



Lying in Wait: Modeling the Control of Bacterial Infections via Antibiotic-Induced Proviruses

Sara M. Clifton,^a Ted Kim,^b Jayadevi H. Chandrashekhar,^b George A. O'Toole,^c Zoi Rapti,^{a,d} Rachel J. Whitaker^{b,d}

^aDepartment of Mathematics, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

^bDepartment of Microbiology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

^cDepartment of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, New Hampshire, USA

^dCarl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

ABSTRACT Most bacteria and archaea are infected by latent viruses that change their physiology and responses to environmental stress. We use a population model of the bacterium-phage relationship to examine the role that latent phage play in the bacterial population over time in response to antibiotic treatment. We demonstrate that the stress induced by antibiotic administration, even if bacteria are resistant to killing by antibiotics, is sufficient to control the infection under certain conditions. This work expands the breadth of understanding of phage-antibiotic synergy to include both temperate and chronic viruses persisting in their latent form in bacterial populations.

IMPORTANCE Antibiotic resistance is a growing concern for management of common bacterial infections. Here, we show that antibiotics can be effective at subinhibitory levels when bacteria carry latent phage. Our findings suggest that specific treatment strategies based on the identification of latent viruses in individual bacterial strains may be an effective personalized medicine approach to antibiotic stewardship.

KEYWORDS bacteria, bacteriophage, temperate, phage, chronic, latent, lytic, lysogenic, *Pseudomonas aeruginosa*, cystic fibrosis, resistance, population dynamics, mathematical model, antibiotic resistance, latent infection, mathematical modeling

A worldwide growth of antibiotic resistance threatens the efficacy of antibiotic treatments for common infections, driving medical professionals to seek alternative treatments (1). Infections by *Pseudomonas aeruginosa* alone represent about 10% of nosocomial infections, are a leading cause of death among patients with cystic fibrosis (CF), and have been deemed a serious threat on the United States Centers for Disease Control and Prevention watch list for antibiotic resistance (2–4). Despite the increasing trend of multidrug resistance, antibiotic regimes remain the consensus first treatment for *P. aeruginosa* infection (5). As a last resort and as an attempt to prevent the evolution of resistance in *P. aeruginosa*, clinicians have turned to combination therapies (6) with bacteriophage (viruses) and antibiotics to treat recalcitrant bacteria.

Synergy between phage and antibiotic treatment (PAS) is now rising in interest for treatment of *P. aeruginosa* and other recalcitrant bacteria (7–9). Combination phage therapy uses viruses that kill bacteria (often in phage cocktails) and different types of antibiotics either at the same time or in series to clear bacteria and prevent the evolution of new resistant phenotypes (10–18). Although preexisting proviruses are highly prevalent in *P. aeruginosa* infections and appear to be induced by certain antibiotic treatments, synergy has not been considered in the context of temperate virus induction. Here, we investigate the role that phages play during antibiotic treatment when they are already present in the system. We show that, even without

Citation Clifton SM, Kim T, Chandrashekhar JH, O'Toole GA, Rapti Z, Whitaker RJ. 2019. Lying in wait: modeling the control of bacterial infections via antibiotic-induced proviruses. *mSystems* 4:e00221-19. <https://doi.org/10.1128/mSystems.00221-19>.

Editor Katrine L. Whiteson, University of California, Irvine

Copyright © 2019 Clifton et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Sara M. Clifton, smc567@illinois.edu.

Received 1 April 2019

Accepted 30 August 2019

Published 1 October 2019

deliberate phage therapy, phages may play a critical role in antibiotic treatment, especially if the bacteria are antibiotic resistant.

Background. Bacteriophages are viruses that infect bacteria and hijack cell functions in order to reproduce. Just as bacteria have evolved many strategies to evade infection, phages have developed multiple strategies to circumvent cell defenses. Phages can be characterized by their lifestyles (obligately lytic, temperate, or chronic) within the host (19). Lytic viruses replicate within the host and kill host cells by bursting them open to release new particles. Temperate viruses have a lytic cycle but can also integrate into host genomes, where they remain latent until they are induced to replicate (19). In chronic infection, productive host cells shed new phages that bud from the cell without killing the bacterium (20). Both temperate and chronic viruses have a lysogenic (latent lytic or latent chronic) cycle in which phage DNA is incorporated into the bacterium's genome, and the cell transmits the phage's genetic material (prophage) to daughter cells vertically (21).

Comparative genomics among closely related bacterial strains has uncovered a plethora of proviruses of both temperate and chronic lifestyles (22–24). The large genome of the opportunistic pathogen *P. aeruginosa* is no exception (25–27). Each sequenced strain reveals multiple proviral genomes of both the temperate and chronic lifestyles, each in both active and inactive (latent) forms (28). These proviruses change bacterial fitness and environmental response, sometimes conferring competitive advantage, virulence, and antibiotic resistance (29–32).

Stressful environmental conditions (e.g., radiation, heat, and sublethal antibiotics) may trigger the cell to induce latent prophage and begin phage production (33–37). The induction of such latent phages is proposed to be one of the mechanisms behind the synergistic effect of antibiotics and phage infection (37, 38). The environmental conditions, especially dynamic antibiotic dosing regimes, under which these phage types may coexist are not well understood. We therefore develop a population model to understand the impact of antibiotics on the bacterium-phage system with multiple phage strategies and antibiotic resistance. We address conditions under which the bacterium-phage-antibiotic ecosystem results in control of the bacterial infection (14).

Previous work. Many mathematical models of bacterium-phage systems exist at various levels of complexity. The simplest models include only one phage strategy (lysis); in this simple scenario, either all bacteria are affected by the phage (39) or some bacteria are resistant to infection (40). More complex models study the competition between two different phage strategies, such as lysis and lysogeny (41) or lysis and productive chronic infection (42). The scope of many studies is extended to also include interactions among bacteria, phages, the host's immune response, and/or antibiotic treatment. The immune response and antibiotic agent have been modeled implicitly by modifying the rates of change of bacteria and phages (40) or explicitly by adding compartments governing antibiotic and immune response rate of change (43–45).

Other distinctions among models of bacterial infections can be made based on how bacteria reproduce. Mechanistic models incorporate a limited nutrient as an additional compartment (45–47), while more phenomenological models assume that bacteria grow logistically (39, 41, 48, 49). Furthermore, many models are used to study bacterial evolution of resistance to either phages (45, 47) or antibiotics (50). These models are either deterministic (47) or stochastic (45, 50).

Phage and antibiotic synergy has been investigated experimentally using phage isolated from wastewater or other sources. Attention has primarily been paid to the breadth of killing that lytic phage exhibit on a diversity of *P. aeruginosa* strains, while little attention has been given to other parts of the phage lifestyle. Accordingly, models for phage-antibiotic synergy incorporate only the killing aspects of viruses (14). These models suggest that pretreatment with phage decreases the bacteria to a low-enough level that antibiotics can extinguish bacterial populations; they do not yet consider potential for phage to spread within a population and be induced by antibiotic treatment at a later time.

Consideration has been given to the impact of antibiotic treatment on the mobilization of temperate phage genetic material (including antibiotic resistance genes) between cells via transduction (51, 52). However, to our knowledge, no mathematical models of bacterium-phage interaction have analyzed the competition between temperate and chronic phage strategies in an environment with pulses of antibiotic stress, as would happen during treatment. Filling this knowledge gap is critical to understanding the impact of antibiotic treatment on a patient infected with the bacterium *P. aeruginosa*.

RESULTS

First, we examine the model without antibiotic administration. Without external stress, the bacterial population eventually stabilizes at carrying capacity, with doubly infected productive bacteria dominating the population (Fig. 1). Because we have assumed that infection by one phage type does not prevent infection by a different type (i.e., no cross-infection exclusion) and that coinfection does not impose a fitness cost on bacteria, eventually all bacteria are infected with both phages.

Productive bacteria dominate the population because, initially, populations of bacteria latently infected with temperate phage increase faster than those latently infected with chronic phage due to the early rapid proliferation of temperate phage. Subsequently the productive strains dominate since they are formed at a much higher frequency on secondary infection than either latent infection. With a substantial population of chronically infected bacteria producing phage at steady state, the ratio of free chronic phage to bacteria stabilizes at approximately 10:1. Although little is known about the proportion of phage types seen in either clinical or wild settings, it is known that both temperate and chronic strains are often found in the same environment (53). Figure 2 shows a visualization of the dominant path through the model system without antibiotics.

Antibiotic treatment. Next, we examine the model where all bacteria are sensitive to antibiotics (i.e., bacteria are not resistant to the antibiotic's intended killing mechanism, namely, inhibiting bacterial DNA replication [54]) using baseline parameter values (see Table 2). For the purpose of illustration, we choose the period of antibiotic treatment $T = 7.3$, which is one antibiotic dose every 24 h; this is a typical clinical dosing protocol (55). When all bacteria are sensitive to antibiotics, periodic administration of antibiotic leads to periodic dips in bacterial populations and periodic spikes in induced free phage (Fig. 3). During antibiotic treatment, the total bacterial population remains well below the carrying capacity, and the ratio of free phage to bacteria is around 20:1 on average and about 30:1 at most. These values are consistent with existing studies of bacterium-to-phage ratios (28, 56).

Figure 1 shows that without antibiotic administration, productive bacteria that are latently carrying the temperate phage are the dominant bacterial strain due to their high frequency of formation in early stages. With each antibiotic dose, the productive bacteria are replaced with strains doubly infected by latent phage, which eventually dominate the system (Fig. 3). This phenomenon occurs because most bacteria that are latently infected with temperate virus (including $P_{CT}^{(T)}$) respond to antibiotic stress by inducing lysis, which brings the number of bacteria to a very low number. The drop in bacterial population allows the doubly latently infected bacteria (unencumbered by phage production) to grow slightly faster than productive bacteria and eventually dominate the population. Antibiotic administration resets the population structure from one set by initial relative frequencies of latent and active infection to one that is set by relative fitness (growth rate). The number of free chronic phage decreases over time because latently infected strains cannot become productive in this model.

To control an infection, there are two primary parameters that can be independently varied: antibiotic administration period T and antibiotic efficacy κ . The antibiotic dosing period and deadliness required to control an infection depend on other model parameters, especially the amplitude of stress caused by antibiotics and the metabolic decay rate of the antibiotic (Fig. 4). Antibiotics must be administered more frequently if

