent bacteria transitions between infection states, and dotted arrows represent virus production or loss. The green boxes illustrate actions which may occur when environmental stressors, such as sublethal antibiotics, are introduced to the system (Color figure online)

The total bacterial population is composed of susceptible, lytic, latent temperate, productive, and latent chronic bacteria. We assume that the total bacterial population grows logistically to a carrying capacity K (Zwietering et al. 1990). The susceptible bacteria grow at an intrinsic rate r_S and can become infected by chronic or temperate viruses.

The susceptible bacteria are infected by the temperate viruses at a rate η_T . When infected, there is a probability f_T that the bacterium enters a latent lytic state in which viruses are not being produced, but the viral DNA is integrated into the bacterium’s genome and passed on to later generations (Harper et al. 2014). The latent lytic bacteria reproduce at a rate r_T and recover from (or evade) infection at a rate γ_T .

Alternatively, there is a probability $(1 - f_T)$ that the bacteria will instead enter the lytic lifestyle following infection. The bacteria in this state die due to lysis (bursting of cell) at a rate δ . When the bacteria lyse, they produce β_T temperate viruses per bacterium. Finally, bacteria can recover from (or evade) infection at a rate γ_l . In both cases of evasion or recovery, the bacteria become susceptible to phage infection once again and do not develop phage resistance.

Similar to temperate infections, the susceptible bacteria may be infected by the chronic viruses at a rate η_C . When infected, there is a probability f_C that a bacterium will enter the latent chronic state in which the bacterium reproduces at a rate r_C and recovers from (or evades) infection at a rate γ_C .

Alternatively, there is a probability $(1 - f_C)$ that the bacteria will instead become productive upon infection, reproducing at a rate r_P while producing viruses at a rate β_C . In contrast with lytic infections, productive infections do not cause cell death. Productive bacteria recover from (or evade) infection at a rate γ_P .

Outside the bacterial cells, free temperate and chronic viruses degrade naturally at a rate μ_T and μ_C , respectively (Heldal and Bratbak 1991). Once a bacterium is infected, we assume it will exclude both superinfection by the same viral type and cross-infection by viruses of other types (De Smet et al. 2017). Although we assume infection rates for temperate and chronic viruses are constant, the infection rates may depend on the bacterial population; many relevant bacteria form biofilms at high density that protect the population from infection (Harper et al. 2014). For simplicity, we have also assumed that lysogen frequencies are constant, but some studies have demonstrated that bacterial density may impact lysogeny rates (Hargreaves et al. 2014; Silpe and Bassler 2018). These simplifications are necessary for the analytic tractability of steady state and bifurcation analysis (see Model Behavior and Analysis section and Clifton et al. 2021).

2.1 Antibiotics

We introduce antibiotics into the system at times t_i ; specifically, these are the times at which the antibiotics become bioavailable, which depends on the mode of delivery. We assume that antibiotic-induced stress spikes at times t_i and decays exponentially at a rate k , consistent with typical antibiotic metabolism in the human system (Naber et al. 1973; Bax et al. 1989; Fish and Chow 1997). The antibiotic concentration within the system is then

$$a(t) = A \sum_{i=1}^N H(t - t_i) e^{-k(t-t_i)}, \quad (1)$$

where t is the current time, t_i are the antibiotic dose times, A is the maximum concentration of one antibiotic dose, N is the total number of antibiotic doses, H is the Heaviside function, and k is the decay rate of antibiotics in the system.

When the system is stressed by antibiotics, there are three major responses. First, antibiotics can induce the production of latent temperate or latent chronic viruses at a rate $\epsilon a(t)$, where $a(t)$ is the antibiotic concentration (Fothergill et al. 2011; Ptashne 1986; Nanda et al. 2015). This response occurs with bacteria that are antibiotic-susceptible or antibiotic-resistant (Fisher et al. 2017; Monack et al. 2004; Stewart and Costerton 2001; James et al. 2012; Redgrave et al. 2014; Valencia et al. 2017; Brazas and Hancock 2005). However, not all classes of antibiotics induce virus production, so this model is only applicable to antibiotics (such as quinolones) known to do so (Zhang et al. 2000; Comeau et al. 2007). To simplify the model, we ignore spontaneous induction because it is a rare phenomenon (Nanda et al. 2015; Garro and Law 1974; Cortes et al. 2019).

