Quantitative Models of Phage-Antibiotics Combination Therapy

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Abstract

The spread of multi-drug resistant (MDR) bacteria is a global public health crisis. Bacteriophage therapy (or "phage therapy") is a potential alternative approach to treat MDR infections. However, the effective use of phage therapy may be limited when phage-resistant bacterial mutants evolve and proliferate during treatment. Here, we develop a nonlinear population dynamics model of combination therapy that accounts for the interactions between bacteria, phage and antibiotics for *in-vivo* application given an immune response against bacteria. We simulate the combination therapy model for two strains of *Pseudomonas aeruginosa*, one which is phage-sensitive (and antibiotic resistant) and one which is antibiotic-sensitive (and phage-resistant). We find that combination therapy outperforms either phage or antibiotic alone, and that therapeutic effectiveness is enhanced given interaction with innate immune responses. Notably, therapeutic success can be achieved even at sub-inhibitory concentrations of antibiotic such as ciprofloxacin. These *in-silico* findings provide further support to the nascent application of combination therapy to treat MDR bacterial infections, while highlighting the role of innate immunity in shaping therapeutic outcomes.

I. INTRODUCTION

Multi-drug resistant bacterial infections are a threat to global health. The World Health Organization (WHO) has reported that drug-resistant tuberculosis alone kills 250,000 people each year[1]. Moreover, the United States Centers for Disease Control and Prevention (CDC) have reported 23,000 deaths each year attributed to drug-resistant pathogens, while their European counterparts have reported 25,000 deaths each year resulting from drug-resistant infections[2,3]. The WHO has identified and prioritized twelve pathogens causing common to life-threatening diseases, one of those pathogens is *Pseudomonas aeruginosa* (P.a.). Moreover, the pathogens listed by the WHO show increasing resistance to current antibiotics, while there is a dearth of alternative treatments against such bacteria[1].

Bacterial viruses (i.e., bacteriophage or "phage") can replicate *in situ*, and represent an alternative approach to treat MDR bacterial infections. Phage lysis of bacteria cells can drastically change bacterial population densities. In doing so, phage exert a strong selection pressure on the bacterial population. As a result, phage-resistant mutants can appear and become dominant[4–6], whether via surface-based resistance[4,7] or intracellular mechanisms[8]. The possibility that phage therapy may select for phage-resistant mutants has increased interest in identifying strategies to combine phage with other therapeutics, e.g., antibiotics[4,6,7,9–11]. However, the realized outcomes of combination strategies are varied, ranging from successes *in vitro*[9] and *in vivo*[4,11] to failure given *in vitro* settings[6].

In most cases, the mechanism underlying potential phage-antibiotic interactions are unknown. There are exceptions, for example P.a. phage OMKO1 binds the multidrug efflux pump, MexAB/MexXY[7]. This fact suggests that combination treatment of P.a. with OMKO1 and antibiotics can lead to an evolutionary trade-off between phage and antibiotic resistance[7,11]. Phage-resistant mutants show impairments of the multidrug efflux pump, MexAB/MexXY[7], specifically they show reduced functionality (or loss) of outer membrane porin M, OprM. This protein is part of the efflux pump complex and works as the cell receptor of the phage OMKO1. Mutations in the gene encoding OprM impair the phage attachment site and restore the sensitivity to some classes of antibiotics, including ciprofloxacin[7]. Therefore, the combination of phage OMKO1 and antibiotics against P.a. is a potential way to limit the spread of resistance to phage and antibiotic. Moreover, the genetic trade-off resulting from phage-OMKO1 therapy may help to resolve the discrepancy between *in vitro* and *in vivo* outcomes of the combined therapies due to the success of OMKO1 when combined with antibiotics in both contexts[7,11].

Therapeutic application of phage and antibiotics *in vivo* necessarily involves interactions with a new class of antimicrobial agents: effector cells within the innate immune response. Recent work has shown that phage and innate immune cells, specifically neutrophils, combine synergistically to clear otherwise fatal respiratory infections in which neither phage nor the innate immune response could eliminate alone[5]. This 'immunophage synergy' is hypothesized to result from density-dependent feedback mechanisms[12]. Phage lysis decreases bacterial densities such that the activated immune response can clear bacteria; without phage the bacterial densities increase to sufficiently high levels that are outside the range of control by immune cells. However, the potential role of the innate immune response in the context of phage-antibiotic combination therapy remains largely

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unexplored.

Here, we develop and analyze a mathematical model of phage-antibiotic combination therapy that builds on the synergistic interactions between phage, antibiotic, and immune cells. In doing so we extend a mathematical model of immunophage synergy[12] to take into account the pharmacodynamics and pharmacokinetics of an antibiotic, e.g., ciprofloxacin. At the core of the combination therapy model is its multiple-targeting approach, the phage target phage-sensitive (antibiotic-resistant) bacteria while the antibiotic targets phage-resistant (antibiotic-sensitive) mutants[7,11]. Critically, immune effector cells can target both bacterial strains. As we show, combination therapy successfully clears infections insofar as immune responses are active. Our proof-of-principle model highlights the role of immune responses in developing and assessing the effectiveness of phage-based therapeutics for treatment of MDR pathogens, particularly MDR P.a. that exhibit evolutionary trade-offs.

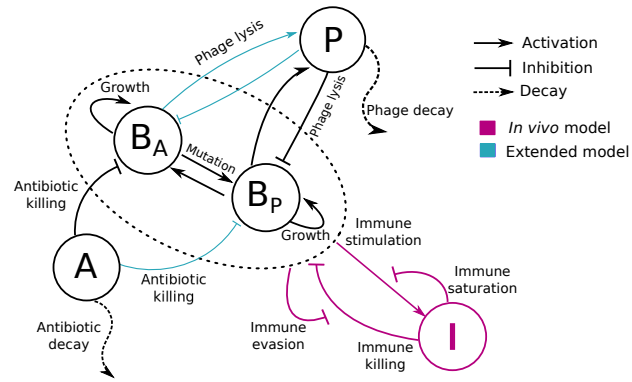

FIG. 1: **Schematic of the phage-antibiotic combination therapy model**. Antibiotic-sensitive bacteria (B_A) and phage-sensitive bacteria (B_P) are targeted by antibiotic (A) and the phage (P), respectively. Host innate immune response interactions (pink arrows) are included in the *in vivo* model. Innate immunity (I) is activated by the presence of bacteria and attacks both bacterial strains. Furthermore, in model versions accounting for (blue arrows), B_A and B_P are targeted by both antibiotic and phage but in quantitatively different levels.