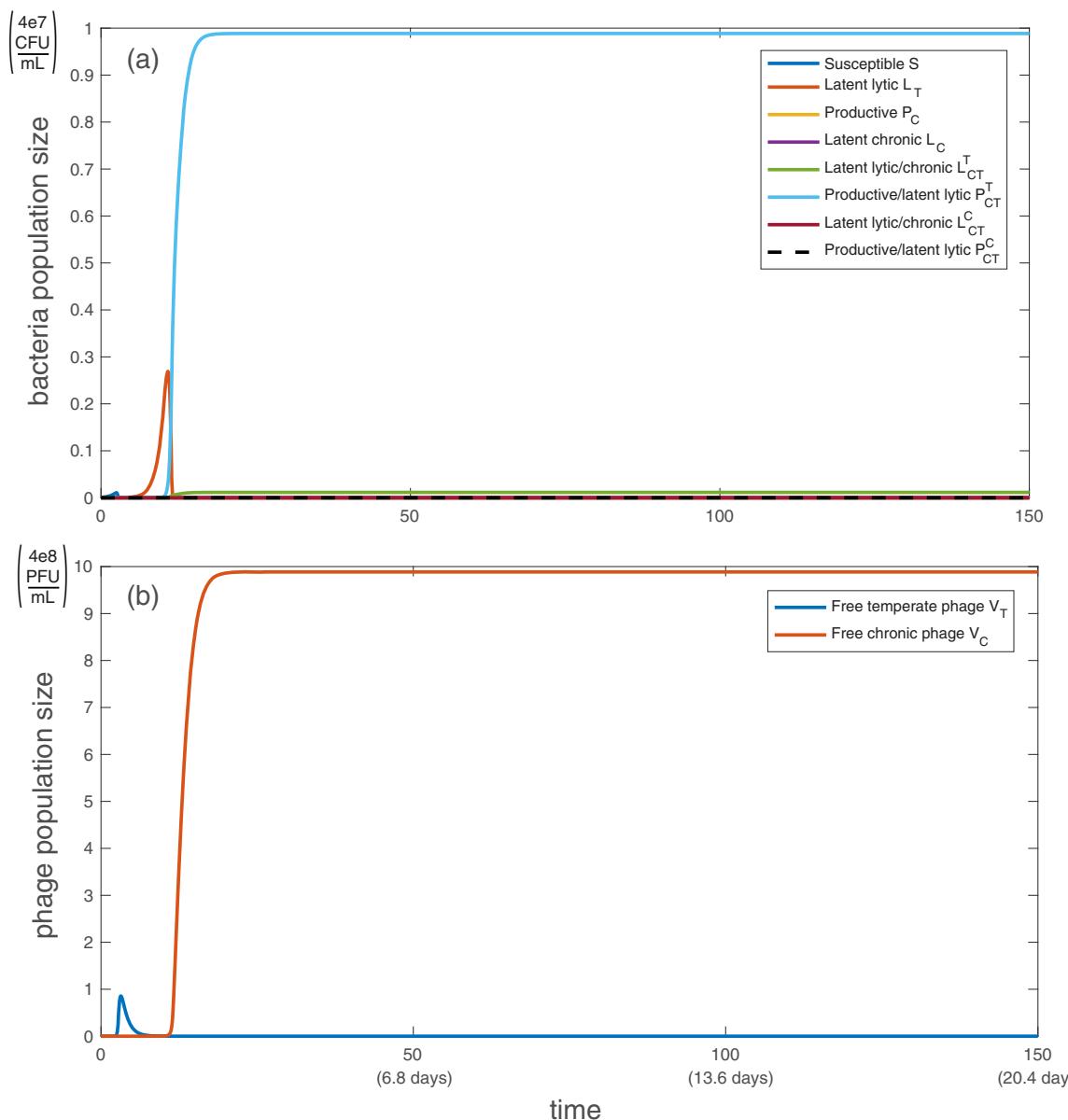


FIG 1 Simulation of population dynamics with no antibiotic administration: bacterial population (a) and free phage population (b). Without antibiotics, the dominant bacterial strain is producing chronic virus while also latently infected with temperate phage (P_{CT}^T), and the only free phage are chronic (V_C). All bacteria and phage types are described in Table 1. All parameter values are taken from the baselines in Table 2, with $h_\eta = 1/2$, $h_\beta = 1$, $h_\gamma = 1$. Note that both axes are linear, not logarithmic. Initially, $S(0) = 1e-3$, $V_T(0) = V_C(0) = 1e-7$, according to the work of Sinha et al. (41).

antibiotics are less effective at killing bacteria either directly or via induced lysis, or if antibiotics are metabolized more quickly (Fig. 4a). On the other hand, antibiotics must be more effective in order to control an infection if antibiotics are administered less frequently, if antibiotic stress induces lysis less effectively, or if antibiotics are metabolized more quickly (Fig. 4b). See Text S2 in the supplemental material for technical details on the sensitivity analysis.

Antibiotic resistance. If all bacteria are resistant to antibiotics ($\kappa = 0$), then the population dynamics are qualitatively similar to those when bacteria are sensitive to antibiotics. In both cases, antibiotic administration causes doubly latently infected bacteria to dominate the system. However, when all bacteria are antibiotic resistant, the total bacterial population and phage populations are noticeably larger (Fig. 5).

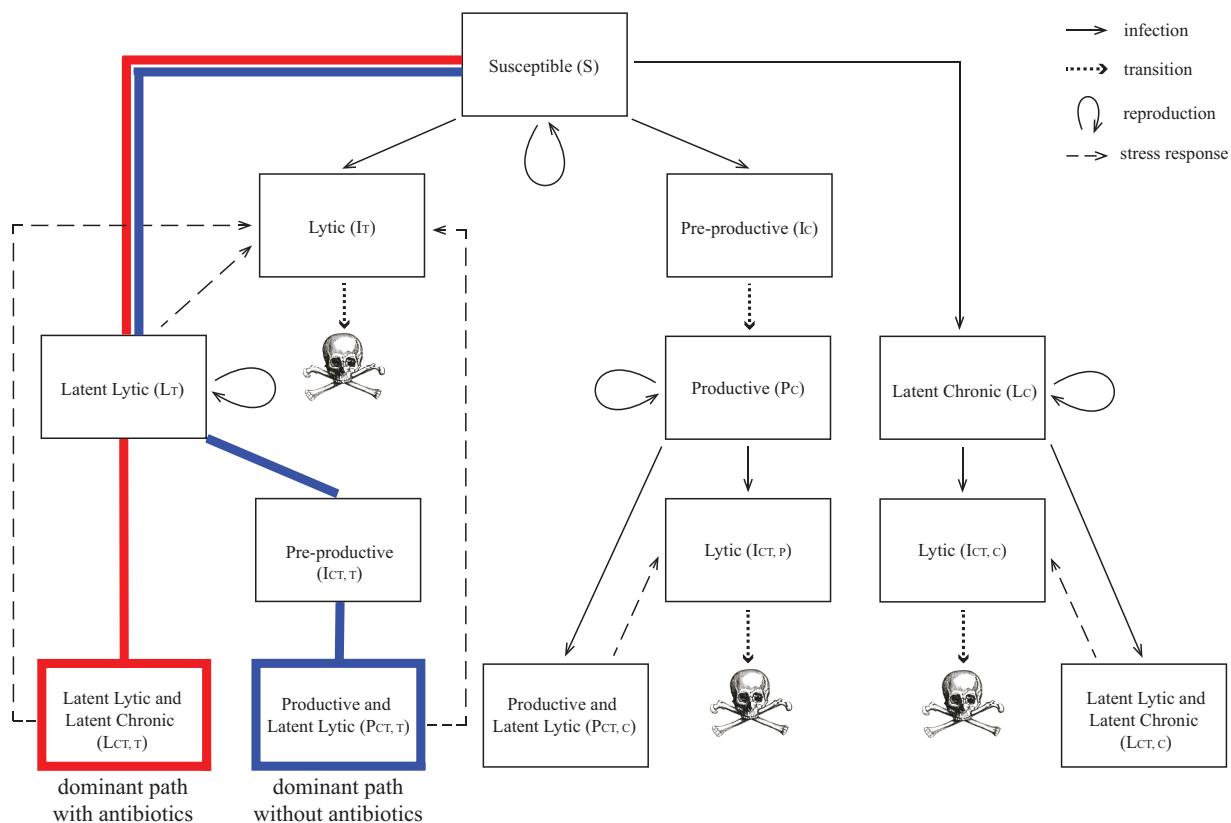


FIG 2 Full flowchart of bacterium-phage system, corresponding to model system (equations S1 to S15 in Text S1), with results superimposed. The dominant path through the model compartments without antibiotics is shown in blue, while the dominant path with periodic antibiotic dosing is shown in red. Skull sketch courtesy of Dawn Hudson (CC0).

Pharmacological implications with antibiotic resistance. The main concern when treating an infection with antibiotics is the size of the bacterial population. Therefore, we investigate the total bacterial population under a range of antibiotic dosing frequencies (Fig. 6). We compute the average total bacterial population over the first 300 bacterial reproductive cycles (40.8 days), and we find that both antibiotics and temperate phage are critical to controlling the infection and work synergistically even when bacteria are antibiotic resistant. We define infection control to be an average bacterial population less than 10% of carrying capacity (i.e., 1-log decrease in bacterial levels compared with placebo).

If only chronic phage are present in the system (see Fig. S1a in the supplemental material), effective antibiotics are required to control the infection. If all bacteria are sensitive to antibiotics, the presence of chronic phage controls the infection slightly better than if there are no chronic phage due to the cost of production during productive infection.

If only temperate phage are present in the system (Fig. S1b), infection is controlled even when bacteria are resistant. In fact, the efficacy of temperate phage alone is similar to the efficacy of antibiotics alone. With both effective antibiotics and temperate phage, the number of antibiotic doses required to keep the infection under control is cut in half compared with antibiotics alone or temperate phage alone.

If both phages are present in the system (Fig. 6), infection control is marginally better than if only temperate phage are present (Fig. S1b). These results demonstrate the synergy between temperate phage and antibiotics even in resistant populations. No deliberate combination therapy may be needed to treat these infections because temperate phage are commonly found in natural populations of *P. aeruginosa* bacteria (53).

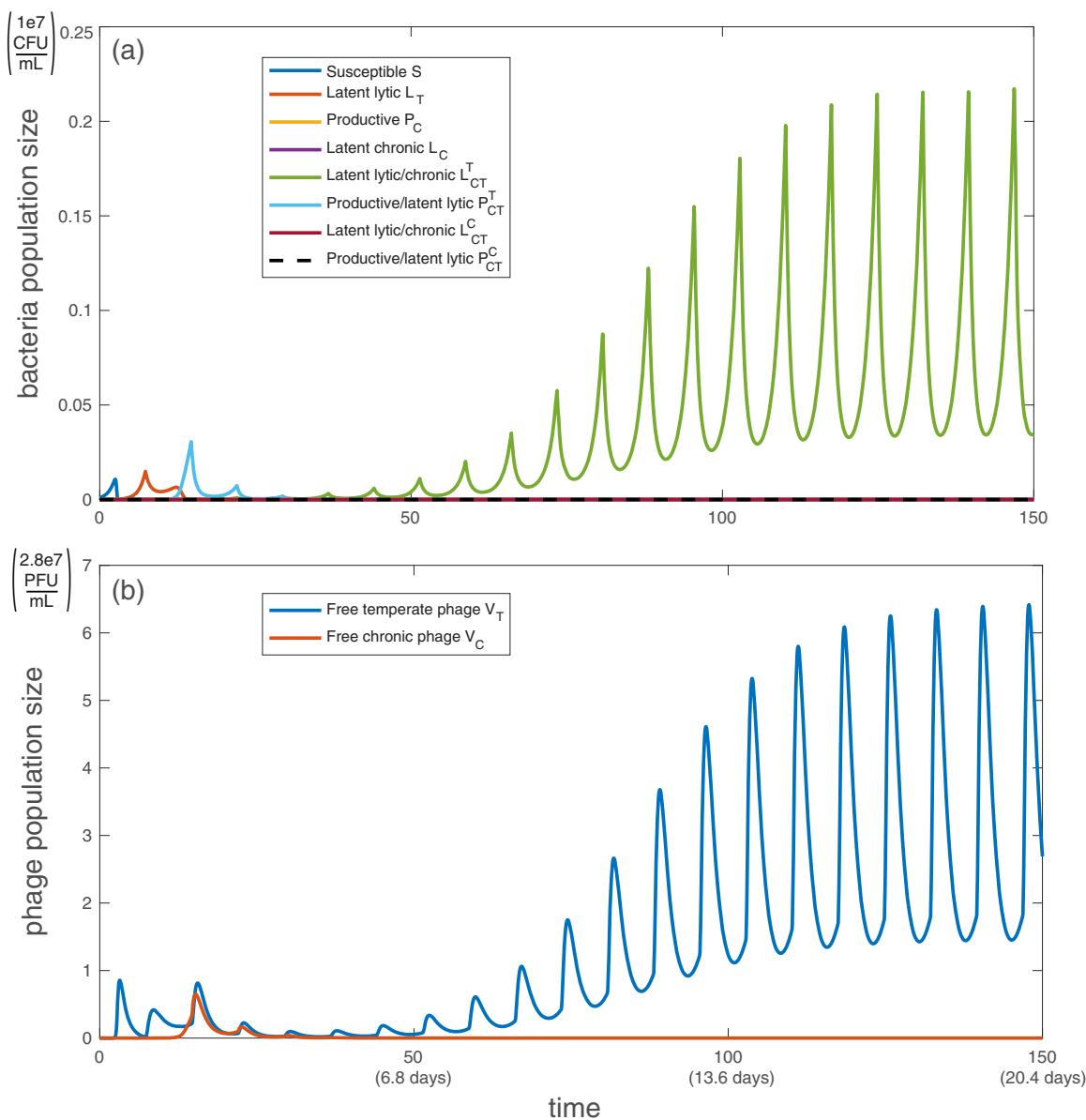


FIG 3 Simulation of population dynamics with no antibiotic resistance: bacterial population (a) and free phage population (b). All bacteria and phage types are described in Table 1. All parameter values are taken from the baselines in Table 2, with $h_\eta = 1/2$, $h_\beta = 1$, $h_\gamma = 1$ (see Text S2 in the supplemental material for more details). Antibiotics are administered periodically every $T = 7.3$ bacterial reproductive cycles (once-daily dose). Note that both axes are linear, not logarithmic. Initially, $S(0) = 10^{-3}$, $V_T(0) = V_C(0) = 10^{-7}$, according to the work of Sinha et al. (41).