Second, antibiotic-susceptible bacteria are killed at a rate monotonically increasing with the antibiotic concentration, typically in the form of a Hill function (Levin and Udekwi 2010; Regoes et al. 2004). For simplicity, we take the antibiotic death rate to be directly proportional to the antibiotic concentration ($\kappa a(t)$), but all qualitative results hold under more realistic functional responses. Antibiotic-resistant bacteria are not killed directly by the antibiotics (Fisher et al. 2017; Monack et al. 2004; Stewart and Costerton 2001).

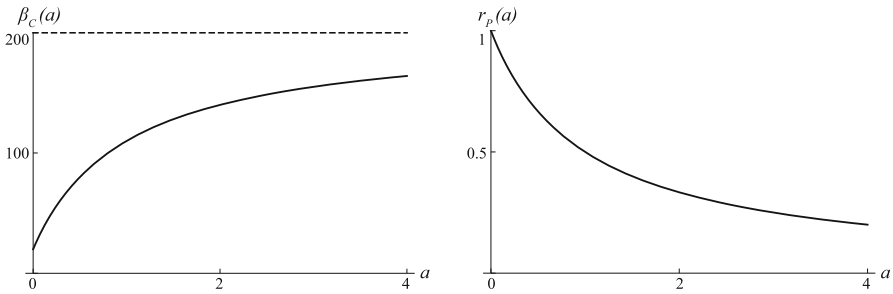


Fig. 2 Functional forms of chronic virus production rates (left) and productive bacteria growth rates (right). With no system stress (no antibiotics), the chronic phage production rate is β_C and the bacterial growth rate is r_p . As stress increases, the phage production rate saturates to β_{\max} and the bacterial growth rate approaches 0

Finally, studies show that productive bacteria stressed by sublethal antibiotics increase viral production while decreasing cell reproduction (Hagens et al. 2006; Secor et al. 2015). We incorporate the increased viral production with a saturating functional response; when there is no antibiotic stress in the system ($a = 0$), viruses are produced at a rate β_C , and as the concentration of antibiotics increases, the viral production rate saturates at β_{\max} (Fig. 2). The functional form of the stress-dependent production rate is

$$\beta_C(a) = \beta_C + \frac{a}{h_\beta + a}(\beta_{\max} - \beta_C), \tag{2}$$

where a is the time-dependent antibiotic concentration, h_b is the concentration at which the production rate is halfway between the minimum and maximum, and β_{\max} is the maximum production rate when stress is maximum.

We also incorporate the decreased cell reproduction as the concentration of antibiotics increases using a saturating functional response. When there are no antibiotics in the system ($a = 0$), the cellular reproduction rate is r_p . As the antibiotic concentration increases, the reproduction rate approaches 0 (Fig. 2). The functional form of the stress-dependent cellular reproduction rate is

$$r_p(a) = r_p \left(1 - \frac{a}{h_r + a} \right), \tag{3}$$

where a is the time-dependent antibiotic concentration and h_r is the concentration at which the reproduction rate is half of the maximum.

The dynamical system capturing the described model behavior is

$$\dot{S} = \underbrace{S(1 - N)}_{\text{growth}} - \underbrace{\eta_T SV_T - \eta_C SV_C}_{\text{infection}} + \underbrace{\gamma_T L_T + \gamma_I I_T + \gamma_P P_C + \gamma_C L_C}_{\text{recovery}} - \underbrace{\kappa a(t) S}_{\text{antibiotic death}} \tag{4}$$

$$\dot{I}_T = \underbrace{\eta_T(1 - f_T)SV_T}_{\text{infection}} - \underbrace{\delta I_T}_{\text{lysis}} - \underbrace{\gamma_I I_T}_{\text{recovery}} + \underbrace{\epsilon a(t)L_T}_{\text{induction}} - \underbrace{\kappa a(t)I_T}_{\text{antibiotic death}} \tag{5}$$

Table 1 Description of model variables in bacteria–virus system (4–10)