II. COMBINATION THERAPY MODEL

The combination therapy model is a system of nonlinear, ordinary differential equations representing the interactions among, bacteria, phage, antibiotics, and the innate immune system (see Figure 1). Two strains of bacteria are included, one of which is phage-sensitive (B_P) and the other of which is antibiotic-sensitive (B_A). The strains B_P and B_A reproduce given density limitation. B_P is infected and lysed by phage but resists the antibiotic, while B_A population is killed by the antibiotic but is resistant to phage (for an *in vitro* model of bacteriophage therapy with fully susceptible and resistant types see[13]). We do not consider double-resistant mutants in our model due to the evolutionary trade-off between resistance against phage and antibiotics observed for P.a.[7]. Phage replicate inside the host B_P and decay in the environment. The antibiotic is administered at a constant concentration, then it is metabolized and removed at a fixed rate. The model is:

$$\dot{B}_P = r_P B_P \left(1 - \frac{B_{tot}}{K_C}\right)(1-\mu_1) + \mu_2 r_A B_A \left(1 - \frac{B_{tot}}{K_C}\right) - \frac{\varepsilon I B_P}{1 + \frac{B_{tot}}{K_D}} - B_P F(P) \quad (1)$$

$$\dot{B}_A = r_A B_A \left(1 - \frac{B_{tot}}{K_C}\right)(1-\mu_2) + \mu_1 r_P B_P \left(1 - \frac{B_{tot}}{K_C}\right) - \frac{\varepsilon I B_A}{1 + \frac{B_{tot}}{K_D}} - K_{kill} \frac{A^H}{EC_{50}^H + A^H} B_A, \quad (2)$$

$$\dot{P} = \tilde{\beta} B_P F(P) - \omega P, \quad (3)$$

$$\dot{I} = \alpha I \left(1 - \frac{I}{K_I}\right)\left(\frac{B_{tot}}{B_{tot} + K_N}\right), \quad (4)$$

$$\dot{A} = A_I - \theta A \quad (5)$$

In this model, phage-sensitive bacteria grow at a maximum rate r_P and have a carrying capacity K_C, while antibiotic-sensitive bacteria (B_A) grow at a maximum rate r_A and have a carrying capacity K_C. Both strains experience the same degree of density dependence, i.e., $B_{tot} = B_P + B_A$. Phage infect and lyse B_P bacteria at a rate $F(P)$. Antibiotic killing is approximated by a Hill function with the nonlinear coefficient H[14–17]. The maximum antibiotic killing rate is K_{kill}, while EC_{50} is the concentration of the antibiotic, here modeled as ciprofloxacin, at which the antibiotic effect is half as the maximum. Phage P replicate with a burst size $\beta - 1 = \tilde{\beta}$ and decay at a rate ω. We assume that antibiotic dynamics are relatively fast, and use a quasi-steady state approximation of $A^* = A_I/\theta$.

In the event that immune response are active (e.g., in the *in vivo* scenario), then host innate immune response, I, is activated by the presence of bacteria and grows with a maximum rate α. K_N is a half-saturation constant, i.e., the bacterial density at which the growth rate of the immune response is half its maximum. Bacteria grow and are killed by the innate immunity with a maximum killing rate, ϵ. However, at high bacterial concentration ($B_P + B_A > K_D$) bacteria can evade the immune response and reduce the immune killing efficiency[5,12].

Throughout, we consider three modalities of phage infection, $F(P)$, linear, heterogeneous mixing, and phage saturation. The linear phage infection modality assumes a well-mixed environment, where phage easily encounter and infect bacteria, so the infection rate $F(P) = \phi P$ is proportional to the phage density, where ϕ is the linear adsorption rate. The heterogeneous mixing model accounts for spatial heterogeneity, $F(P) = \tilde{phi} P^{\gamma}$[5]. Where $\tilde{\phi}$ is the nonlinear adsorption rate and $\gamma < 1$ the power-law exponent[18]. The third modality assumes that at high phage density multiple phages adsorb to a sin-

gle bacterium so phage infection follows a saturating Hill function, $F(P) = \frac{\phi P}{1+\frac{P}{P_C}}$. Here, ϕ is the adsorption rate and P_C is the phage density at which the infection rate is half-saturated[5].

Note that in later stages, we consider an 'extended' combination therapy model (Figure 1 (blue arrows)) in which bacterial strains are sensitive to both phages and antibiotic in quantitatively distinct levels. The full set of equations for this extension are found in the Supplementary Materials. In addition, a full description of parameter choices are described in the Methods.

III. RESULTS

A. Differential outcomes of single phage therapy

We begin by exploring the dynamics arising from adding a single phage at a density of 7.4×10^8 PFU/g two hours after infections caused by either phage-sensitive or phage-resistant bacteria. When the infection is caused by a phage-sensitive bacteria ($B_P = 7.4 \times 10^7$ CFU/g), phage lysis reduced B_P density to the point where the immune response alone could control this bacterial population. Despite the emergence of phage-resistant mutants (B_A), total bacterial population remained low and the innate immunity effectively controlled the infection. On the other hand, when the infection was caused by phage-resistant mutants ($B_A = 7.4 \times 10^7$ CFU/g), the phage could not target B_A so the bacterial population grew unimpeded. The immune response was overwhelmed by the rapid growth of B_A which then reached a density of $\sim 10^{10}$ CFU/g after 12h, leading to a persistent infection despite an activated immune response (similar to the outcomes described in[12]).

This initial analysis illustrates how therapeutic outcomes given application of a single phage type may be strongly dependent on the initial bacterial inoculum. As expected, single phage therapy fails to clear the infection when the bacterial inoculum is mistargeted (Fig. 2 bottom). In the next section we evaluate infection dynamics in response to the combined application of phage and antibiotics – similar to that in multiple *in vitro* and *in vivo* studies of phage-antibiotic treatment of MDR *P.a.*.[7,11].