DISCUSSION

The model presented here shows that temperate phage infection makes antibiotic treatment of bacterial infections both more effective and more efficient, whether or not the bacteria are susceptible to the antibiotics. When bacteria are sensitive to antibiotics, then antibiotic treatments need not be as frequent if temperate phage are present. Even if some or all bacterial strains are antibiotic resistant, antibiotics may still be able to control the infection in the presence of phages by triggering phage induction and cell lysis. For the rest of the discussion, we will assume that an infection is controlled if the average total bacterial population remains below 10% of carrying capacity over 300 bacterial reproductive cycles; in clinical terms, control is a 1-log difference between *P. aeruginosa* density in sputum for patients given antibiotics versus placebo over 40.8 days.

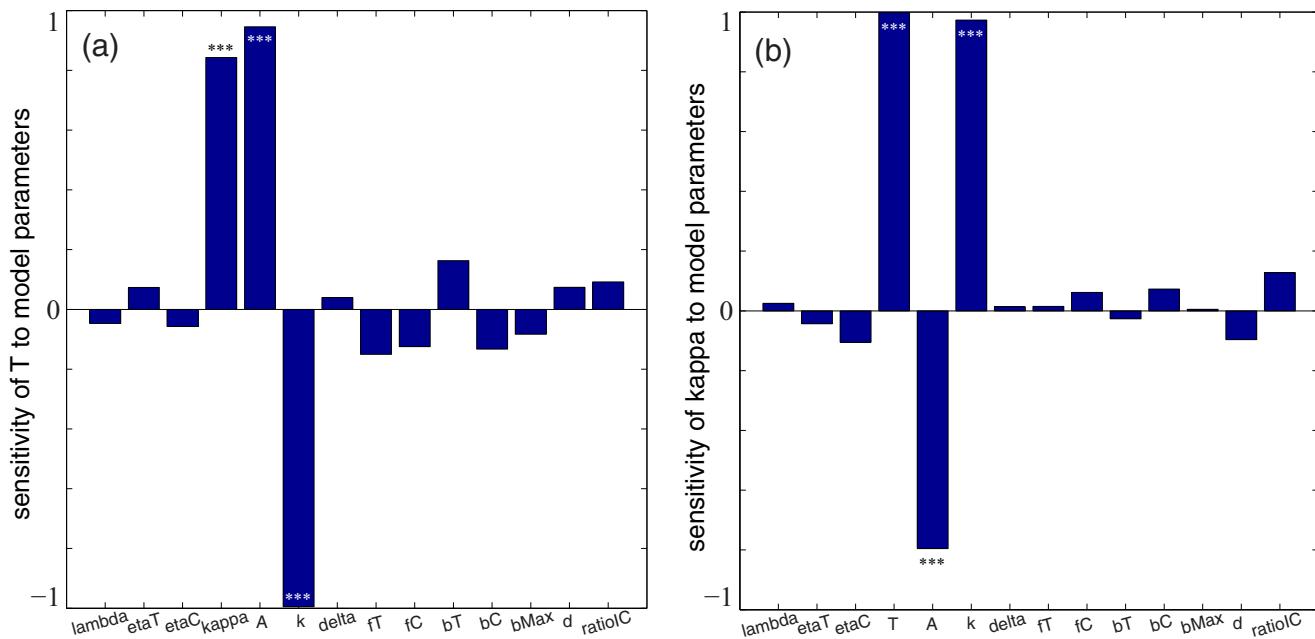


FIG 4 Sensitivity of the antibiotic dosing period T required to control the infection (a) and the antibiotic deadliness κ required to control the infection (b). The sensitivity analyses use Latin hypercube sampling (LHS) of parameter space and partial rank correlation coefficients (PRCC) (92). Infection control is an average total bacterial population below 10% of carrying capacity over 300 bacterial reproductive cycles. All parameter values are taken near the baselines in Table 2, with $h_\eta = 1/2$, $h_\beta = 1$, $h_\gamma = 1$. Initially, $S(0) = 1e-3$, $V_r(0) = 1e-7$, $V_c(0) = \text{ratio } l_c \times 1e-7$. The number of simulations is $n = 150$. Asterisks indicate significance (***, $P < 0.001$; no asterisks, $P > 0.05$). See Text S2 in the supplemental material for technical details.

For *P. aeruginosa* bacterial infections that respond to antibiotics, the model predicts that standard antibiotic doses need to be administered approximately every 12.1 h if no phage are present but only once every 25.1 h if temperate phage are present (Fig. 6). If bacteria are all antibiotic resistant, then temperate phages are required to control the infection, and antibiotic dosing is required every 12.6 h to sufficiently induce lysis.

These findings are consistent with clinical evidence; patients with cystic fibrosis (CF) given aerosolized levofloxacin twice daily experienced a nearly 10-fold decrease in *P. aeruginosa* density (our definition of infection control) over the treatment period compared with the placebo group (57). The study did not investigate the presence of phage but did note that approximately 60% of *P. aeruginosa* isolates showed resistance to levofloxacin, supporting our prediction that dosing should fall between once and twice daily depending on the susceptibility of the bacteria to antibiotics. Our findings are also consistent with existing antibiotic dosing protocols; although aerosolized quinolones are no longer approved for CF patients, intravenous (i.v.) and oral doses are commonly recommended on a once-, twice-, or three-times-daily schedule (55, 58).

While chronic phages are marginally beneficial in controlling infections, they are not able to control an infection without either temperate phages or effective antibiotics. In fact, chronic phages may actually inhibit control of infections by disrupting the human immune response (59, 60), a detail not yet incorporated into our model.

Like all models, our model has limitations. In the interest of simplicity, we have ignored the possibility of multiple infections by the same phage type. However, many phages that infect *P. aeruginosa* produce superinfection exclusion proteins that effectively prevent multiple infections by the same phage type (61, 62). We also do not include the exclusion of one phage type by the other. Little is known about cross-resistance to phage infection; it is often assumed to be uncommon, but including cross-resistance may dramatically impact the model predictions. If cross-resistance is in fact common, it is possible that phage-antibiotic synergy breaks down for some range of model parameters; we leave this analysis for future study.

Also, our model assumes that antibiotics induce phage, so this model is applicable

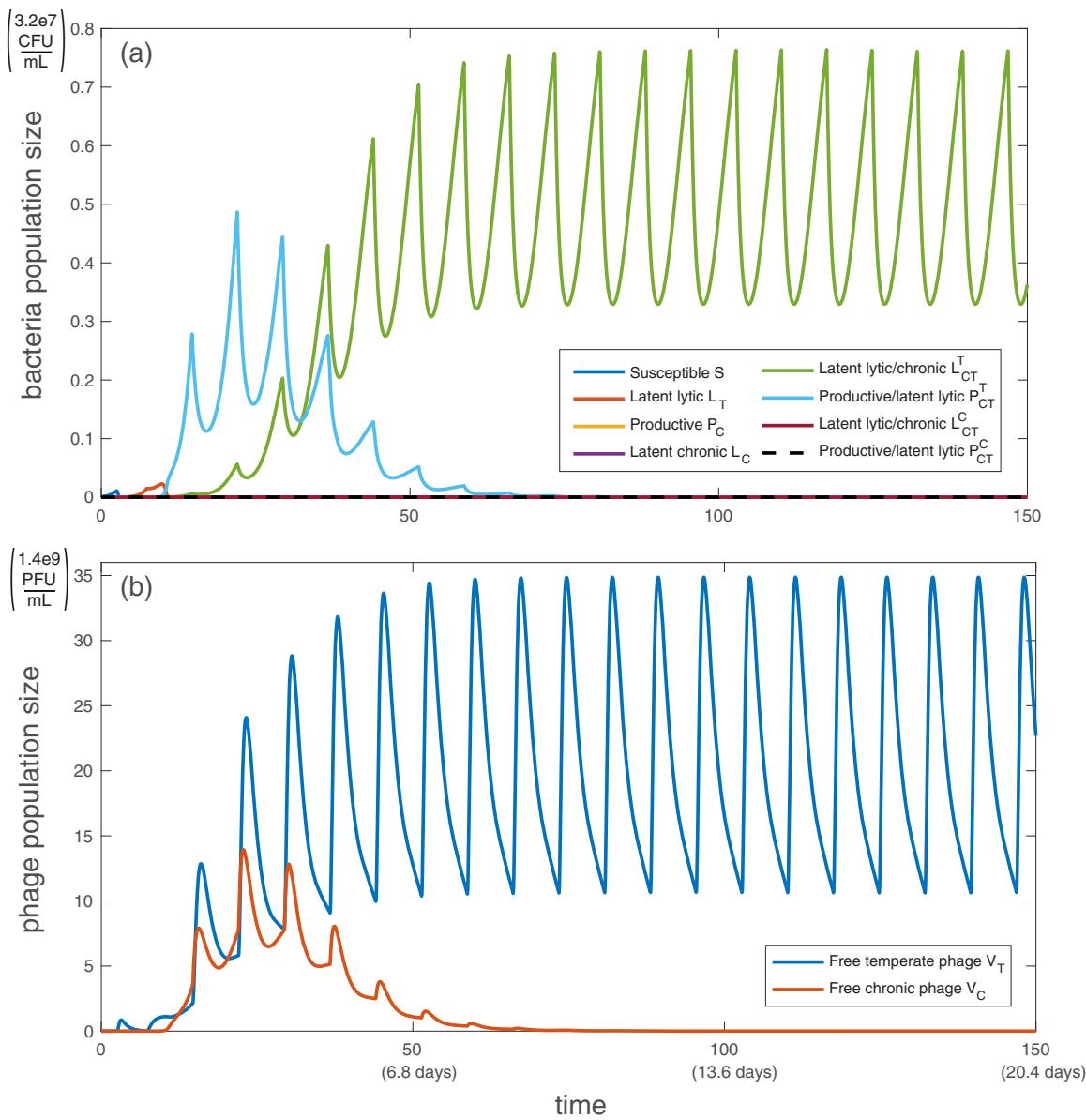


FIG 5 Simulation of population dynamics with complete antibiotic resistance: bacterial population (a) and free phage population (b). All bacteria and phage types are described in Table 1. All parameter values are taken from the baselines in Table 2, with $h_\eta = 1/2$, $h_\beta = 1$, $h_\gamma = 1$, and $\kappa = 0$ for all bacteria (see supplemental material for more details). Antibiotics are administered periodically every $T = 7.3$ bacterial reproductive cycles (once-daily dose). Initially, $S(0) = 1e-3$, $V_T(0) = V_C(0) = 1e-7$, according to the work of Sinha et al. (41).

with only quinolone antibiotics like levofloxacin and ciprofloxacin (34). However, drugs from this class of antibiotics are commonly used to treat *P. aeruginosa* infections (57, 63).