Variable	Meaning	Units
S	Susceptible bacteria as a proportion of carrying capacity	Unitless
I_T	Lytic bacteria as a proportion of carrying capacity	Unitless
L_T	Latent lytic bacteria as a proportion of carrying capacity	Unitless
P_C	Productive bacteria as a proportion of carrying capacity	Unitless
L_C	Latent chronic bacteria as a proportion of carrying capacity	Unitless
N	All bacteria ($S + I_T + L_T + P_C + L_C$)	Unitless
V_T	Ratio of free temperate viruses to bacteria carrying capacity	$\frac{\text{PFU}}{\text{CFU}}$
V_C	Ratio of free chronic viruses to bacteria carrying capacity	$\frac{\text{PFU}}{\text{CFU}}$
t	Time rescaled by intrinsic growth rate	Unitless

In the system, all population densities are rescaled to be proportional to the carrying capacity and the timescale is rescaled such that all rates are relative to the susceptible bacteria’s growth rate

$$\dot{L}_T = \underbrace{r_T L_T (1 - N)}_{\text{growth}} + \underbrace{\eta_T f_T S V_T}_{\text{infection}} - \underbrace{\gamma_T L_T}_{\text{recovery}} - \underbrace{\epsilon a(t) L_T}_{\text{induction}} - \underbrace{\kappa a(t) L_T}_{\text{antibiotic death}} \tag{6}$$

$$\dot{P}_C = \underbrace{r_p(a) P_C (1 - N)}_{\text{growth}} + \underbrace{(1 - f_C) \eta_C S V_C}_{\text{infection}} - \underbrace{\gamma_P P_C}_{\text{recovery}} + \underbrace{\epsilon a(t) L_C}_{\text{induction}} - \underbrace{\kappa a(t) P_C}_{\text{antibiotic death}} \tag{7}$$

$$\dot{L}_C = \underbrace{r_C L_C (1 - N)}_{\text{growth}} + \underbrace{f_C \eta_C S V_C}_{\text{infection}} - \underbrace{\gamma_C L_C}_{\text{recovery}} - \underbrace{\epsilon a(t) L_C}_{\text{induction}} - \underbrace{\kappa a(t) L_C}_{\text{antibiotic death}} \tag{8}$$

$$\dot{V}_T = \underbrace{\beta_T \delta I_T}_{\text{burst}} - \underbrace{\eta_T S V_T}_{\text{adsorption}} - \underbrace{\mu_T V_T}_{\text{degradation}} \tag{9}$$

$$\dot{V}_C = \underbrace{\beta_C(a) P_C}_{\text{production}} - \underbrace{\eta_C S V_C}_{\text{adsorption}} - \underbrace{\mu_C V_C}_{\text{degradation}} \tag{10}$$

In the model system (4–10), we rescale all population densities to be proportional to the bacterial carrying capacity, and we rescale the timescale such that all rates are relative to the susceptible bacteria’s growth rate. See Supplemental Materials for the rescaling process. All model variables are listed in Table 1, and all model parameters are listed in Table 2.

2.2 Model Behavior and Analysis

When antibiotics are not introduced to the system, the model exhibits four steady states: coexistence (all populations exceed 0), temperate strategy only ($V_C = P_C = L_C = 0$), chronic strategy only ($V_T = I_T = L_T = 0$), and susceptible only (all populations, except S , are 0) (Clifton et al. 2021). Transitions between steady states occur via transcritical bifurcations as the lysogeny fractions f_T and f_C vary (Clifton et al. 2021). All model parameters are selected from the literature for

Table 2 Description of model parameters in bacteria–virus system (4–10)