B. Phage-antibiotic therapy treatment dynamics in immunocompetent hosts

We simulated the combined effects of phage (7.4×10^8 PFU/g) and antibiotics (assuming 2.5×MIC of ciprofloxacin for the B_A strain) in two different infection settings. First, when an immunocompetent host was infected with phage-sensitive bacteria, the infection was cleared before ∼36 hours due to the combined killing effect of phage, antibiotic, and innate immunity. The

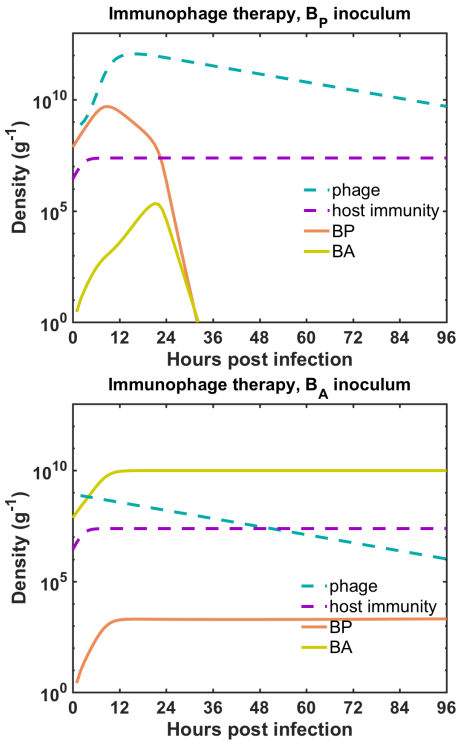

FIG. 2: **Dynamics of the immunophage therapy model against two different bacterial inoculum**. We simulate the phage therapy model developed by[12] against two infection settings. In the first infection setting (a), a phage-sensitive bacterial inoculum, B_P (orange solid line), is challenged with phage (blue dashed line) in an immunocompetence context. In the second scenario (b), antibiotic-sensitive bacteria, B_A (green solid line), are challenged with phage in presence of an active immune response (purple dashed line). The initial bacterial density and the initial phage dose are, $B_0 = 7.4 \times 10^7$ CFU/g and $P_0 = 7.4 \times 10^8$ PFU/g, respectively. The simulation run is 96 hours with phage being administered 2 hours after the infection. The bacterial carrying capacity is, $K_C = 10^{10}$ CFU/g.

dominant bacterial population, B_P, was targeted by the phage while the antibiotic targeted B_A. The combined effects of phage and antibiotic reduced total bacterial density to the point where innate immunity eliminate the bacterial infection. Second, when the host was infected with antibiotic-sensitive bacteria, the pathogen was cleared (before ∼12 hours) due to the combined effect of phage, antibiotic, and innate immunity. The antibiotic facilitated the decrease of B_A while phage maintained B_P concentration low, easing the innate immunity control over the infection. The resulting infection clearance in the phage-resistant case (Fig. 3 bottom) stands in stark contrast to the previous outcome of the single phage therapy model (Fig. 2 bottom). Overall, the results suggest that a curative outcome is possible when phage are combined with antibiotics in an immunocompetent host – even when the phage is initially mistargeted to the dom-

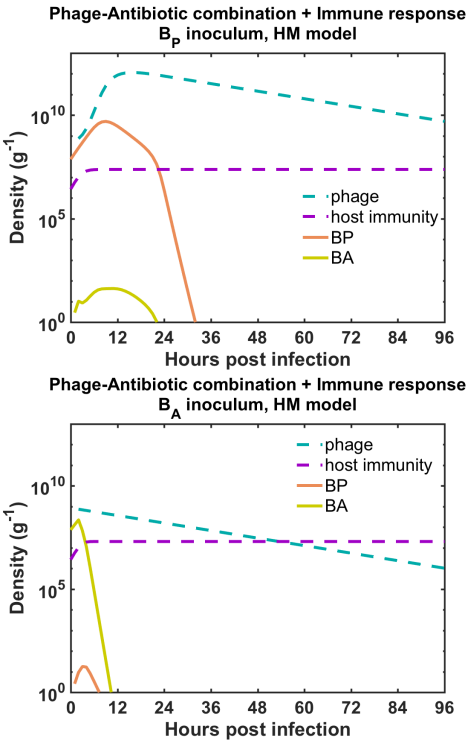

FIG. 3: **Outcomes of the phage-antibiotic combination therapy model for two different infection settings**. We simulate the combined effects of phage and antibiotics in an immunocompetent host infected with phage-sensitive bacteria (a), B_P (orange solid line). In (b), the host is infected with antibiotic-sensitive bacteria, B_A (green solid line). The dynamics of the phages (blue dashed line) and innate immunity (purple dashed line) are shown for each infection setting. Initial bacterial density and phage dose are, $B_0 = 7.4 \times 10^7$ CFU/g and $P_0 = 7.4 \times 10^8$ PFU/g, respectively. The simulation run is 96 hours (4 days). Antibiotic and phage are administered 2 hours after the beginning infection. Ciprofloxacin is maintained at a constant concentration of 0.0350 μg/ml during the simulation. The carrying capacity of the bacteria is $K_C = 10^{10}$ CFU/g.

inant bacterial strain. However what remains unclear is the extent to which successful treatment is driven by phage and antibiotics alone or, in part, because of the synergistic interactions with the innate immune response.

C. Phage-antibiotic combination therapy requires innate immunity to robustly clear the pathogen

In this section we assess the dependency of combination therapy on the immune response. To do so, we evaluate the combination therapy while setting $I = 0$. This is meant to mimic conditions of severe immunodeficiency. In order to further assess outcomes, we also consider multiple functional forms for phage-bacteria interactions - including the phage-saturation, heterogeneous mixing, and linear infection models (see Methods for more details). Unlike the results from the prior section, we find that application of phage and antibiotics does not robustly eliminate the bacterial population.

First, when a phage-sensitive bacterial inoculum was challenged with the combination therapy, the pathogen persisted in two of three infection models. Bacteria persist in the HM (Fig. 4 top-left) and PS (Fig. 4 upper-middle) models, while the combination of phage and antibiotic successfully eliminates the bacterial population in the LI model (Fig. 4 top-right). Although the combination of phage and antibiotic did not eliminate the bacterial population in the HM and PS models, the combination strategy still reduced the bacterial concentration relative to the carrying capacity ($K_C = 10^{10}$ CFU/g).

Second, when an antibiotic-sensitive bacterial inoculum was challenged with phage and antibiotic, bacteria persisted in two of three infection models, similar to the previous phage-sensitive case. Bacteria persist in the HM (Fig. 4 bottom-left) and PS (Fig. 4 lower-middle) models, while bacterial population is eliminated in the LI model (Fig. 4 bottom-right). Inclusion of antibiotics facilitated a decrease in B_A and the spread of B_P leading to coexistence between bacteria and phage. Furthermore, the elimination of bacteria in the LI model took longer ($\sim$24 hours) compared to the previous phage-sensitive case.

The outcomes of the combination therapy model suggest that, in absence of innate immunity, infection clearance is not achieved in two of three phage infection models. Pathogen clearance is only achieved in the linear infection case, that is, when we assume a well-mixed environment. On the other hand, when we assume spatial heterogeneity or phage saturation, a coexistence state between phage and bacteria arises from the tripartite dynamics between phage, bacteria, and antibiotic. Such coexistence state is inconsistent with the expected antimicrobial effect of the combination therapy[7] – and points to a potentially unrealized role of the immune response in the effectiveness of phage-antibiotic combination therapy..