In addition, some phage are able to detect bacterial population density, which appears to affect the frequency of lysogeny (64, 65). If this process applies to *P. aeruginosa* and its phages, a more sophisticated model would incorporate a density-dependent latency probability: $f_T(B_{tot})$ and $f_C(B_{tot})$.

The model additionally assumes that bacteria resistant to antibiotics are still susceptible to lysis via phage induction, but this phenomenon depends on the mechanism of antibiotic resistance. There are many mechanisms of resistance to quinolones and fluoroquinolones. However, subinhibitory concentrations of several antibiotics are known to induce SOS but not result directly in cell death (34, 61, 66–68). Therefore, we model the impact of phage induction on *P. aeruginosa* population size with and without antibiotic resistance.

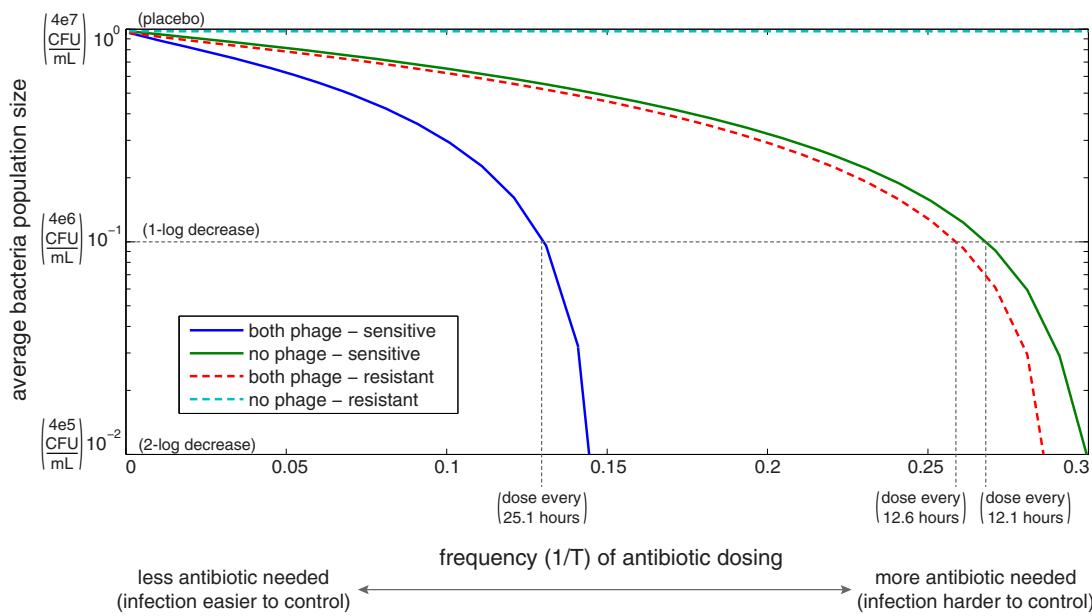


FIG 6 Average total bacterial population for a range of periodic antibiotic dosing protocols. All parameter values are taken at the baselines in Table 2, with $h_\eta = 1/2$, $h_\beta = 1$, $h_\gamma = 1$, $t_{\max} = 300$ (see the supplemental material for more details). Solid lines indicate that all bacteria are sensitive to antibiotics, and dashed lines indicate that all bacteria are resistant. Note that the vertical axis is logarithmic, while the horizontal axis is linear. Nondimensional units are supplemented with standard units parenthetically. Initially, $S(0) = 1e-3$, $V_r(0) = V_c(0) = 1e-7$, unless otherwise noted.

Because this model does not include an evolutionary dynamics component, the results presented here are applicable only to acute exacerbations. If bacterium/phage evolution were integrated into this model, it might be able to explain longer-term dynamics seen in chronic infections in humans (28).

Also, all latent chronic infection states are final such that virus production cannot be induced by stress. We believe that changing the model structure to accommodate chronic phage induction might change the number of productive bacteria but would not change the overall impact of antibiotic synergy, which primarily occurs with temperate infections.

Finally, the quantitative results presented in Fig. 6 depend significantly on how effective antibiotics are at killing bacteria directly versus killing via phage induction (κ in our model). To our knowledge, no study has experimentally measured the relative number of bacteria killed by the intended antibiotic mechanism versus phage induction, so we assume that antibiotics kill via each method equally quickly. If antibiotics directly kill bacteria much more quickly ($\kappa > 1$), then antibiotic resistance is more detrimental to infection control than lack of phages. If antibiotics trigger phage induction much more quickly ($\kappa < 1$), then a lack of phages is more detrimental to infection control than antibiotic resistance. Experimental work is needed to determine a reasonable range for κ and test whether it is an evolvable trait.

Conclusion. Antibiotic resistance threatens the efficacy of standard treatments for many dangerous and common infections. Using *P. aeruginosa* infections as motivation, we present a theoretical case for using antibiotics that trigger phage induction (e.g., quinolones) to treat bacterial infections. We show that if bacteria are antibiotic resistant, then using antibiotics in the presence of phages can still control the infection. If bacteria are susceptible to antibiotics, then the presence of phages allows for less-frequent antibiotic dosing, which reduces the risk for antibiotic resistance in the future. In either case, the natural presence of phages in bacterial populations allows for more effective treatment of common bacterial infections. These, strain-dependent responses to antibiotics suggest the importance of personalized medicine approaches to treatment of infectious disease.

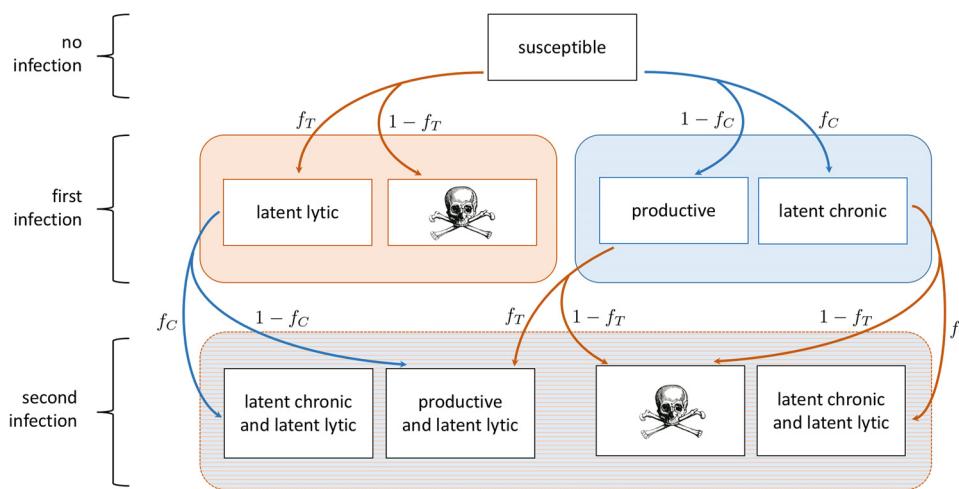


FIG 7 Flowchart of bacterium-phage system with both temperate (orange) and chronic (blue) phages. Boxes indicate a bacterial state, and arrows indicate an infection by phage. If a bacterium is infected by temperate phage, the probability of going latent lytic is f_T . If a bacterium is infected by chronic phage, the probability of becoming latent chronic is f_C . Skull sketch courtesy of Dawn Hudson (CC0).

As a final perspective, we remember that phage induction and bacterial death may occur across the microbiome of individual hosts treated with antibiotics. The impact of these dynamics in a community context must be considered carefully for the stability of the microbiome ecosystem as a whole.

MATERIALS AND METHODS

Modeling framework. Consider a system of two competing types of bacteriophage (e.g., see references 41 and 42): one temperate phage V_T with lytic and latent lytic stages and one chronic phage V_C with productive and latent stages. During the productive phase of the chronic lifestyle, phage particles are released through budding and do not kill the host bacterium. Each phage attacks one strain of bacteria that is initially susceptible to infection by either phage type. Figure 7 shows an overview of the process; Fig. 8 shows the complete modeling framework.

We assume the total bacterial population B_{tot} grows logistically at a rate γ to a carrying capacity K (69). Each phage infects susceptible bacteria S at a rate η . Bacteria infected by the temperate phage V_T will either become latently infected L_T with probability f_T or will enter a lytic state I_T with probability $(1 - f_T)$. Bacteria in the lytic state produce phage and burst (with burst size β_T) at a rate δ . (This modeling choice circumvents the necessity of a delay differential equation.) While in the lytic state, the phage hijacks cell functions, and the cell cannot reproduce (70, 71). Bacteria do not move between lytic and latent states unless there is a perturbation or stress to the system where viruses are induced.

Bacteria infected by the chronic phage V_C will either become latently infected L_C with probability f_C or will enter a preproductive state I_C with probability $(1 - f_C)$. Bacteria in the preproductive state stop reproducing and prepare to manufacture phage with delay rate δ . After the production delay, the preproductive bacteria enter the productive state P_C and continue reproducing at a potentially reduced rate $\lambda\gamma$, and begin producing phage at a rate β_C without cell death (72). As above, after chronic phage enter the latent or productive state in a cell, they will not change state. Latent chronic phage cannot be induced by stress to become productive; however, productively infected strains produce more phage under stress and reproduce more slowly. We note that biologically, productively infected strains can revert to latent infection and latent hosts can induce chronic virus production.

Once a bacterium is infected, we assume that it will exclude superinfection by the same phages but may be infected by phages of the other type (73). If a bacterium that is latently infected by the temperate phage is additionally infected with the chronic phage, the bacterium will either become latently infected with both phages ($L_{CT}^{(T)}$) with probability f_C or will enter a preproductive state $I_{CT}^{(T)}$ with probability $(1 - f_C)$. Bacteria in the preproductive state stop reproducing and prepare to manufacture phage with delay rate δ . After the production delay, the infected bacteria enter the productive state $P_{CT}^{(T)}$ and continue reproducing at a potentially reduced rate $\lambda\gamma$, and begin producing phage at a rate β_C without cell death (72).

Similarly, if a bacterium that is latently infected with a chronic phage is infected with the temperate phage, it will either become latently infected ($L_{CT}^{(C)}$) with probability f_T or will enter a lytic state $I_{CT}^{(C)}$ with probability $(1 - f_T)$. Bacteria in the lytic state produce phage and burst (with burst size β_T) at a rate δ . While in the lytic state, the phage hijacks cell functions, and the cell cannot reproduce.