Parameter	Meaning	Range ^a	Baseline	Sources
r_T, r_C	Growth rates of (respectively) latent lytic and latent chronic bacteria, relative to the susceptible bacteria growth rate ^b	[0.5, 3] ^c	1	Shapiro et al. (2016)
$r_P(a)$	Growth rate of productive bacteria, a decreasing function of the antibiotic concentration $a(t)$	[0.5, 3] ^c	1	Shapiro et al. (2016)
η_T, η_C	Infection rate of (respectively) temperate and chronic viruses	[0.38, 14.7] ^d	1	Sinha et al. (2017)
$\gamma_T, \gamma_P, \gamma_C$	Recovery/evasion rates of (respectively) latent lytic, productive, and latent chronic bacteria	[0, 1000] ^e	0.67 ^f	Brüssow et al. (2004) and Horvath and Barrangou (2010)
γ_I	Recovery/evasion rates of lytic bacteria	[0, 1000]	0 ^g	Brüssow et al. (2004) and Horvath and Barrangou (2010)
δ	Rate at which lytic infection leads to bursting	[1.5, 7.8] ^h	4	Yu et al. (2017) and El Didamony et al. (2015)
f_T	Lysogen frequency for temperate viruses	[0, 0.9]	0.01	Calendar (2006), Oppenheim and Adhya (2007) and Volkova et al. (2014)
f_C	Lysogen frequency for chronic viruses	[0, 0.9]	0 ^f	Calendar (2006), Oppenheim and Adhya (2007), Volkova et al. (2014) and Clifton et al. (2021)
β_T	Burst size for bacteria infected with V_T	[10, 1000]	100	Yu et al. (2017), El Didamony et al. (2015), Latino et al. (2014), Schrader et al. (1997), Ceyssens et al. (2010), Garbe et al. (2011) and You et al. (2002)
$\beta_C(a)$	Viral production rate for bacteria infected with V_C , an increasing function of antibiotic concentration $a(t)$	[5, 200]	20	Clifton et al. (2019)
μ_T, μ_C	Degradation rate of (respectively) free temperate viruses and free chronic viruses	[0.9, 3.6] ⁱ	1	Heldal and Bratbak (1991)
κ	Death rate per concentration of antibiotics	[0, 3.5]	1	Spalding et al. (2018) and Grillon et al. (2016a)
ϵ	Lysis rate per concentration of antibiotics	[0, 2]	1	Spalding et al. (2018) and Grillon et al. (2016a)

Table 2 continued

Parameter	Meaning	Range ^a	Baseline	Sources
A	Maximum concentration of one standard antibiotic dose (μg/mL)	[0, 10] ^j	1	Grillon et al. (2016b) and Fong et al. (1986)
k	Metabolic decay rate of antibiotic within the system	[1e-3, 0.6] ^k	0.3	Spalding et al. (2018), Zhanel et al. (2006) and Wingender et al. (1984)
N	Number of standard antibiotic doses	-	-	-
h_{β}, h_r	Stress level at which growth rate is half the maximum	-	1	-

^aAll parameter ranges are taken for the human pathogens *P. aeruginosa* or *E. coli* and their viruses, unless otherwise noted

^bGrowth rate is approximately $5.1e-3 \text{ min}^{-1}$ for *P. aeruginosa* grown in vitro (Spalding et al. 2018), but is highly variable in human hosts (Kopf et al. 2016)

^cEstimates based on *E. coli* and M13 phage

^dEstimates based on *E. coli* and λ phage

^eA wide range of evasion and recovery rates has been found, depending on the mechanism. We average over all intracellular mechanisms that do not result in phage resistance

^festimated from viral steady state density in Clifton et al. (2021)

^gselected to be 0 to simplify model analysis; allowing $\gamma_I = \gamma_T$ produces qualitatively similar results, so the increased model complexity is not justified

^hLow estimate is for PAXYB1 phage and PAO1 host, high estimate is for φ PSZ1 phage and PAO1 host

ⁱLow estimate is for viruses extracted from Raunefjorden, high estimate is for viruses extracted from Bergen Harbor (strains unknown)

^jMaximum blood serum concentration in human subjects for standard doses of ciprofloxacin and levofloxacin

^kAntibiotic is levofloxacin

the human pathogens *P. aeruginosa* or *E. coli* and their viruses, where possible (see Supplemental Materials for technical details). No parameters are fitted in this study. For the baseline parameters in Table 2, the coexistence state is stable for any initial condition, which is consistent with the observation that viruses of both lifestyles are present in natural and clinical environments (Mosquera-Rendón et al. 2016; Spencer et al. 2003; Kung et al. 2010; James et al. 2015).

3 Results

Because patients infected with dangerous bacterial infections, particularly *P. aeruginosa*, are typically treated with antibiotics at the time of bacterial detection (Højby et al. 2015, 2017), we investigate the effects of antibiotic administration on the bacteria–phage ecosystem. Specifically, we measure the time-averaged bacterial population level at steady state as a function of the frequency of antibiotic administration for the baseline parameters in Table 2 (Fig. 3). We explore both the scenario in which all bacteria are susceptible to antibiotics and the scenario in which all bacteria are resistant to antibiotics ($\kappa = 0$) for the duration of the numerical experiment. In each of those scenarios, we investigate the impact of each virus lifestyle on the bacterial population by simulating four cases: (1) no viruses are present, (2) only temperate viruses are present, (3) only chronic viruses are present, and (4) both viruses are present.