D. Outcomes of the combination therapy model are robust to the bacterial composition of the inoculum and the concentration of antibiotic

Thus far we have simulated two extreme infection inoculum scenarios involving exclusively phage-sensitive bacteria or exclusively antibiotic-sensitive bacteria.Next, we consider the effects of combination therapy on mixed bacterial inoculum containing both B_P and B_A. To do so, we performed a robustness analysis of four (*in silico*) therapy models, i.e., antibiotic-only, antibiotic-innate immunity, phage-antibiotic, and phage-antibiotic combination in presence of innate immunity. For each model, we varied the concentration of the antibiotic and the bacterial composition of the inoculum. Outcomes from the different therapeutics are consistent with previous results

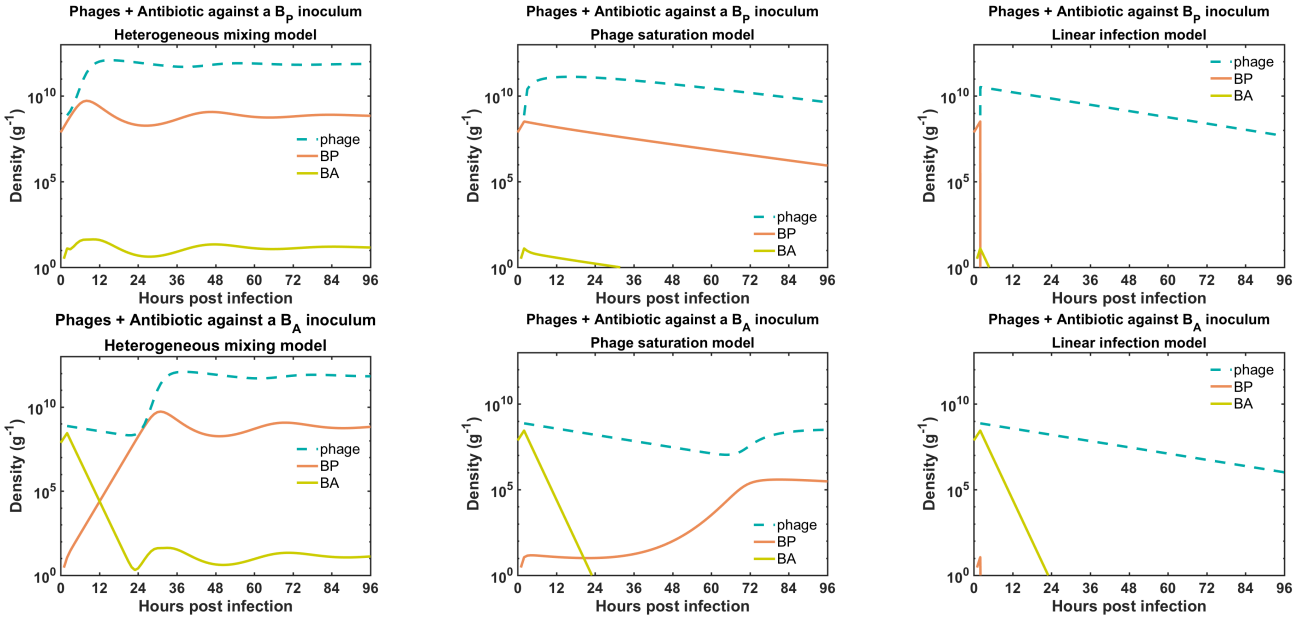

FIG. 4: **Bacterial dynamics given joint exposure to phages and antibiotic**. We simulate bacterial growth for 96 hours in exposure to phage (blue dashed line) and antibiotic (data not shown) added two hours after the beginning of the inoculation. The combination of phage and antibiotic is tested against two different bacterial inoculum. The first inoculum consisted of exclusively phage-sensitive bacteria (a-c), B_P (orange solid line). The second inoculum consisted of antibiotic-sensitive bacteria (d-f), B_A (green solid line). Additionally, we test three different models of phage infection, heterogeneous mixing (a, d), phage saturation (b, e) and linear infection models (c, f). The initial bacterial density and phage dose are, $B_0 = 7.4 \times 10^7$ CFU/g and $P_0 = 7.4 \times 10^8$ PFU/g, respectively. Ciprofloxacin is maintained at a constant concentration of 2.5×MIC (i.e., 0.0350 $\mu g/ml$) during the simulations.

obtained using a fixed set of initial conditions (Table I). We find that model outcomes are robust to variations in the initial conditions (i.e., inoculum composition and concentration of ciprofloxacin).

Treatment			Outcome
A	P	I	
1	0	0	Infection via BP proliferation
1	0	1	Infection via BP proliferation
1	1	0	Infection via BP coexistence with phage
1	1	1	Curative

TABLE I: **Summary of therapeutic outcomes given a combination of antibiotics (A), phage (P), and immunity (I)**. The presence or absence of different antimicrobial agents is represented with 1 or 0, respectively.

First, we evaluated the killing effect of the antibiotic against mixed bacterial inoculum. We find that the pathogen persisted ($\sim 10^{10}$ CFU/g) for all different inoculum and concentrations of antibiotic. The antibiotic targeted B_A while B_P grew unimpeded in the absence of phage, such that B_P predominated after 96 hrs. In contrast, antibiotics and innate immunity (Fig. 5 top-right) could eliminate bacterial inoculum with high percentages of antibiotic-sensitive bacteria ($> 90\%$ of $\mathrm{B_A}$). Pathogen clearance was observed even for sub-inhibitory concentrations of ciprofloxacin. As is apparent, the antibiotic on its own cannot clear the infection, and therapeutic outcomes are only modestly improved in a narrow region of inoculum space.

Second, we assessed the effects of combining antibiotic with phage against mixed bacterial inoculum. The phage-antibiotic combination strategy failed to clear the infection for all combinations of initial conditions – consistent with the infection scenarios of the prior section. Nonetheless, bacterial concentration was $\sim$10 times smaller due to phage killing (dark blue area in Fig. 5 bottom-left) compared to the bacterial concentration from the antibiotic-only therapy (bright yellow area of Fig. 5 top-left). After 96 hours of combined treatment, the phage-sensitive population was predominant at above MIC levels while antibiotic-sensitive bacteria populated the sub-MIC levels. In contrast, a robust pathogen clearance was achieved when the phage-antibiotic combination strategy was supplemented with active innate immunity (Fig. 5 bottom-right). The synergistic interactions between phage, antibiotic, and innate immunity led to clearance of the infection for the majority of initial conditions. The clearance region even spanned sub-inhibitory concentrations of ciprofloxacin.