If a productive bacterium is then infected with the temperate phage, the bacterium will become latently infected with temperate phage ($P_{CT}^{(C)}$) with probability f_T . Otherwise, the productive bacterium will enter a lytic state $I_{CT}^{(P)}$ with probability $(1 - f_T)$. Bacteria in the lytic state produce phage and burst (with

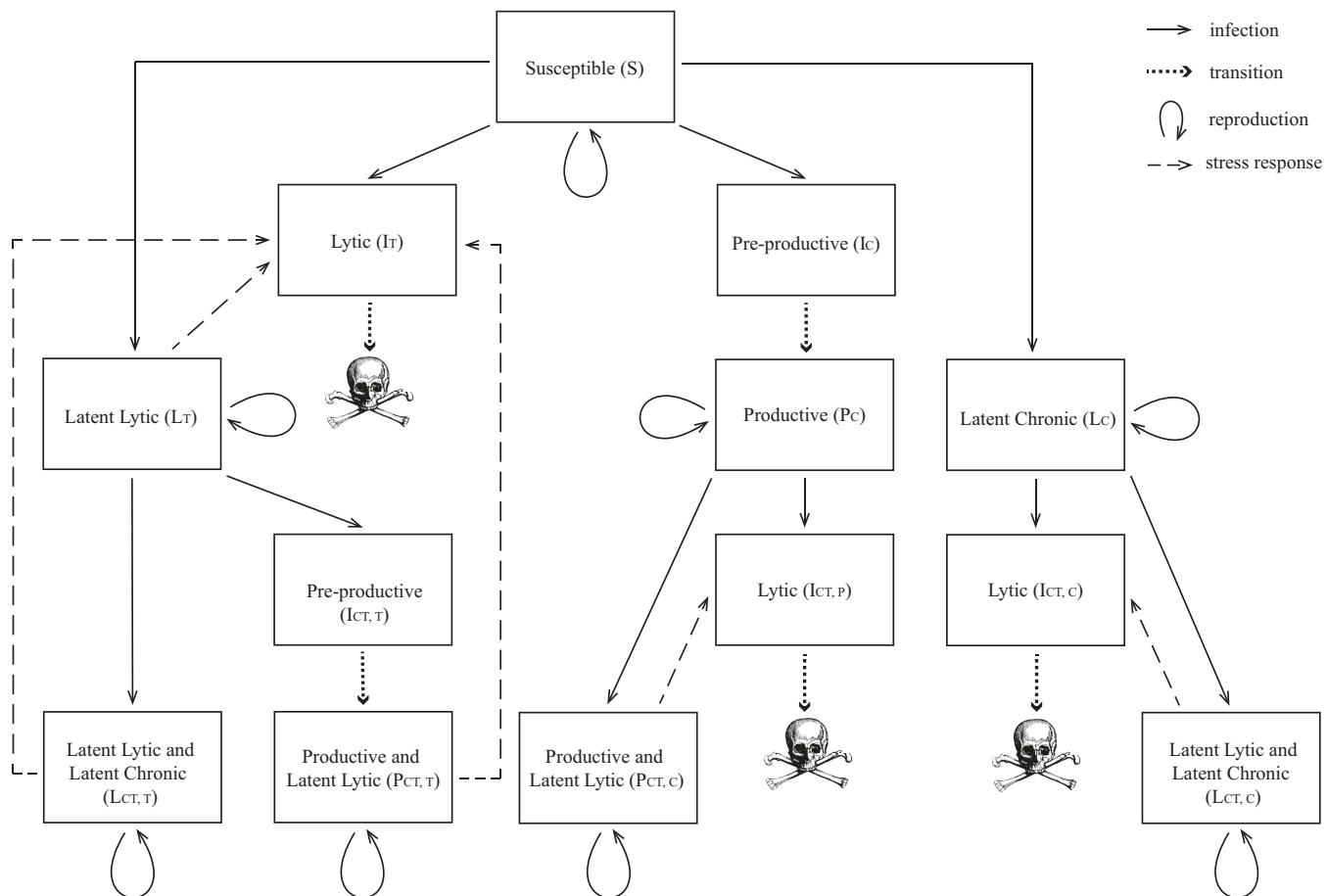


FIG 8 Full flowchart of bacterium-phage system, corresponding to model system (see equations S1 to S15 in Text S1). Skull sketch courtesy of Dawn Hudson (CC0).

burst size β_r at a rate δ . While in the lytic state, the phage hijacks cell functions, and the cell cannot reproduce.

As shown in Fig. 7, without the addition of new susceptible bacteria, this infection process results quickly in a population of cells that phenotypically are either doubly infected by both phages in the latent state or producing the chronic virus and latently infected with temperate phage.

Infection. Many models of bacterium-phage interaction assume that a mass action process governs infection (41, 44), but *P. aeruginosa*-phage infection rates are not well approximated by a mass action process (74, 75). More realistically, infection rates decrease as population growth activates quorum-sensing and biofilm formation (76). One way to accommodate this infection process is to replace a mass action term with a Michaelis-Menten or Hollings type II functional response. In this case, all infection and absorption rates are proportional to the nonlinear response

$$r(V, B) = \frac{VB}{h_\eta + B} \quad (1)$$

where V is the phage of interest, B is the bacterium of interest, and h_η is the bacterial population at which the infection rate is half of the maximum. For small bacterial populations ($B \approx 0$), infection is approximately a mass action process. As the bacterial population grows, the infection rate saturates (Fig. 9a).

Antibiotics. Because patients infected with *P. aeruginosa* are typically treated with antibiotics at the time of bacterial detection (77, 78), we must incorporate the effects of antibiotic doses administered at times t_i on the bacterium-phage ecosystem. We assume that system stress spikes at times t_i (when antibiotics become bioavailable) and decays exponentially, consistent with typical antibiotic metabolism in the human system (79–81). The functional form of stress is then

$$s(t, \{t_i\}) = A \sum_{i=1}^N H(t - t_i) \exp(-k(t - t_i)) \quad (2)$$

where t is the current time, $\{t_i\}$ is a list of antibiotic dose times, A is the amplitude of stress due to 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. For inhaled or intravenous antibiotics, the dose times are the exact times

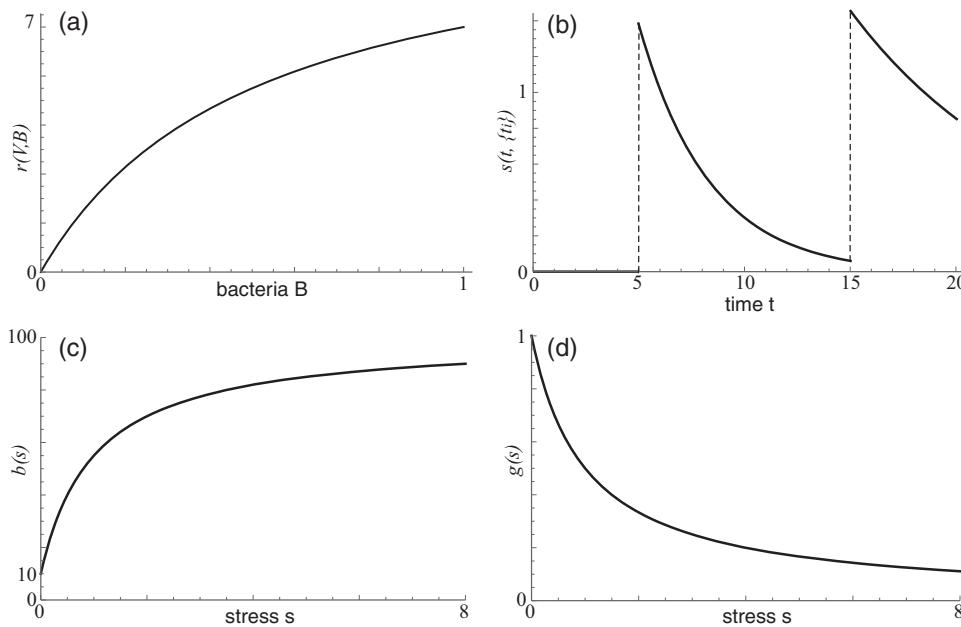


FIG 9 Sketches of the functions for infection $r(V,B)$ with phage density $V = 10$ (a), antibiotic stress $s(t, \{t_i\})$ with $\{t_i\} = \{5,15\}$ (b), phage production $b(s)$ (c), and cell reproduction multiplier $g(s)$ (d). Parameter values are taken from the baselines in Table 2.

of antibiotic administration. For oral antibiotics, $\{t_i\}$ are the times at which the antibiotics become bioavailable in the bloodstream (Fig. 9b).

When the system is stressed, the following three processes occur. (i) Bacteria that are susceptible to the antibiotics die at a rate proportional to the amount of antibiotic in the system (82). If certain strains of bacteria are resistant to antibiotics, then they will not be killed directly by antibiotics (83–85). (ii) Bacteria that are infected by temperate phage induce phage production at a rate equal to the stress (34, 86, 87). In other words, stress measures the rate at which latent lytic bacteria induce phage. Note that not all antibiotics induce phage (34), so we focus only on the types of antibiotics known to do so (e.g., quinolones like levofloxacin and ciprofloxacin) (8, 88). We assume that even antibiotic-resistant bacteria induce viruses in the presence of antibiotics, which has been demonstrated for several classes of antibiotics (34, 61, 66–68). (iii) Productive bacteria increase phage production and decrease cell reproduction (89, 90). A simple way to incorporate increased phage production during system stress is with

TABLE 1 Description of model variables in bacterium-phage system^a

Variable	Meaning
S	Density of susceptible bacteria
I_T	Density of lytic bacteria preparing to burst
I_C	Density of preproductive bacteria preparing to manufacture phage
L_T	Density of latent lytic bacteria
P_C	Density of productive bacteria
L_C	Density of latent chronic bacteria
$I_{CT}^{(T)}$	Density of latent lytic bacteria that have entered preproductive state
$I_{CT}^{(P)}$	Density of productive bacteria that have become lytic
$I_{CT}^{(C)}$	Density of latent chronic bacteria that have become lytic
$L_{CT}^{(T)}$	Density of latent chronic and latent lytic bacteria (first infection, V_T ; second infection, V_C)
$P_{CT}^{(T)}$	Density of productive and latent lytic bacteria (first infection, V_T ; second infection, V_C)
$L_{CT}^{(C)}$	Density of latent chronic and latent lytic bacteria (first infection, V_C ; second infection, V_T)
$P_{CT}^{(C)}$	Density of productive and latent lytic bacteria (first infection, V_C ; second infection, V_T)
B_{tot}	Density of all bacteria
V_T	Density of free temperate phage
V_C	Density of free chronic phage
V_{tot}	Density of all free phage
t	Time normalized by bacterial reproduction rate

^aSee equations S1 to S15 in Text S1 in the supplemental material. Due to nondimensionalization of density and time, all variables and parameters are nondimensional; all densities are relative to the bacterial carrying capacity, and all rates are relative to the growth rate of bacteria under ideal conditions.

TABLE 2 Description of model parameters in bacterium-phage system^m

Parameter	Meaning	Range	Baseline	Reference(s)
γ	Growth rate of bacteria under ideal conditions, normalized to 1 ^a	1	1 (5.1e-3 min ⁻¹)	93, 94
λ	Proportion growth rate change due to productive chronic infection	(0.5, 3) ^b	1	72
K	Carrying capacity of bacteria, normalized to 1 ^c	1	1 (4e7 CFU/ml)	95, 96
η	Infection rate	(0, 40)	20 (0.10 min ⁻¹) ^d	41
κ	Bacterial death rate due to antibiotic, relative to antibiotic lysis induction rate	(0, 3.5) ^e	1	93, 97
A	Amplitude of stress (rate at which antibiotic induces lysis) introduced with one antibiotic dose	(0, 2)	1.1 (5.6e-3 min ⁻¹) ^f	93, 98
k	Metabolic decay rate of antibiotic within the system	(1e-3, 0.6) ^g	0.3 (1.7e-3 min ⁻¹) ^h	93, 99, 100
$\{t_i\}$	Vector of antibiotic administration times			55
δ	Rate at which infection leads to phage production (eclipse and rise phase)	(1.5, 7.3) ⁱ	4 (2.0e-2 min ⁻¹)	101, 102
f_T	Fraction of bacteria infected with V_T that become latently infected	(0, 1)	0.01	103, 104
f_C	Fraction of bacteria infected with V_C that become latently infected	(0, 1)	0.01 ^j	
β_T	Burst size for bacteria infected with V_T	(10, 1,000)	100	101, 102, 105-109
β_C	Phage production rate for bacteria infected with V_C	(5, 200)	10 (5.1e-2 min ⁻¹) ^k	
β_{\max}	Maximum phage production rate for bacteria infected with V_C under maximum stress	(10, 10,000)	100 (0.51 min ⁻¹)	34
d	Rate of free phage degradation	(0.9, 3.6) ^l	1 (5.1e-3 min ⁻¹)	110

^aGrowth rate is approximately 5.1e-3 min⁻¹ for *P. aeruginosa* grown *in vitro* but is highly variable in cystic fibrosis patients.