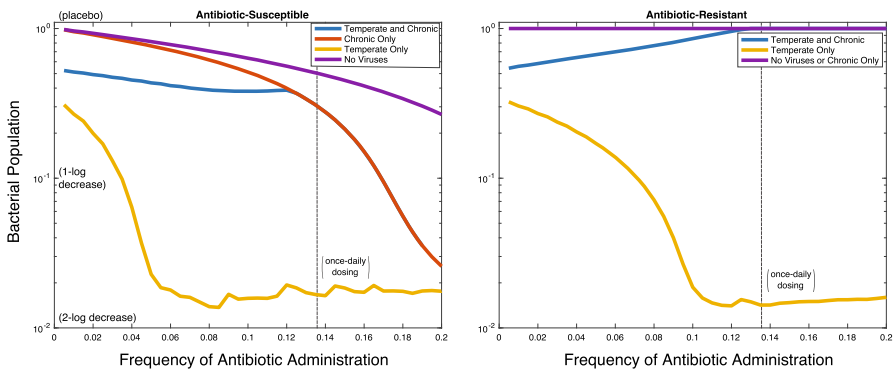


Fig. 3 Simulation of the effect of antibiotic administration frequency on the time-averaged bacterial population at steady state when infected with various bacteriophages in the human host when, for the duration of the numerical simulation, all bacteria are susceptible to antibiotics (left) or all bacteria are resistant to antibiotics (right). Time is scaled by the growth rate of *P. aeruginosa* ($5.1e-3 \text{ min}^{-1}$), so 0.136 in unitless frequency units is approximately one dose per 24 h. In the system, all population densities are rescaled to be proportional to the carrying capacity. Thus, the “placebo” condition (no antibiotic treatment) is the bacterial population at carrying capacity (1 in unitless density) (Color figure online)

3.1 Phage–Antibiotic Synergy is Predicted When Bacteria are Susceptible to Antibiotics

Figure 3 illustrates that regardless of the antibiotic frequency, the bacterial population is largest when not infected with viruses. When the SOS response is triggered in bacteria without free phages or proviruses, no lysis from rapid phage production occurs. Instead, the bacteria only die from antibiotics.

When chronic phages are present, the average bacterial population decreases more as antibiotic frequency increases because antibiotic stress reduces the bacterial growth rate. The presence of both temperate and chronic viruses has an even larger negative impact on the bacterial population than when only chronic viruses are present, at least when the frequency of antibiotics is below 0.12 (approximately once per 8 bacterial cell divisions). When no antibiotics are present, the chronic bacterial population reaches carrying capacity because the bacteria are not lysing or being killed by antibiotics. However, when chronic and temperate viruses are present with no antibiotics, the temperate viruses can still lyse the bacteria. This accounts for the difference in bacterial population when frequency of antibiotic administration is zero. The two populations converge when the antibiotic frequency is greater than 0.12 because chronic viruses out-compete temperate viruses due to the increase in stress-induced chronic virus production rate.

Antibiotics have the largest effect on the bacterial population infected with only temperate viruses. When infected only with temperate viruses, the bacterial population substantially decreases as the frequency of antibiotics increases because latent bacteria are being induced by antibiotics into the lytic lifestyle (killing the cells). The apparent noise in population levels when the frequency is greater than 0.06 (approximately once every 17 bacterial cell divisions) is likely due to pseudo-chaos in the system. Studies show that chaos is commonly seen in periodically forced predator-prey models (Tang and Chen 2003; Taylor et al. 2013). Further exploration of this phenomenon is beyond the scope of this paper.

3.2 Phage–Antibiotic Antagonism is Possible When Bacteria are Resistant to Antibiotics

When no phages are present in the system or when only chronic viruses are present in the system, antibiotic-resistant bacterial populations remain at carrying capacity as the frequency of antibiotics increases. The bacterial population reaches carrying capacity when no viruses are present because there exists no mechanism (antibiotic or virus) to kill the bacteria. The bacterial population also reaches carrying capacity rapidly when only chronic viruses are present because chronic viruses do not kill their host in order to reproduce; although chronic infection slows bacterial reproduction, the effect is negligible near steady state.