Finally, we note that these results apply to the case of dynamics arising among extreme phenotypes. In reality, phage-sensitive strains retain some sensitivity to antibiotics and antibiotic-sensitive strains can be infected at

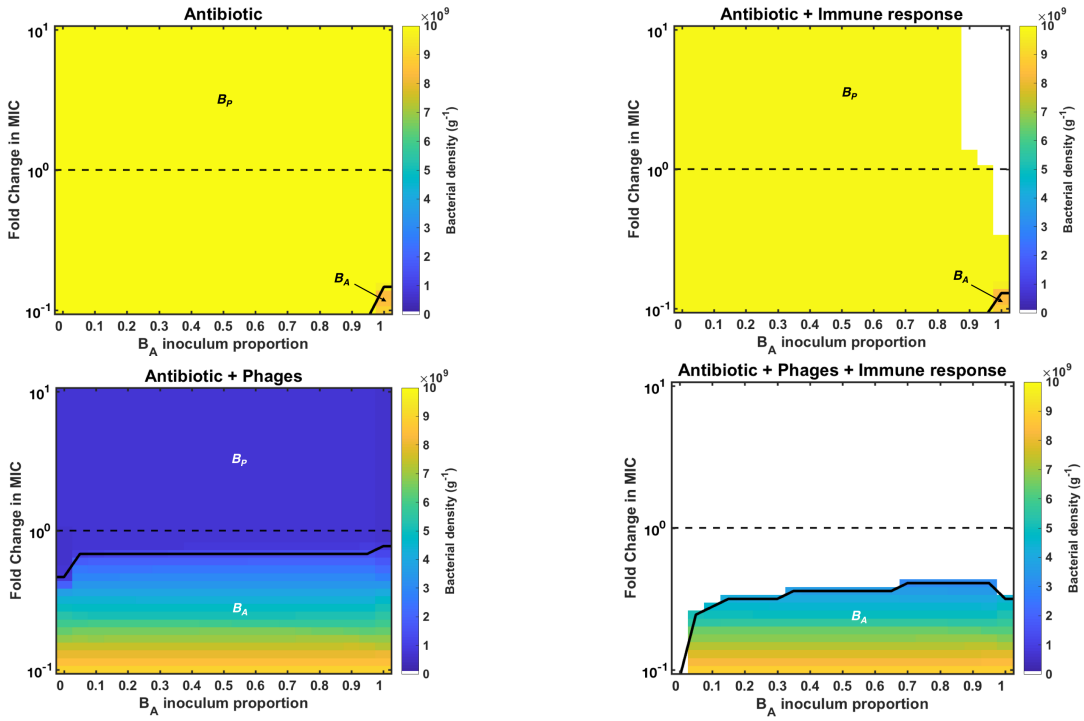

FIG. 5: **Outcomes of the robustness analysis for different antimicrobial strategies**. We simulate the exposure of bacteria to different antimicrobial strategies, such as antibiotic-only (a), antibiotic + innate immunity (b), phage + antibiotic (c), and phage-antibiotic combination in presence of innate immunity (d). The heatmaps show the bacterial density at 96 hours post infection. Colored regions represent bacteria persistence (e.g., blue areas $\sim 10^9$ CFU/g and yellow areas $\sim 10^{10}$ CFU/g) while the white regions represent pathogen clearance. We vary the concentration of ciprofloxacin (MIC = 0.014 μg/ml), ranging from 0.1 MIC (0.0014 μg/ml) to 10 MIC (0.14 μg/ml), and the bacterial composition of the inoculum ranging from 100% phage-sensitive bacteria (0% B_A) to 100% antibiotic-sensitive bacteria (100% B_A). Initial bacterial density and phage dose (c-d) are, $B_0 = 7.4 \times 10^7$ CFU/g and $P_0 = 7.4 \times 10^8$ PFU/g, respectively. Phage and antibiotic are administered two hours after the beginning of the infection.

reduced levels by phage[7,19,20]. We repeated the robustness analysis, using the extended model that incorporates quantitatively different levels of phage-infectivity and antibiotic-sensitivity of both strains (see Supplementary Materials). Partial resistance model outcomes are qualitatively consistent with previous outcomes of the extreme resistance model (contrast Fig. S2 with Fig. 5). Overall, our model analysis suggests that robust, curative success of phage-antibiotic combination therapy could be driven, in part, by a largely unrealized synergy with the immune response.

IV. DISCUSSION

We have developed a combination therapy model that combines phage and antibiotics against a mixed-strain infection of *Pseudomonas aeruginosa*. The model suggests that infection clearance arises from nonlinear synergistic interactions between phage, antibiotic, and innate immunity. Moreover, the infection clearance shows robustness to variations in the concentration of antibiotic, the bacterial composition of the inoculum, and model assumptions. In contrast, when innate immunity effects are removed from the model, the phage-antibiotic combination therapy is predicted to fail to eliminate the infection. This suggests that combined therapy may depend critically on immune response for resolving bacterial infections.

The mathematical model developed here includes multiple feedback mechanisms, but in doing so focuses on a homogeneous environment. In doing so, the model implicitly accounts for spatial heterogeneity and phage saturation effects, but it lacks explicit spatial structure. Spatial structure can be relevant therapeutically. For example, during chronic infections spatially organized bacterial aggregates of P.a. protect themselves against phage killing by producing exopolysaccharides[21]. Furthermore, modelling efforts have shown that spatial structure affects the therapeutic success of phage therapy[22] and phage-antibiotic combination therapy[23]. For example, structured environments limit phage dispersion and amplification, promoting bacterial survival and resistance acquisition[22,23]. Moreover, the heterogeneous distribution of antibiotic creates spatial refuges (of low or null antimicrobial presence) where bacteria survive and

resistant mutants arise[23]. Hence, multiples lines of evidence from experiments and computational models suggest that spatial structure effects could negatively affect the therapeutic success of phage-antibiotic combination therapy. The current model also neglects the complex features of immune response termination[24] and interactions with commensal microbes[25], both priority areas for future work.

In conclusion, the phage-antibiotic combination therapy model developed here describes our initial efforts to explore how host immunity modulates infection outcomes. As we have shown: immune clearance of pathogens may lie at the core of the curative success of combination treatments. If so, this additional synergy may help to resolve the resistance problem and also guide use of sub-MIC concentrations of antibiotics. When combined in an immunocompetent context, we find that phage-antibiotic combination therapy is robust to quantitatively and qualitatively distinct resistance profiles. These findings reinforce findings that phage and antibiotics can be used to treat a certain class of MDR *P.a.* pathogens in patients. Model results also highlight the role of the immune response in realizing curative success - which will be relevant to expanding combination theory for a range of clinical applications.