^bEstimates based on *Escherichia coli* and M13 phage.

^cStable bacterial density in sputum is highly variable in patients with cystic fibrosis; a study of viable *P. aeruginosa* densities in sputum of 12 patients not undergoing treatment ranged from 5.3e3 CFU/ml to 1.8e11 CFU/ml; log differences between control/placebo and treatment are more commonly reported. We select a carrying capacity near the geometric mean of that range; see the supplemental material for details.

^dEstimate based on *E. coli* and λ phage; see the supplemental material for details.

^eEstimate for antibiotic levofloxacin (upper limit on death rate may include death by phage induction).

^fEstimated from *in vitro* experiment using antimicrobial peptides and meropenem; see the supplemental material for details.

^gLow estimate is for meropenem *in vitro*; high estimate is for ciprofloxacin *in vivo* (human).

^hAntibiotic is levofloxacin (half-life approximately 6.9 h); see the supplemental material for details.

ⁱLow estimate is for PAXYB1 phage and PAO1 host, and high estimate is for PAK_P3 phage and PAO1 host; see the supplemental material for details.

^jGuess based on temperate phage.

^kGuess based on author experience.

^lLow estimate is for phage extracted from Raunefjorden, and high estimate is for phage extracted from Bergen Harbor (strains unknown).

^mSee equations S1 to S15 in Text S1 in the supplemental material. Due to nondimensionalization of density and time, all variables and parameters are nondimensional; all densities are relative to the bacterial carrying capacity, and all rates are relative to the growth rate of bacteria under ideal conditions. Commonly used density and time units are noted in parentheses for baseline rates.

a Hollings-like functional response. With no system stress, the phage production rate is β_C and with increasing system stress, the phage production rate saturates at β_{\max} :

$$b(s) = \beta_C + \frac{s}{h_{\beta} + s} (\beta_{\max} - \beta_C) \quad (3)$$

where s is the time-dependent stress level (equation 2) in the system, h_{β} is the stress level at which the production rate is halfway between the minimum and maximum, and β_{\max} is the maximum production rate when stress is maximal (Fig. 9c). We assume that bacteria that are latently infected with the chronic virus do not induce phage production, although there is evidence that this occurs in real-world systems.

Similarly, a simple way to incorporate decreased cell reproduction during system stress is with a Hollings-like functional response. With no system stress, the cell reproduction rate is $\lambda\gamma$, and with increasing system stress, reproduction slows by a factor of $g(s)$, and the cell eventually stops reproducing:

$$g(s) = 1 - \frac{s}{h_{\gamma} + s} \quad (4)$$

where s is the time-dependent stress level (equation 2) in the system and h_{γ} is the stress level at which the growth rate is half the maximum. As stress increases, the bacterium eventually stops reproducing (Fig. 9d).

Tables 1 and 2 show variable and parameter definitions, respectively. See Text S2 in the supplemental material for a discussion on parameter selection. See equations S1 to S15 in Text S1 for the dynamical systems model.

Data availability. All software (Matlab.m files) is publicly available from the Illinois Data Bank at <https://databank.illinois.edu/datasets/IDB-9721455> (91).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/mSystems.00221-19>.

TEXT S1, PDF file, 0.1 MB.

TEXT S2, PDF file, 0.2 MB.

FIG S1, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

This work was funded in part by the National Science Foundation grant DMS-1815764 (Z.R.), the Cystic Fibrosis Foundation grant WHITAK16PO (R.J.W.), an Allen Distinguished Investigator Award (R.J.W.), and National Institutes of Health grant R37 AI83256-06 (G.A.O.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

The authors declare no competing interests.

REFERENCES

1. Ventola CL. 2015. The antibiotic resistance crisis: part 1: causes and threats. *P T* 40:277–283.
2. Jarvis WR, Martone WJ. 1992. Predominant pathogens in hospital infections. *J Antimicrob Chemother* 29:19–24. https://doi.org/10.1093/jac/29.suppl_A.19.
3. Hancock RE, Speert DP. 2000. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and impact on treatment. *Drug Resist Updat* 3:247–255. <https://doi.org/10.1054/drup.2000.0152>.
4. Centers for Disease Control and Prevention. 2013. Antibiotic resistance threats in the United States, 2013. Centers for Disease Control and Prevention, Atlanta, GA.
5. Driscoll JA, Brody SL, Kollef MH. 2007. The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections. *Drugs* 67: 351–368. <https://doi.org/10.2165/00003495-200767030-00003>.
6. Kanj SS, Sexton DJ. 2019. Principles of antimicrobial therapy of *Pseudomonas aeruginosa* infections. UpToDate Inc, Waltham, MA.
7. Lin Y, Chang RYK, Britton WJ, Morales S, Kutter E, Chan HK. 2018. Synergy of nebulized phage PEV20 and ciprofloxacin combination against *Pseudomonas aeruginosa*. *Int J Pharm* 551:158–165. <https://doi.org/10.1016/j.ijpharm.2018.09.024>.
8. Comeau AM, Tétart F, Trojet SN, Prere MF, Krisch H. 2007. Phage-antibiotic synergy (PAS): β -lactam and quinolone antibiotics stimulate virulent phage growth. *PLoS One* 2:e799. <https://doi.org/10.1371/journal.pone.0000799>.
9. Kutter E, De Vos D, Gvasalia G, Alavidze Z, Gogokhia L, Kuhl S, Abedon ST. 2010. Phage therapy in clinical practice: treatment of human infections. *Curr Pharm Biotechnol* 11:69–86. <https://doi.org/10.2174/138920110790725401>.
10. Jansen M, Wahida A, Latz S, Krüttgen A, Häfner H, Buhl EM, Ritter K, Horz HP. 2018. Enhanced antibacterial effect of the novel T4-like bacteriophage KARL-1 in combination with antibiotics against multi-drug resistant *Acinetobacter baumannii*. *Sci Rep* 8:14140. <https://doi.org/10.1038/s41598-018-32344-y>.
11. Valério N, Oliveira C, Jesus V, Branco T, Pereira C, Moreirinha C, Almeida A. 2017. Effects of single and combined use of bacteriophages and antibiotics to inactivate *Escherichia coli*. *Virus Res* 240:8–17. <https://doi.org/10.1016/j.virusres.2017.07.015>.
12. Moulton-Brown CE, Friman VP. 2018. Rapid evolution of generalized resistance mechanisms can constrain the efficacy of phage-antibiotic treatments. *Evol Appl* 11:1630–1641. <https://doi.org/10.1111/eva.12653>.
13. Torres-Barceló C, Hochberg ME. 2016. Evolutionary rationale for phages as complements of antibiotics. *Trends Microbiol* 24:249–256. <https://doi.org/10.1016/j.tim.2015.12.011>.
14. Chaudhry WN, Concepción-Acevedo J, Park T, Andleeb S, Bull JJ, Levin BR. 2017. Synergy and order effects of antibiotics and phages in killing *Pseudomonas aeruginosa* biofilms. *PLoS One* 12:e0168615. <https://doi.org/10.1371/journal.pone.0168615>.
15. Knezevic P, Curcin S, Aleksić V, Petrusic M, Vlaski L. 2013. Phage-antibiotic synergism: a possible approach to combatting *Pseudomonas aeruginosa*. *Res Microbiol* 164:55–60. <https://doi.org/10.1016/j.resmic.2012.08.008>.
16. Torres-Barceló C, Arias-Sánchez FI, Vasse M, Ramsayer J, Kaltz O, Hochberg ME. 2014. A window of opportunity to control the bacterial pathogen *Pseudomonas aeruginosa* combining antibiotics and phages. *PLoS One* 9:e106628. <https://doi.org/10.1371/journal.pone.0106628>.
17. Oechslin F, Piccardi P, Mancini S, Gabard J, Moreillon P, Entenza JM, Resch G, Que YA. 2017. Synergistic interaction between phage therapy and antibiotics clears *Pseudomonas aeruginosa* infection in endocarditis and reduces virulence. *J Infect Dis* 215:703–712. <https://doi.org/10.1093/infdis/jiw632>.
18. Torres-Barceló C, Franzon B, Vasse M, Hochberg ME. 2016. Long-term effects of single and combined introductions of antibiotics and bacteriophages on populations of *Pseudomonas aeruginosa*. *Evol Appl* 9:583–595. <https://doi.org/10.1111/eva.12364>.
19. Weinbauer MG. 2004. Ecology of prokaryotic viruses. *FEMS Microbiol Rev* 28:127–181. <https://doi.org/10.1016/j.femsre.2003.08.001>.
20. Rakonjac J. 2012. Filamentous bacteriophages: biology and applications. In eLS. Wiley Online Library. John Wiley & Sons, Inc, Hoboken, NJ.
21. Lwoff A. 1953. Lysogeny. *Bacteriol Rev* 17:269.
22. Davies EV, Winstanley C, Fothergill JL, James CE. 2016. The role of temperate bacteriophages in bacterial infection. *FEMS Microbiol Lett* 363:fnw015. <https://doi.org/10.1093/femsle/fnw015>.
23. Roux S, Hallam SJ, Woyke T, Sullivan MB. 2015. Viral dark matter and virus–host interactions resolved from publicly available microbial genomes. *Elife* 4:e08490. <https://doi.org/10.7554/elife.08490>.
24. Mathee K, Narasimhan G, Valdes C, Qiu X, Matewish JM, Koehrsen M, Rokas A, Yandava CN, Engels R, Zeng E, Olavarietta R, Doud M, Smith RS, Montgomery P, White JR, Godfrey PA, Kodira C, Birren B, Galagan JE, Lory S. 2008. Dynamics of *Pseudomonas aeruginosa* genome evolution. *Proc Natl Acad Sci U S A* 105:3100–3105. <https://doi.org/10.1073/pnas.0711982105>.
25. Mosquera-Rendón J, Rada-Bravo AM, Cárdenas-Brito S, Corredor M, Restrepo-Pineda E, Benítez-Páez A. 2016. Pangenome-wide and molecular evolution analyses of the *Pseudomonas aeruginosa* species. *BMC Genomics* 17:45. <https://doi.org/10.1186/s12864-016-2364-4>.
26. Spencer DH, Kas A, Smith EE, Raymond CK, Sims EH, Hastings M, Burns JL, Kaul R, Olson MV. 2003. Whole-genome sequence variation among multiple isolates of *Pseudomonas aeruginosa*. *J Bacteriol* 185: 1316–1325. <https://doi.org/10.1128/JB.185.4.1316-1325.2003>.
27. Kung VL, Ozer EA, Hauser AR. 2010. The accessory genome of *Pseudomonas aeruginosa*. *Microbiol Mol Biol Rev* 74:621–641. <https://doi.org/10.1128/MMBR.00027-10>.
28. James CE, Davies EV, Fothergill JL, Walshaw MJ, Beale CM, Brockhurst MA, Winstanley C. 2015. Lytic activity by temperate phages of *Pseudomonas aeruginosa* in long-term cystic fibrosis chronic lung infections. *ISME J* 9:1391. <https://doi.org/10.1038/ismej.2014.223>.
29. Torres-Barceló C. 2018. The disparate effects of bacteriophages on antibiotic-resistant bacteria. *Emerg Microbes Infect* 7:168. <https://doi.org/10.1038/s41426-018-0169-z>.
30. Davies EV, James CE, Brockhurst MA, Winstanley C. 2017. Evolutionary diversification of *Pseudomonas aeruginosa* in an artificial sputum model. *BMC Microbiol* 17:3. <https://doi.org/10.1186/s12866-016-0916-z>.
31. Davies EV, James CE, Williams D, O'Brien S, Fothergill JL, Haldenby S, Paterson S, Winstanley C, Brockhurst MA. 2016. Temperate phages both mediate and drive adaptive evolution in pathogen biofilms. *Proc Natl Acad Sci U S A* 113:8266–8271. <https://doi.org/10.1073/pnas.1520056113>.
32. Wang X, Kim Y, Ma Q, Hong SH, Pokusaeva K, Sturino JM, Wood TK. 2010. Cryptic prophages help bacteria cope with adverse environments. *Nat Commun* 1:147. <https://doi.org/10.1038/ncomms1146>.
33. Rokney A, Kobiler O, Amir A, Court DL, Stavans J, Adhya S, Oppenheim AB. 2008. Host responses influence on the induction of lambda prophage. *Mol Microbiol* 68:29–36. <https://doi.org/10.1111/j.1365-2958.2008.06119.x>.
34. Fothergill JL, Mowat E, Walshaw MJ, Ledson MJ, James CE, Winstanley C. 2011. Effect of antibiotic treatment on bacteriophage production by a cystic fibrosis epidemic strain of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 55:426–428. <https://doi.org/10.1128/AAC.01257-10>.
35. López E, Domenech A, Ferrández MJ, Fries MJ, Ardanuy C, Ramirez M, García E, Liñares J, de la Campa AG. 2014. Induction of prophages by fluoroquinolones in *Streptococcus pneumoniae*: implications for emer-

gence of resistance in genetically-related clones. *PLoS One* 9:e94358. <https://doi.org/10.1371/journal.pone.0094358>.