The behaviors of the antibiotic-resistant bacterial population change when both viruses are in the system and when only temperate viruses are in the system. The bacterial population infected by only temperate viruses decreases as the frequency of antibiotics increases. Decreasing at an increasing rate, we see that the bacterial

population levels off at a frequency of 0.1 (approximately once every 10 bacterial cell divisions). This phenomenon occurs because, as the frequency of antibiotics increases, more latent temperate viruses are induced into the lytic cycle. The dynamics of the antibiotic-resistant bacteria infected with temperate phages are similar to when the bacteria are antibiotic-susceptible; however, when the bacteria are antibiotic-resistant, it requires a higher frequency of antibiotics to substantially decrease the bacterial population.

Contrary to expectations, as antibiotic frequency increases, the antibiotic-resistant bacterial population infected with both temperate and chronic viruses increases. At a frequency of roughly 0.13 (approximately once per 7 bacterial cell divisions), the bacterial population infected with temperate and chronic viruses reaches carrying capacity. While antibiotics are not directly killing the antibiotic-resistant bacteria through the intended mechanisms, they cause the induction of the SOS response in bacteria. This induction triggers the production of chronic viruses and lysis of temperate infected bacteria. At higher antibiotic frequencies, all temperate infected bacteria eventually lyse, leaving only bacteria that have been infected by chronic viruses. Chronic viruses, having out-competed temperate viruses (a natural predator of bacteria), leave bacteria invulnerable to control. This phenomenon has clinical implications in that, contrary to the intended outcome, antibiotics can counterintuitively increase the bacterial population.

3.3 Sensitivity Analysis

While we have used *P. aeruginosa* infections as a case study, this model is theoretically applicable to any ecosystem with bacteria, temperate viruses, and chronic viruses. Therefore, we conduct a sensitivity and uncertainty analysis to understand the impact of each model parameter on the clinical outcome of interest: bacteria population density. Because the relationships between the parameters and the bacterial population size are generally nonlinear but monotonic, we use Latin Hypercube Sampling (LHS) with $n = 500$ samples of parameter space and partial rank correlation coefficients (PRCC). Following Marino et al. (2008), the sensitivity analysis indicates that the parameters κ , T , β_{\max} , and $\eta = \eta_T = \eta_C$ have a significant ($p < 0.05$) impact on the bacterial population (Fig. 4). The antibiotic death rate (κ) has the largest magnitude PRCC, indicating that a small increase in κ will result in the largest decrease in the bacterial population. The period of antibiotic administration (T), the infection rate $\eta = \eta_T = \eta_C$ (which we assume for simplicity are equal for viral lifestyles), and the maximum chronic virus production rate β_{\max} also have a negative correlation with the bacterial population size.

For any particular bacteria–phage–antibiotic system, the parameters κ , T , and η are likely well characterized. It is less likely that β_{\max} will be well characterized because chronic viruses have not been studied as extensively as lytic and temperate viruses. We wish to understand the impact of β_{\max} on the bacterial population when the population is antibiotic-resistant and infected with both temperate and chronic viruses; this scenario is the most common for patients infected with *P. aeruginosa* (James et al. 2015; Burgener et al. 2019; Geller et al. 2011). Figure 5 shows that

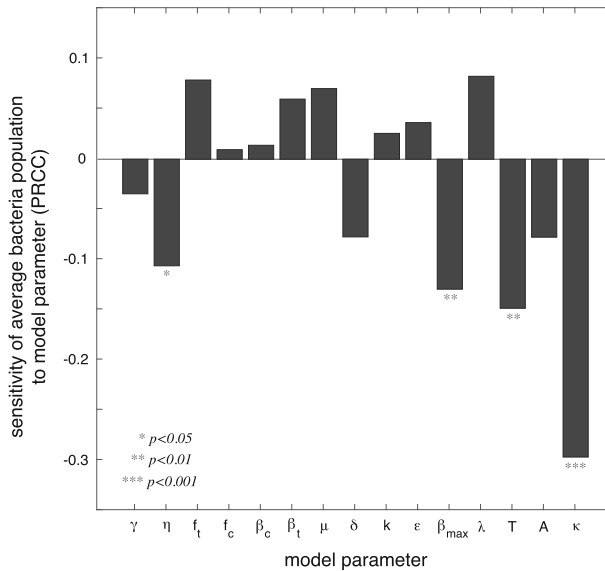


Fig. 4 Sensitivity of parameters on the average bacteria population. The sensitivity analysis uses Latin Hypercube Sampling (LHS) of parameter space and partial rank correlation coefficients (PRCC) (Marino et al. 2008). All parameter values are taken near the baselines in Table 2. Initially, $S(0) = 1e-3$, $V_T(0) = V_C(0) = 1e-7$. Asterisks indicate significance with $n = 500$ simulations. See Supplemental Materials for technical details

as β_{max} decreases, the slope of bacterial population as a function of frequency of antibiotic decreases until it ultimately becomes negative around $\beta_{max} = 70$. This shows that virus competition is only detrimental to infection control if chronic virus production is sufficiently increased by antibiotic stress.