V. METHODS

A. Model simulation

The numerical integration of the combination therapy model is carried out using ODE45 in MATLAB. We obtain the temporal dynamics of two bacterial strains, phage, antibiotic, and innate immune response. Moreover, we set an extinction threshold of 1 g^{-1}; hence, when B_P or B_A densities are ≤ 1 CFU/g at any time during the simulation, we set their densities to 0 CFU/g. We run all the simulations for 96 hours (4 days), we consider this time span as biologically plausible.

B. Robustness analysis

We perform a robustness analysis of the phage-antibiotic combination therapy model by varying the initial conditions. Hence, we vary the concentration of antibiotic from sub-MIC concentrations (0.1 MIC) to above MIC concentrations (10 MIC). We use the MIC of ciprofloxacin (0.014μg/ml) for PAPS phage-resistant strain as a reference[11]. Moreover, we vary the bacterial composition of the inoculum by increasing the bacterial density of one strain (e.g., B_A) by 5% and decreasing the density of the other by 5%. Then we select a pair of initial conditions and run the model 96 hrs. Finally, we calculate total bacterial density, $B_{total} = B_A + B_P$.

C. Parameter Estimation

The values of the parameters used in the simulations of the combination therapy model are shown in Table II-III. Most of the parameter estimation was carried out in a previous work (see *Parameter Estimation* section in[5]). The parameter values of the functions describing the pharmacodynamics and pharmacokinetics of ciprofloxacin were found in literature[14,26]. Furthermore, for accounting for partial resistant strains, we searched in the literature the minimum inhibitory concentration (MIC) of the phage-sensitive strain, B_P, and the parameter δ_P which modulates the level of phage resistance of the antibiotic-sensitive strain, B_A.

For describing the pharmacodynamics of ciprofloxacin,[14] used the following Emax model,

$$K_{kill}\frac{A^H}{EC_{50}^H + A^H}$$

. Initially, they grew P.a. *in vitro* at different concentrations of ciprofloxacin (CP), generating growth curves for each concentration of CP. Then, the slopes of the growth curves were calculated; once the growth rates were obtained they generate a concentration vs growth-rate curve. Finally, the Emax model was fitted to the concentration vs growth-rate curve and the parameters, K_{kill}, EC_{50} and H, were estimated using non-linear least square regression.

In this study, the EC_{50} parameter was re-calculated using the MIC values of CP for P.a. PAPS strains. Following the definition of the MIC, i.e., the minimum concentration of antibiotic at which bacterial growth is prevented, we calculate the parameter EC_{50} by isolating the variable in the following equation,

$$r_P - K_{kill}\frac{MIC_{B_P}^H}{EC_{50}^H + MIC_{B_P}^H} = 0$$

Where r_P is the growth rate of the phage-sensitive strain, K_{kill} is the maximum killing rate of the antibiotic, and MIC_{B_P}, the minimum inhibitory concentration of CP for phage-sensitive strain B_P. We use the same procedure for calculating the EC_{50} of CP for the antibiotic-sensitive strain, B_A, .

The elimination rate, θ, of CP was obtained from[26]. Serum samples of mice infected with P.a. and treated with CP were taken over four hours. Then, the concentration of CP was measured in each sample. Finally, they performed a linear regression analysis of the terminal phase of the time vs concentration curve to obtain the elimination rate, θ.

For the probabilities of mutation, μ_1 and μ_2, we use the same values that were obtained from[27]. Therefore, the probability of generating a phage-sensitive mutant per cell division, μ_2, is the same as the probability of producing an antibiotic-sensitive mutant per cellular division, μ_1.

Parameters of the combination therapy model	Value	Estimated from
r_P, maximum growth rate of phage-sensitive (antibiotic-resistant) bacteria	0.75 h^{-1}	*P. aeruginosa* murine pneumonia model[28]
K_C, carrying capacity of bacteria	1×10^{10} CFU/g	Assuming ∼4 times above the typical bacterial density (2.4×10^9 CFU/g) in wild-type mice 24h post infection
β, burst size of phage	100	Estimated from[5]
ω, decay rate of phage	0.07 h^{-1}	Estimated from[5]
ϵ, killing rate parameter of immune response	8.2×10^{-8} g/(h cell)	Set such that ϵK_I gives the maximum granulocyte killing rate[28]
α, maximum growth rate of immune response	0.97 h^{-1}	Fitting of neutrophil recruitment data[29]
K_I, maximum capacity of immune response	2.4×10^7 cell/g	Fitting of neutrophil recruitment data[29]
K_I, maximum capacity of immune response (immunodeficient mice)	Same as I_0	No innate immune activation
K_D, bacteria concentration at which immune response is half as effective	4.1×10^7 CFU/g	Corresponds to lethal dose of about 5.5×10^6 CFU/lungs
K_N, bacteria concentration when immune response growth rate is half its maximum	10^7 CFU/g	In vitro data of TLR5 response to PAK strain[30]
B_0, initial bacteria density (in presence or absence of the innate immune response)	7.4×10^7 CFU/g	Total inoculum of 10^7 CFU
P_0, initial phage dose (in presence or absence of the innate immune response)	7.4×10^8 PFU/g	Total phage dose of 10^8 PFU
I_0, initial immune response	2.7×10^6 cell/g	Fitting of neutrophil recruitment data[29]
I_0, initial immune response (immunodeficient mice)	0 cell/g	Assuming no primary innate immunity
Heterogeneous mixing (HM) model parameters		
$\tilde{\phi}$, nonlinear phage adsorption rate	5.4×10^{-8} $(g/PFU)^{\gamma}$ h^{-1}	Estimated from[5]
γ, power law exponent in phage infection rate	0.6	Estimated from[5]
Phage saturation (PS) model parameters		
ϕ, linear phage adsorption rate	5.4×10^{-8} (g/PFU) h^{-1}	Estimated from[5]
P_C, Phage concentration at which phage infection rate is half-saturated	1.5×10^7 PFU/g	Estimated from[5]
Linear phage infection (LI) model parameters		
ϕ, linear phage adsorption rate	5.4×10^{-8} (g/PFU) h^{-1}	Estimated from[5]

TABLE II: Microbiology and phage-associated parameter values.