36. Martínez-García E, Jatsenko T, Kivisaar M, de Lorenzo V. 2015. Freeing *Pseudomonas putida* KT 2440 of its proviral load strengthens endurance to environmental stresses. *Environ Microbiol* 17:76–90. <https://doi.org/10.1111/1462-2920.12492>.
37. Kaur S, Harjai K, Chhibber S. 2012. Methicillin-resistant *Staphylococcus aureus* phage plaque size enhancement using sublethal concentrations of antibiotics. *Appl Environ Microbiol* 78:8227–8233. <https://doi.org/10.1128/AEM.02371-12>.
38. Kim M, Jo Y, Hwang YJ, Hong HW, Hong SS, Park K, Myung H. 2018. Phage-antibiotic synergy via delayed lysis. *Appl Environ Microbiol* 84: e02085-18. <https://doi.org/10.1128/AEM.02085-18>.
39. Weitz JS, Dushoff J. 2008. Alternative stable states in host-phage dynamics. *Theor Ecol* 1:13–19. <https://doi.org/10.1007/s12080-007-0001-1>.
40. Payne RJ, Jansen VA. 2001. Understanding bacteriophage therapy as a density-dependent kinetic process. *J Theor Biol* 208:37–48. <https://doi.org/10.1006/jtbi.2000.2198>.
41. Sinha V, Goyal A, Svenningsen SL, Semsey S, Krishna S. 2017. In silico evolution of lysis-lysogeny strategies reproduces observed lysogeny propensities in temperate bacteriophages. *Front Microbiol* 8:1386. <https://doi.org/10.3389/fmicb.2017.01386>.
42. Gulbudak H, Weitz JS. 2019. Heterogeneous viral strategies promote coexistence in virus-microbe systems. *J Theor Biol* 462:65–84. <https://doi.org/10.1016/j.jtbi.2018.10.056>.
43. Levin BR, Bull J. 1996. Phage therapy revisited: the population biology of a bacterial infection and its treatment with bacteriophage and antibiotics. *Am Nat* 147:881–898. <https://doi.org/10.1086/285884>.
44. Leung CYJ, Weitz JS. 2017. Modeling the synergistic elimination of bacteria by phage and the innate immune system. *J Theor Biol* 429: 241–252. <https://doi.org/10.1016/j.jtbi.2017.06.037>.
45. Levin BR, Bull JJ. 2004. Population and evolutionary dynamics of phage therapy. *Nat Rev Microbiol* 2:166. <https://doi.org/10.1038/nrmicro822>.
46. Ankromah P, Levin BR. 2014. Exploring the collaboration between antibiotics and the immune response in the treatment of acute, self-limiting infections. *Proc Natl Acad Sci U S A* 111:8331–8338. <https://doi.org/10.1073/pnas.1400352111>.
47. Bohannan BJM, Lenski RE. 2000. Linking genetic change to community evolution: insights from studies of bacteria and bacteriophage. *Ecol Lett* 3:362–377. <https://doi.org/10.1046/j.1461-0248.2000.00161.x>.
48. Tam VH, Schilling AN, Poole K, Nikolaou M. 2007. Mathematical modelling response of *Pseudomonas aeruginosa* to meropenem. *J Antimicrob Chemother* 60:1302–1309. <https://doi.org/10.1093/jac/dkm370>.
49. Webb GF, D'Agata EMC, Magal P, Ruan S. 2005. A model of antibiotic-resistant bacterial epidemics in hospitals. *Proc Natl Acad Sci U S A* 102:13343–13348. <https://doi.org/10.1073/pnas.0504053102>.
50. Levin BR, Baquero F, Johnsen PJ. 2014. A model-guided analysis and perspective on the evolution and epidemiology of antibiotic resistance and its future. *Curr Opin Microbiol* 19:83–89. <https://doi.org/10.1016/j.mib.2014.06.004>.
51. Touchon M, de Sousa JAM, Rocha EP. 2017. Embracing the enemy: the diversification of microbial gene repertoires by phage-mediated horizontal gene transfer. *Curr Opin Microbiol* 38:66–73. <https://doi.org/10.1016/j.mib.2017.04.010>.
52. Stanczak-Mrozek KI, Laing KG, Lindsay JA. 2017. Resistance gene transfer: induction of transducing phage by sub-inhibitory concentrations of antimicrobials is not correlated to induction of lytic phage. *J Antimicrob Chemother* 72:1624–1631. <https://doi.org/10.1093/jac/dkx056>.
53. Winstanley C, Langille MG, Fothergill JL, Kukavica-Ibrulj I, Paradis-Bleau C, Sanschagrin F, Thomson NR, Winsor GL, Quail MA, Lennard N, Bignell A, Clark L, Seeger K, Saunders D, Harris D, Parkhill J, Hancock RE, Brinkman FS, Levesque RC. 2008. Newly introduced genomic prophage islands are critical determinants of in vivo competitiveness in the Liverpool Epidemic Strain of *Pseudomonas aeruginosa*. *Genome Res* 19:12–23. <https://doi.org/10.1101/gr.086082.108>.
54. Aldred KJ, Kerns RJ, Osherooff N. 2014. Mechanism of quinolone action and resistance. *Biochemistry* 53:1565–1574. <https://doi.org/10.1021/bi5000564>.
55. Stockmann C, Sherwin CM, Zobell JT, Young DC, Waters CD, Spigarelli MG, Ampofo K. 2013. Optimization of anti-pseudomonal antibiotics for cystic fibrosis pulmonary exacerbations: III. Fluoroquinolones. *Pediatr Pulmonol* 48:211–220. <https://doi.org/10.1002/ppul.22667>.
56. Knowles B, Silveira CB, Bailey BA, Barott K, Cantu VA, Cobián-Güemes AG, Coutinho FH, Dinsdale EA, Felts B, Furby KA, George EE, Green KT,
- Gregoracci GB, Haas AF, Haggerty JM, Hester ER, Hisakawa N, Kelly LW, Lim YW, Little M, Luque A, McDole-Somera T, McNair K, de Oliveira LS, Quistad SD, Robinett NL, Sala E, Salamon P, Sanchez SE, Sandin S, Silva GGZ, Smith J, Sullivan C, Thompson C, Vermeij MJA, Youle M, Young C, Zgliczynski B, Brainard R, Edwards RA, Nulton J, Thompson F, Rohwer F. 2016. Lytic to temperate switching of viral communities. *Nature* 531: 466. <https://doi.org/10.1038/nature17193>.
57. Geller DE, Flume PA, Staab D, Fischer R, Loutit JS, Conrad DJ. 2011. Levofloxacin inhalation solution (MP-376) in patients with cystic fibrosis with *Pseudomonas aeruginosa*. *Am J Respir Crit Care Med* 183: 1510–1516. <https://doi.org/10.1164/rccm.201008-1293OC>.
58. Proesmans M, Vermeulen F, Boulanger L, Verhaegen J, De Boeck K. 2013. Comparison of two treatment regimens for eradication of *Pseudomonas aeruginosa* infection in children with cystic fibrosis. *J Cyst Fibros* 12:29–34. <https://doi.org/10.1016/j.jcf.2012.06.001>.
59. Sweere JM, Van Belleghem JD, Ishak H, Bach MS, Popescu M, Sunkari V, Kaber G, Manasherob R, Suh GA, Cao X, de Vries CR, Lam DN, Marshall PL, Birukova M, Katzenelson E, Lazzareschi DV, Balaji S, Keswani SG, Hawn TR, Secor PR, Ballyk PL. 2019. Bacteriophage trigger antiviral immunity and prevent clearance of bacterial infection. *Science* 363: eaat9691. <https://doi.org/10.1126/science.aat9691>.
60. Burgener EB, Sweere JM, Bach MS, Secor PR, Haddock N, Jennings LK, Marvig RL, Johansen HK, Rossi E, Cao X, Tian L, Nedelec L, Molin S, Ballyk PL, Milla C. 2019. Filamentous bacteriophages are associated with chronic *Pseudomonas* lung infections and antibiotic resistance in cystic fibrosis. *Sci Transl Med* 11:eaau9748. <https://doi.org/10.1126/scitranslmed.aau9748>.
61. James CE, Fothergill JL, Kalwij H, Hall AJ, Cottell J, Brockhurst MA, Winstanley C. 2012. Differential infection properties of three inducible prophages from an epidemic strain of *Pseudomonas aeruginosa*. *BMC Microbiol* 12:216. <https://doi.org/10.1186/1471-2180-12-216>.
62. Heo YJ, Chung IY, Choi KB, Lau GW, Cho YH. 2007. Genome sequence comparison and superinfection between two related *Pseudomonas aeruginosa* phages, D3112 and MP22. *Microbiology* 153:2885–2895. <https://doi.org/10.1099/mic.0.2007/007260-0>.
63. Hodson M, Butland R, Roberts C, Smith M, Batten J. 1987. Oral ciprofloxacin compared with conventional intravenous treatment for *Pseudomonas aeruginosa* infection in adults with cystic fibrosis. *Lancet* 329:235–237. [https://doi.org/10.1016/S0140-6736\(87\)90062-6](https://doi.org/10.1016/S0140-6736(87)90062-6).
64. Hargreaves KR, Kropinski AM, Clokie MR. 2014. What does the talking?: quorum sensing signalling genes discovered in a bacteriophage genome. *PLoS One* 9:e85131. <https://doi.org/10.1371/journal.pone.0085131>.
65. Silpe JE, Bassler BL. 2018. A host-produced quorum-sensing autoinducer controls a phage lysis-lysogeny decision. *Cell* 176:268–280.e13. <https://doi.org/10.1016/j.cell.2018.10.059>.
66. Redgrave LS, Sutton SB, Webber MA, Piddock LJ. 2014. Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. *Trends Microbiol* 22:438–445. <https://doi.org/10.1016/j.tim.2014.04.007>.
67. Valencia EY, Esposito F, Spira B, Blázquez J, Galhardo RS. 2017. Ciprofloxacin-mediated mutagenesis is suppressed by subinhibitory concentrations of amikacin in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 61:e02107-16. <https://doi.org/10.1128/AAC.02107-16>.
68. Brazas MD, Hancock RE. 2005. Ciprofloxacin induction of a susceptibility determinant in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 49:3222–3227. <https://doi.org/10.1128/AAC.49.3222-3227.2005>.
69. Zwietering MH, Jongenburger I, Rombouts FM, van 't Riet K. 1990. Modeling of the bacterial growth curve. *Appl Environ Microbiol* 56: 1875–1881.
70. Tabib-Salazar A, Liu B, Shadrin A, Burchell L, Wang Z, Wang Z, Goren MG, Yosef I, Qimron U, Severinov K, Matthews SJ, Wigneshwaran S. 2017. Full shut-off of *Escherichia coli* RNA-polymerase by T7 phage requires a small phage-encoded DNA-binding protein. *Nucleic Acids Res* 45:7697–7707. <https://doi.org/10.1093/nar/gkx370>.
71. St-Pierre F, Endy D. 2008. Determination of cell fate selection during phage lambda infection. *Proc Natl Acad Sci U S A* 105:20705–20710. <https://doi.org/10.1073/pnas.0808831105>.
72. Shapiro JW, Williams ES, Turner PE. 2016. Evolution of parasitism and mutualism between filamentous phage M13 and *Escherichia coli*. *PeerJ* 4:e2060. <https://doi.org/10.7717/peerj.2060>.
73. De Smet J, Hendrix H, Blasdel BG, Danis-Włodarczyk K, Lavigne R. 2017. *Pseudomonas* predators: understanding and exploiting phage-host interactions. *Nat Rev Microbiol* 15:517. <https://doi.org/10.1038/nrmicro.2017.61>.