4 Discussion

Previous modeling efforts have shown that phage induction in the presence of antibiotic stress results in phage–antibiotic synergy even if bacteria are resistant to antibiotics, assuming that cross-infection by different viral strains occurs (Clifton et al. 2019). In our study, we instead include the fact that bacteria infected by one virus type may exclude other viruses (De Smet et al. 2017), and we find that phage–antibiotic synergy is no longer inevitable. In Clifton et al. (2019), all bacteria are eventually infected by both temperate and chronic viruses due to cross-infection, effectively eliminating true competition between the viral lifestyles. When true viral competition is introduced, chronic viruses thrive in an environment with antibiotic treatment of an antibiotic-resistant bacterial population; antibiotics clear bacteria infected with latent temperate viruses via induction, allowing bacteria with chronic viral infections to fill the void.

Interestingly, the failure of phage–antibiotic synergy is not necessarily due to a host’s compromised immune system or phage resistance, as shown in other studies (Rodriguez-Gonzalez et al. 2020; Tazzyman and Hall 2015). Without including

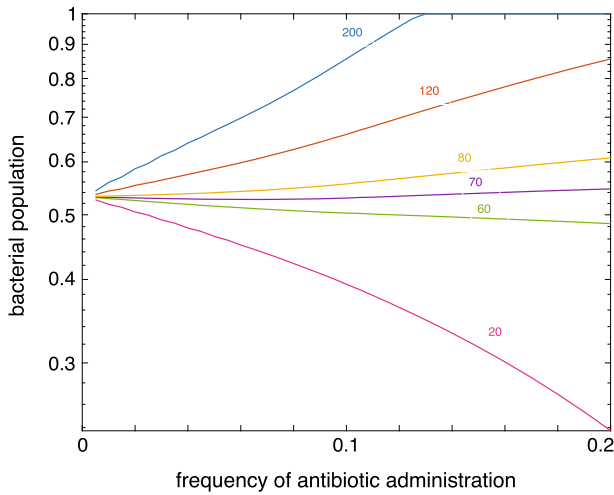


Fig. 5 Impact of β_{\max} (value labeled on curves) on a resistant bacterial population when bacteria exist in an environment with temperate and chronic phages. As β_{\max} decreases, the effect of antibiotic frequency on bacterial population flips from a positive slope (i.e., phage–antibiotic antagonism) to a negative slope (i.e., phage–antibiotic synergy) (Color figure online)

modeling complexities, such as stochasticity, phage resistance, evolutionary adaptation, or an immune response, we find that the ecological forces of viral competition alone can compromise phage–antibiotic synergy.

Finally, although phage therapy is being studied as a possible treatment for recalcitrant bacterial infections (Kortright et al. 2019; Doss et al. 2017), antibiotic treatment continues to be the standard of care (Geller et al. 2011; Hodson et al. 1987). Given that phages are naturally present in bacterial populations (Davies et al. 2016; Roux et al. 2015; Mathee et al. 2008; Mosquera-Rendón et al. 2016; Spencer et al. 2003; Kung et al. 2010), we find that phages play an important role in antibiotic treatment even without deliberate phage therapy.

4.1 Medical Implications

The implications of our model can theoretically be applied to any bacterial infection with naturally present temperate and chronic viruses, but the results presented in this paper focus on treatment of *P. aeruginosa* infections in patients with cystic fibrosis. Clinical trials of antibiotics confirm that both temperate and chronic viral lifestyles are present in the lungs of most patients with cystic fibrosis (James et al. 2015; Burgener et al. 2019), and around 60% of *P. aeruginosa* in sputum samples are antibiotic-resistant (Geller et al. 2011). Despite the rise of antibiotic-resistant bacterial infections, antibiotics remain a common treatment for patients with cystic fibrosis (Proesmans et al. 2012). Unfortunately, our model suggests that antibiotic treatment may be counterproductive under the most likely treatment conditions. These results highlight the need for personalized medicine approaches for treating antibiotic-resistant bacterial infection which respect, or even exploit, the underlying ecology within the patient.