1. Accounting for partial resistance in the extended phage-antibiotic combination therapy model

To account for partially resistant strains, we extend our combination therapy model (Equations A1-A5) and include the parameters, EC_{B_P} and δ_P . EC_{B_P} is the half-saturation constant of the antibiotic killing function and modulates the level of antibiotic resistance for B_P. The parameter was calculated based on the MIC of CP for PAPS phage-sensitive strain[7]. Moreover, for modulating the level of phage-resistance of B_A we use $\delta_P < 1$. We find δ_P value by searching on the literature the percentage decrease in phage adsorption rate for phage-resistant mutants (see Table IV).

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Antibiotic (ciprofloxacin) parameters		
K_{kill}, maximum antibiotic killing rate	18.5 h^{-1}	Fitting an Emax model to antibiotic kill curves[14]
EC_{50}, concentration of antibiotic at which the killing rate is half its maximum	0.3697 $\mu g/ml$	Calculated using the MIC of ciprofloxacin for the phage-resistant PAPS strain[7]
EC_{B_P}, concentration of antibiotic at which the killing rate is half its maximum	4.070 $\mu g/ml$	Calculated using the MIC of ciprofloxacin for the phage-sensitive PAPS strain[7]
H, hill coefficient	1	From[14]
MIC, Minimum inhibitory concentration of ciprofloxacin for P.a. PAPS phage-resistant strain	0.014 $\mu g/ml$	From[7]
MIC, Minimum inhibitory concentration of ciprofloxacin for P.a. PAPS phage-sensitive strain	0.172 $\mu g/ml$	From[7]
θ, antibiotic elimination rate from serum samples	0.53 h^{-1}	Estimated from antibiotic concentration vs time curves; concentration of ciprofloxacin was measured in serum samples of P.a.-infected mice[26]
Antibiotic-sensitive bacteria parameters		
μ_1, probability of emergence of antibiotic-sensitive (phage-resistant) mutants per cellular division	2.85×10^{-8}	Estimated from experimental measurements[27]
μ_2, probability of emergence of phage-sensitive (antibiotic-resistant) mutants per cellular division	2.85×10^{-8}	Approximated to the estimates from[27]
r_A, maximum growth rate of antibiotic-sensitive (phage-resistant) bacteria	0.675 h^{-1}	10% trade-off between resistance against phages and growth rate[31]

TABLE III: Additional parameter values associated with the effects of antibiotics.

Phage adsorption rate of phage-sensitive strain (%)	Phage adsorption rate of phage-resistant strain (%)	Fold decreased phage adsorption rate	Ref
31.3%	9.6%	3.2	[19]
73.7%	66.5% to 2.4%	∼1.1 to ∼30	[32]
87.2%	42.8%	2	[32]
92.7%	55.3% and 42.8%	1.6 and 2	[20]

TABLE IV: Phage adsorption rate of phage-sensitive and phage-resistant *Pseudomonas aeruginosa* strains.

Appendix A: Extended *In vivo* Combination Therapy Model

The extended *in vivo* combination therapy model describes a system where two bacterial strains partially sensitive to both, phages and antibiotic, interact with an active host immune response:

$$\dot{B}_P = r_P B_P \left(1 - \frac{B_P + B_A}{K_C}\right)(1-\mu_1) - B_P F(P) + \mu_2 r_A B_A \left(1 - \frac{B_P + B_A}{K_C}\right)$$

$$- \overbrace{K_{kill} \frac{A^H}{EC_{B_P}^H + A^H} B_P}^{\text{Antibiotic killing}} - \overbrace{\frac{\varepsilon I B_P}{1 + \frac{(B_P + B_A)}{K_D}}}^{\text{Immune Killing}}, \tag{A1}$$

$$\dot{B}_A = r_A B_A \left(1 - \frac{B_P + B_A}{K_C}\right)(1-\mu_2) + \mu_1 r_P B_P \left(1 - \frac{B_P + B_A}{K_C}\right) - \overbrace{\delta_P B_A F(P)}^{\text{Lysis}}$$

$$- K_{kill} \frac{A^H}{EC_{50}^H + A^H} B_A - \overbrace{\frac{\varepsilon I B_A}{1 + \frac{(B_P + B_A)}{K_D}}}^{\text{Immune Killing}}, \tag{A2}$$

$$\dot{P} = \tilde{\beta} B_P F(P) - \overbrace{\delta_P \tilde{\beta} B_A F(P)}^{\text{Viral release from } B_A} - \omega P, \tag{A3}$$

$$\dot{I} = \overbrace{\alpha I \left(1 - \frac{I}{K_I}\right)\left(\frac{B_P + B_A}{B_P + B_A + K_N}\right)}^{\text{Immune stimulation}}, \tag{A4}$$

$$\dot{A} = A_I - \theta A \tag{A5}$$

In this model, the parameter EC_{B_P} modulates the level of antibiotic resistance of the phage-sensitive bacteria, B_P (Equation A1) and $0 \leq \delta_P \leq 1$ modulates the level of phage-resistance of B_A (Equation A2). Finally, we add a new viral release term in the phage dynamics that accounts for the phage infection of antibiotic-sensitive bacteria (Equation A3).

Appendix B: Supplementary Text and Analysis: bacterial dynamics of the partial resistance model given exposure to phage and antibiotic

We simulate the partial resistance model under an immunocompetent and immunodeficient context. When the combined therapy was applied under an immunodeficient context, the pathogen persisted regardless of the initial bacterial inoculum. The antibiotic dose was ineffective against the partially-sensitive B_P leading to a phage-bacteria coexistence state. On the other hand, the addition of the immune response into the previous immunodeficiency state facilitated the elimination of the infection for both inoculum levels, phage-sensitive (Fig. S1, bottom-left) and the antibiotic-sensitive (Fig. S1, bottom-right).

The combined synergistic effect between phage, antibiotic, and innate immune response lead to infection clearance. Our results suggest that even for bacterial strains that remain partially sensitive to both phages and antibiotic, the presence of the host innate immunity is still necessary to clear the infection. Hence, the outcomes of the phage-antibiotic combination therapy model are robust to model extensions that account for partially resistant strains.