74. Simmons M, Drescher K, Nadell CD, Bucci V. 2018. Phage mobility is a core determinant of phage-bacteria coexistence in biofilms. *ISME J* 12:531. <https://doi.org/10.1038/ismej.2017.190>.

75. Vidakovic L, Singh PK, Hartmann R, Nadell CD, Drescher K. 2018. Dynamic biofilm architecture confers individual and collective mechanisms of viral protection. *Nat Microbiol* 3:26. <https://doi.org/10.1038/s41564-017-0050-1>.

76. Harper DR, Parracho HM, Walker J, Sharp R, Hughes G, Werthén M, Lehman S, Morales S. 2014. Bacteriophages and biofilms. *Antibiotics* 3:270–284. <https://doi.org/10.3390/antibiotics3030270>.

77. Høiby N, Bjarnsholt T, Moser C, Jensen PØ, Kolpen M, Qvist T, Aanaes K, Pressler T, Skov M, Ciofu O. 2017. Diagnosis of biofilm infections in cystic fibrosis patients. *APMIS* 125:339–343. <https://doi.org/10.1111.apm.12689>.

78. Høiby N, Bjarnsholt T, Moser C, Bassi G, Coenye T, Donelli G, Hall-Stoodley L, Hola V, Imbert C, Kirketerp-Møller K, Lebeaux D, Oliver A, Ullmann A, Williams C. 2015. ESCMID guideline for the diagnosis and treatment of biofilm infections 2014. *Clin Microbiol Infect* 21:S1–S25. <https://doi.org/10.1016/j.cmi.2014.10.024>.

79. Naber KG, Westenfelder SR, Madsen PO. 1973. Pharmacokinetics of the aminoglycoside antibiotic tobramycin in humans. *Antimicrob Agents Chemother* 3:469–473. <https://doi.org/10.1128/aac.3.4.469>.

80. Bax R, Bastain W, Featherstone A, Wilkinson D, Hutchison M. 1989. The pharmacokinetics of meropenem in volunteers. *J Antimicrob Chemother* 24:311–320. https://doi.org/10.1093/jac/24.suppl_A.311.

81. Fish DN, Chow AT. 1997. The clinical pharmacokinetics of levofloxacin. *Clin Pharmacokinet* 32:101–119. <https://doi.org/10.2165/00003088-199732020-00002>.

82. Levin BR, Udekwu KI. 2010. Population dynamics of antibiotic treatment: a mathematical model and hypotheses for time-kill and continuous-culture experiments. *Antimicrob Agents Chemother* 54: 3414–3426. <https://doi.org/10.1128/AAC.00381-10>.

83. Fisher RA, Gollan B, Helaine S. 2017. Persistent bacterial infections and persister cells. *Nat Rev Microbiol* 15:453. <https://doi.org/10.1038/nrmicro.2017.42>.

84. Monack DM, Mueller A, Falkow S. 2004. Persistent bacterial infections: the interface of the pathogen and the host immune system. *Nat Rev Microbiol* 2:747. <https://doi.org/10.1038/nrmicro955>.

85. Stewart PS, Costerton JW. 2001. Antibiotic resistance of bacteria in biofilms. *Lancet* 358:135–138. [https://doi.org/10.1016/s0140-6736\(01\)05321-1](https://doi.org/10.1016/s0140-6736(01)05321-1).

86. Ptashne M. 1986. A genetic switch: gene control and phage [λ]. Cell Press, Cambridge, MA.

87. Nanda AM, Thormann K, Frunzke J. 2015. Impact of spontaneous prophage induction on the fitness of bacterial populations and host-microbe interactions. *J Bacteriol* 197:410–419. <https://doi.org/10.1128/JB.02230-14>.

88. Zhang X, McDaniel AD, Wolf LE, Keusch GT, Waldor MK, Acheson DW. 2000. Quinolone antibiotics induce Shiga toxin-encoding bacteriophages, toxin production, and death in mice. *J Infect Dis* 181:664–670. <https://doi.org/10.1086/315239>.

89. Hagens S, Habel A, Bläsi U. 2006. Augmentation of the antimicrobial efficacy of antibiotics by filamentous phage. *Microb Drug Resist* 12: 164–168. <https://doi.org/10.1089/mdr.2006.12.164>.

90. Secor PR, Sweere JM, Michaels LA, Malkovskiy AV, Lazzareschi D, Katznelson E, Rajadas J, Birnbaum ME, Arrigoni A, Braun KR, Evanko SP, Stevens DA, Kaminsky W, Singh PK, Parks WC, Bollyky PL. 2015. Filamentous bacteriophage promote biofilm assembly and function. *Cell Host Microbe* 18:549–559. <https://doi.org/10.1016/j.chom.2015.10.013>.

91. Rapti Z. 2019. Control of bacterial infections via antibiotic-induced proviruses. Illinois Data Bank. University of Illinois at Urbana-Champaign, Urbana, IL. https://doi.org/10.13012/B2IDB-9721455_V1.

92. Marino S, Hogue IB, Ray CJ, Kirschner DE. 2008. A methodology for performing global uncertainty and sensitivity analysis in systems biology. *J Theor Biol* 254:178–196. <https://doi.org/10.1016/j.jtbi.2008.04.011>.

93. Spalding C, Keen E, Smith DJ, Krachler AM, Jabbari S. 2018. Mathematical modelling of the antibiotic-induced morphological transition of *Pseudomonas aeruginosa*. *PLoS Comput Biol* 14:e1006012. <https://doi.org/10.1371/journal.pcbi.1006012>.

94. Kopf SH, Sessions AL, Cowley ES, Reyes C, Van Sambeek L, Hu Y, Orphan VJ, Kato R, Newman DK. 2016. Trace incorporation of heavy water reveals slow and heterogeneous pathogen growth rates in cystic fibrosis sputum. *Proc Natl Acad Sci U S A* 113:E110–E116. <https://doi.org/10.1073/pnas.1512057112>.

95. Stressmann FA, Rogers GB, Marsh P, Lilley AK, Daniels TW, Carroll MP, Hoffman LR, Jones G, Allen CE, Patel N, Forbes N, Forbes B, Tuck A, Bruce KD. 2011. Does bacterial density in cystic fibrosis sputum increase prior to pulmonary exacerbation? *J Cyst Fibros* 10:357–365. <https://doi.org/10.1016/j.jcf.2011.05.002>.

96. Price KE, Hampton TH, Gifford AH, Dolben EL, Hogan DA, Morrison HG, Sogin ML, O'Toole GA. 2013. Unique microbial communities persist in individual cystic fibrosis patients throughout a clinical exacerbation. *Microbiome* 1:27. <https://doi.org/10.1186/2049-2618-1-27>.

97. Grillon A, Schramm F, Kleinberg M, Jehl F. 2016. Comparative activity of ciprofloxacin, levofloxacin and moxifloxacin against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* assessed by minimum inhibitory concentrations and time-kill studies. *PLoS One* 11:e0156690. <https://doi.org/10.1371/journal.pone.0156690>.

98. Dutreix M, Bailone A, Devoret R. 1985. Efficiency of induction of prophage lambda mutants as a function of reCA alleles. *J Bacteriol* 161: 1080–1085.

99. Zhanell GG, Fontaine S, Adam H, Schurek K, Mayer M, Noreddin AM, Gin AS, Rubinstein E, Hoban DJ. 2006. A review of new fluoroquinolones. *Treat Respir Med* 5:437–465. <https://doi.org/10.2165/00151829-200605060-00009>.

100. Wingender W, Graefe KH, Gau W, Förster D, Beermann D, Schacht P. 1984. Pharmacokinetics of ciprofloxacin after oral and intravenous administration in healthy volunteers. *Eur J Clin Microbiol* 3:355–359. <https://doi.org/10.1007/BF01977494>.

101. Yu X, Xu Y, Gu Y, Zhu Y, Liu X. 2017. Characterization and genomic study of "phiKMV-Like" phage PAXYB1 infecting *Pseudomonas aeruginosa*. *Sci Rep* 7:13068. <https://doi.org/10.1038/s41598-017-13363-7>.

102. El Didamony G, Askara A, Shehata AA. 2015. Isolation and characterization of T7-like lytic bacteriophages infecting multidrug resistant *Pseudomonas aeruginosa* isolated from Egypt. *Curr Microbiol* 70: 786–791. <https://doi.org/10.1007/s00284-015-0788-8>.

103. Calendar RL (ed). 2006. The bacteriophages, 2nd ed. Oxford University Press, New York, NY.

104. Court DL, Oppenheim AB, Adhya SL. 2007. A new look at bacteriophage λ genetic networks. *J Bacteriol* 189:298–304. <https://doi.org/10.1128/JB.01215-06>.

105. Latino L, Essoh C, Blouin Y, Thien HV, Pourcel C. 2014. A novel *Pseudomonas aeruginosa* bacteriophage, Ab31, a chimera formed from temperate phage PAJU2 and *P. putida* lytic phage AF: characteristics and mechanism of bacterial resistance. *PLoS One* 9:e93777. <https://doi.org/10.1371/journal.pone.0093777>.

106. Schrader HS, Schrader JO, Walker JJ, Wolf TA, Nickerson KW, Kokjohn TA. 1997. Bacteriophage infection and multiplication occur in *Pseudomonas aeruginosa* starved for 5 years. *Can J Microbiol* 43:1157–1163. <https://doi.org/10.1139/m97-164>.

107. Ceyssens PJ, Brabban A, Rogge L, Lewis MS, Pickard D, Goulding D, Dougan G, Noben JP, Kropinski A, Kutter E, Lavigne R. 2010. Molecular and physiological analysis of three *Pseudomonas aeruginosa* phages belonging to the "N4-like viruses". *Virology* 405:26–30. <https://doi.org/10.1016/j.virol.2010.06.011>.

108. Garbe J, Bunk B, Rohde M, Schobert M. 2011. Sequencing and characterization of *Pseudomonas aeruginosa* phage JG004. *BMC Microbiol* 11:102. <https://doi.org/10.1186/1471-2180-11-102>.

109. You L, Suthers PF, Yin J. 2002. Effects of *Escherichia coli* physiology on growth of phage T7 in vivo and in silico. *J Bacteriol* 184:1888–1894. <https://doi.org/10.1128/jb.184.7.1888-1894.2002>.

110. Heldal M, Bratbak G. 1991. Production and decay of viruses in aquatic environments. *Mar Ecol Prog Ser* 72:205–212. <https://doi.org/10.3354/meps072205>.