4.2 Limitations and Future Steps

Our conclusions are limited by assumptions made in order to create a simple, analytically tractable model. First we assume that the antibiotics trigger an SOS response in a bacterium which induces the production of latent temperate and latent chronic viruses (Fothergill et al. 2011; Ptashne 1986; Nanda et al. 2015). Not all antibiotics induce phage via the SOS response (Fothergill et al. 2011), so we focus only on the types of antibiotics known to do so (e.g., quinolones like levofloxacin and ciprofloxacin) (Zhang et al. 2000; Comeau et al. 2007). We assume that even antibiotic-resistant bacteria induce viruses in the presence of antibiotics, which has been demonstrated for several classes of antibiotics (Redgrave et al. 2014; Valencia et al. 2017; Brazas and Hancock 2005; James et al. 2012; Fothergill et al. 2011). Thus, the model is applicable to antibiotics known to trigger an SOS response. This is not a major limitation as treatment of *P. aeruginosa* infections often include quinolone antibiotics (Geller et al. 2011; Hodson et al. 1987).

The model also assumes mass action infection of bacteria by phage, but this assumption breaks down at large bacterial densities. Bacteria like *P. aeruginosa* are known to form biofilms at high densities, which effectively saturates the infection rate (Harper et al. 2014). Previous studies have used a Hollings Type II functional response to model the infection rate, and the results are not qualitatively different (Clifton et al. 2019). However, a more sophisticated model would include biofilm formation in the infection assumptions. Antibiotic effectiveness is also known to saturate as the antibiotic concentration increases, often following a Hill function (Levin and Udekwu 2010; Regoes et al. 2004). We ignore this phenomenon for simplicity, but a more directly applicable model would include a realistic antibiotic functional response.

Another limitation of our model is that we do not consider multiplicity of infection, in which multiple infections by the same strain increases the severity of an infection. This is not a significant limitation because when infected with phages, *P. aeruginosa* produces superinfection exclusion proteins which prevent multiple infections by the same phage type (James et al. 2012; Heo et al. 2007).

From the sensitivity analysis, we know that parameters κ , T , β_{\max} , and η have the largest impact on the accuracy of our model. While T (period of antibiotic dosing) is determined by the clinician and κ and η are generally well-characterized for a broad range of phages and antibiotics, β_{\max} has not been definitively estimated experimentally. Our analysis illustrates the importance of having a precise measurement of β_{\max} (Fig. 5). Further experiments are needed to determine a precise value of β_{\max} , which would allow the model to be more readily applicable in a clinical setting.

Finally, our model does not include two important spacial and temporal features important to phage–antibiotic synergy: (1) bacteria and phage do not typically exist in well-mixed environments, and (2) bacteria and viruses evolve over time. Using an individual-based model of the combined effects of antibiotics and phages interacting on a two-dimensional grid (Sousa and Rocha 2019), it was discovered that structured environments (such as bacterial plaques in Sinha et al. 2018) may increase the risk of resistance to both antibiotics and phages. The study also incorporates evolution and co-evolution and finds that antibiotic-sensitive bacteria eventually become extinct (Sousa

and Rocha 2019). Because our model does not incorporate evolutionary dynamics, such as the development of antibiotic or phage resistance, our results are only applicable on short time scales. Our model could serve as the base of a more sophisticated model that includes both ecological and evolutionary dynamics.

5 Conclusion

This model demonstrates the potential for an antagonistic effect of virus competition on antibiotic treatment of resistant bacterial infections. Both temperate and chronic virus lifestyles are often naturally present in human hosts, and antibiotic resistance is a growing concern. Because certain combinations of phages and antibiotics can exhibit a synergistic effect when treating bacterial infections, this work suggests that a more personalized medicine approach may provide better clinical outcomes. Treatment plans tailored to the underlying bacteria–phage ecology that exists within human hosts are critical to controlling bacterial infections, especially when the bacteria are resistant to antibiotics.

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Data Availability All software (MATLAB .m files) are publicly available via the Illinois Data Bank (https://doi.org/10.13012/B2IDB-9460305_V1).

Declarations

Competing interests The authors declare no competing interests.

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