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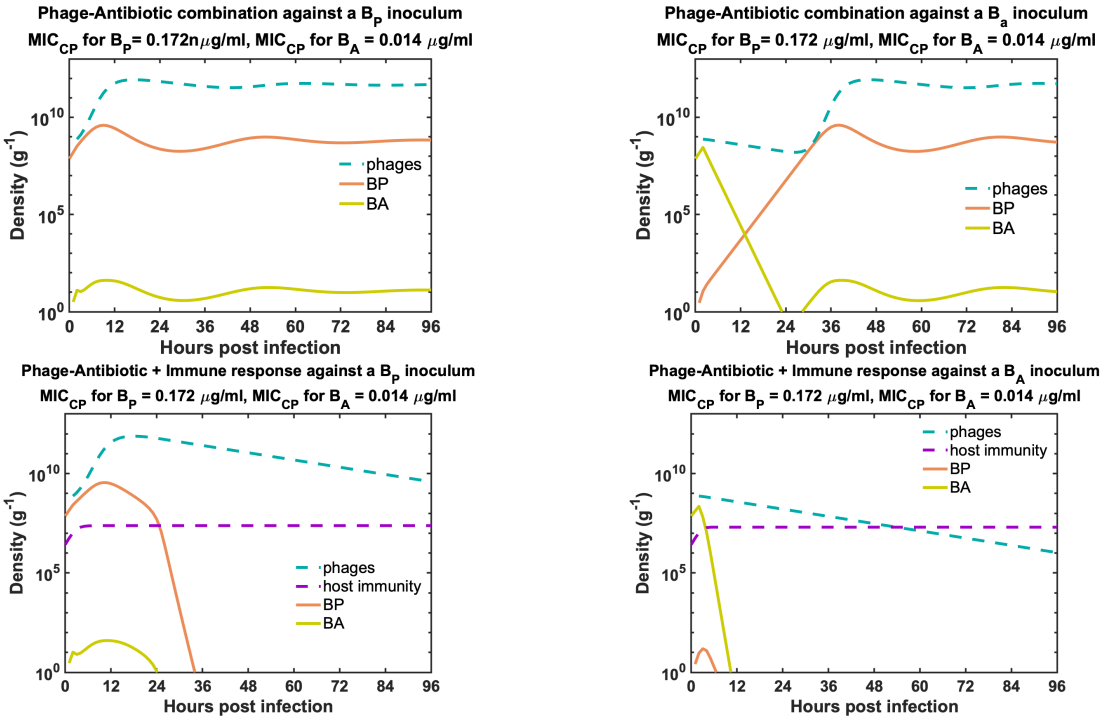

FIG. S1: **Time series of the partial resistance model in presence and absence of host immune response**. We simulate the combination of phage and antibiotic against a phage-sensitive (a) and an antibiotic-sensitive (b) bacterial inoculum in the absence of the immune response. Moreover, we simulate a within-host scenario where the combined therapy interact with the immune response (purple dashed line) and phage-sensitive bacteria (c) or antibiotic-sensitive bacteria (d). Here, phage (blue dashed line) and antibiotic are administered two hours after the infection. Initial conditions are, $B_0 = 7.4 \times 10^7$ CFU/g and $P_0 = 7.4 \times 10^8$ PFU/g. The concentration of antibiotic (0.0350 $\mu g/ml$ = 2.5×MIC for B_A strain) is maintained constant during the simulation, data not shown. The simulation run was 96 hours (4 days).

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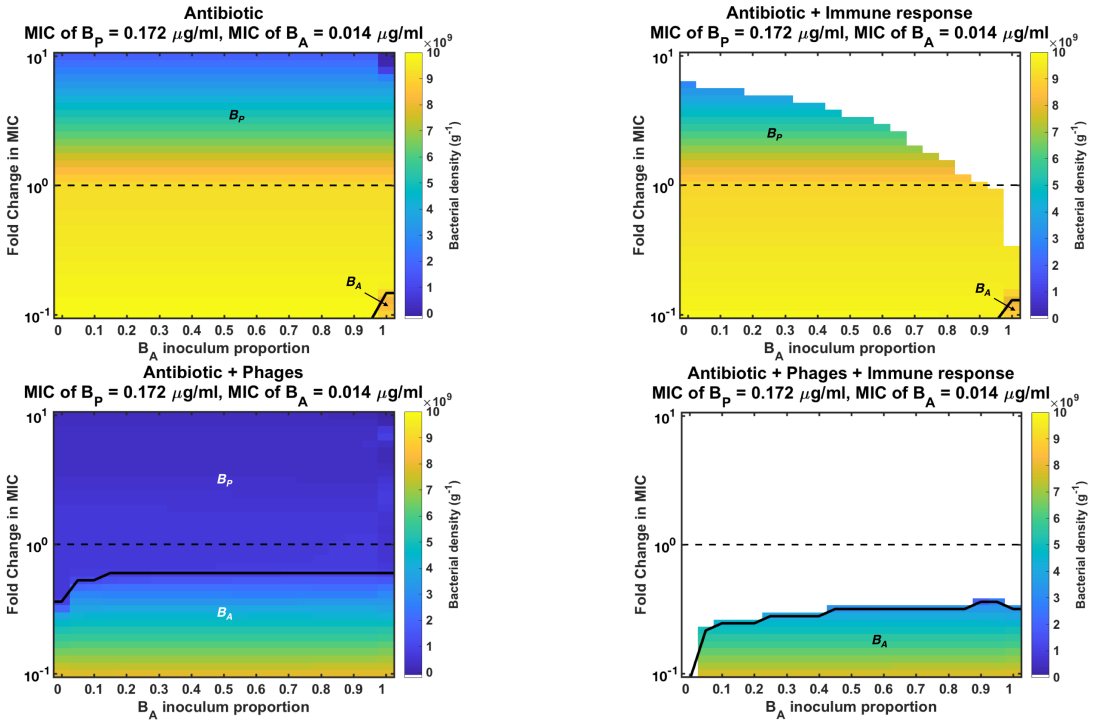

FIG. S2: **Robustness analysis of the extended model for different antimicrobial strategies**. Bacteria grew for 96 hours exposed to different antimicrobial strategies, antibiotic-only (a), antibiotic + innate immunity (b), phages and antibiotic (c), and phage-antibiotic combination plus active innate immunity (d). The heatmaps show the bacterial density at 96 hours post infection. Colored regions represent bacteria persistence while the clearance of the infection is represented by white regions. For the partial resistance model, phage can infect B_A with an infection constant of $\phi\delta_P$, being $\delta_P = 0.1$. Moreover, the phage-sensitive strain, B_P, is slightly sensitive to the antibiotic with an MIC of 0.172 μg/ml. We simulate different concentrations of ciprofloxacin (CP); using the MIC of CP for the B_A strain as a reference (MIC = 0.014 μg/ml). Fold changes in MIC go from 0.1 MIC (0.014 μg/ml) to 10 MIC (0.14 μg/ml). Furthermore, bacterial composition of the inoculum ranges from 100% phage-sensitive bacteria (0% B_A) to 100% antibiotic-sensitive bacteria (100% B_A). Initial bacterial density and phage dose (c-d) are, $B_0 = 7.4 \times 10^7$ CFU/g and $P_0 = 7.4 \times 10^8$ PFU/g, respectively. Phage and antibiotic are administered two hours after the beginning of the infection.

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