Mathematical modeling suggests that benefits of short or long antibiotic treatment depend on details of infection

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Abstract

Antibiotics are the major tool for treating bacterial infections. In the case of acute bacterial infections, which last for several days, a typical recommended treatment is 7-14 days long. Because of the variety of bacterial infections humans are exposed to, for many infections the duration of antibiotic treatment has not been tested in randomized clinical trials. Recently, the necessity of a relatively long antibiotic treatment has been questioned, including with the use of mathematical models suggesting that longer treatment may in fact result in poorer outcomes, for example, in increased antibiotic resistance. Other studies have shown that longer treatment is needed to guarantee bacterial control, lending support to the notion of a definite optimum duration. By using a mathematical model for a generic intracellular bacterial infection, here we show that it is impossible to select for universally optimal treatment duration. In particular, short (3 day) or long (7 day) treatments may be both beneficial depending on the time when the treatment is started, on the metric used to define successful treatment, and on the antibiotic efficacy (defined as the antibiotic kill rate). Our results strongly suggest that generic predictions on the optimality of antibiotic treatment duration are unlikely to be practical. Better quantitative understanding of details of actual within-host dynamics of bacterial infections in humans, of pathology generated during the infection, of the bacterial or immune response thresholds at which patients seek treatment, and of how immunity is involved in infection control should be instrumental to meaningfully guide rational therapy.

Keywords: mathematical model, antibiotic therapy, intracellular infection, antibiotic resistance, optimization

1. Introduction

The treatment of bacterial infections has for many decades relied on the use of antibiotics. Although antibiotics have saved many lives and enabled uncountable medical practices, due to the widespread use of antibiotics in human and animal populations, we are now facing the rise of antibiotic resistance, posing a serious threat to human health and modern medicine [1]. Of particular concern is the rise of multidrug-resistant bacteria, favoured by use of wide-spectrum antibiotics especially in clinical settings [2, 3]. To confront the challenges posed by antibiotic resistance, much research has been devoted to understand the molecular, genetic, and non-genetic mechanisms leading to drug resistance phenotypes in bacteria [4, 5, 6], and to study principles for a more rational administration of antibiotics [7, 8, 9].

While alternative approaches such as anti-virulence [10] or host-directed therapies [11] are also being considered for infections, bringing their own potential adverse outcomes [12], avoiding antibiotic overuse remains an essential step in addressing the antibiotic resistance crisis and reducing the adverse

long-term collateral effects of antibiotic use on human health [13]. In this context, it is important to understand the rational principles by which antibiotics succeed and fail in clearing infections, and whether such principles hold across different scenarios. Until now, despite many studies on the experimental, clinical and theoretical side, we lack a conclusive framework for when moderate treatments are superior to aggressive ones, and what the minimum effective treatment duration should be. Conceptual frameworks are continuously being attempted to assist clinicians with problem recognition and guidance through a logical sequence of questions and steps during antibiotic prescribing [14].

In the case of acute bacterial infections, which last for several days, a typical recommended treatment is 7-14 days long [15]. Because of the variety of bacterial infections humans are exposed to, for many infections the duration of antibiotic treatment has not been tested in randomized clinical trials. Recently, the necessity of a relatively long antibiotic treatment has been questioned [15, 16, 17], including with the use of mathematical models [18, 19] suggesting that longer treatment may in fact result in poorer outcomes, for example, in increased antibiotic resistance. Other studies have shown that longer treatment is needed to guarantee bacterial control [20, 21], suggesting that an optimum duration exists and should be adopted when treating bacterial infections.

An increasing number of clinical studies on bacterial infections are showing that shorter treatment may be as effective as longer treatment, while minimizing adverse effects and resistance risk [22, 23, 15, 17]. Support for shorter durations of therapy than previously administered has been growing for infections such as community-acquired pneumonia, ventilator-associated pneumonia, intraabdominal infections, urinary tract infections, cellulitis, and gram-negative bacteremia [15].

In comparative clinical studies, the definitions of 'short' and 'long' vary, sometimes to reflect a comparison between 3-and 7-day treatment with the same antibiotic, or single-dose vs. multiple-doses over time of two different drugs [21]. Typically, the endpoint from clinical trials is a snapshot measurement at a given period after conclusion of therapy, e.g. bacteriologic outcome or clinical symptom resolution at 7 or 28 days post-therapy. While clinical symptoms and bacteriologic outcome are not always in perfect synchrony, another difficulty hampering reconciliation of infection processes with clinical patient data is the lack of frameworks for monitoring and analyzing whole infection course.

On the theoretical front, models tend to address infection as a multi-factorial dynamic process, resulting from a complex and nonlinear interplay of host, pathogen and intervention characteristics. Successful control is expected to involve a deep understanding of the underlying infection ecology. Mathematical approaches have helped uncover many key aspects of antibiotic resistance dynamics: the role of fitness differences between sensitive and resistant pathogens [24], compensatory mutations [25], the competition dynamics between different sub-populations within host [26], the role of the host immune response and the mode of antibiotics administration [19, 27, 18].

Yet, links between models, data and clinical applications remain scarce. Despite increasing research efforts in recent years, we are far from knowing a-priori what is the best treatment strategy for a given patient [9], and predicting the onward consequences of such treatment for the population. Many studies have attempted the question of comparing different treatment strategies but they have typically focused on dosing strategies, seeking a single criterion for defining optimality, e.g. resistance emergence or resistance selection [24, 28], overlooking other measures such as duration of infection and bacterial load, and neglecting other dimensions of treatment administration such as its timing and duration [29].

In this paper we advocate for a more integrative approach, in addressing this question, by considering simultaneously different criteria, and seeking treatments that optimize each one. We study an intracellular bacterial infection system under antibiotic treatment. The key processes we focus on are target-cell limitation, intracellular replication and extracellular death processes of bacteria, and feedbacks with the host's adaptive immune response. These apply broadly to a wide set of bacterial pathogens that adopt the intracellular lifestyle, including mycobacteria, *Salmonella*, and *Listeria monocytogenes* [30, 31, 32].

For such pathogens, persistence within specialized phagocytic host cells may imply also being protected from the action of antibiotics [33], which are typically more effective against extracellular bacteria. We specifically address the question of the relative effectiveness of short vs. long therapy. Quantifying infection pathology in terms of bacterial burden, infection duration, deviation from homeostatic physiological values, and selection of antibiotic-resistant bacteria, we rank quantitatively the success of specific treatments. Our aim is to investigate how infection processes and antibiotic treatment interact to determine final outcome, and how optimal treatments depend, on one hand, on host and pathogen characteristics, and, on the other hand, on the target criterion for optimization.

2. Results

2.1. Mathematical model

Our model tracks the interplay between extracellular and intracellular bacterial sub-populations during infection, and shares elements with previous models of intracellular infection dynamics [34, 35, 36, Figure 1A], and with models of bacteria-phagocyte interaction within host [29, 37]. The extracellular bacteria are denoted by B, while intracellular bacteria persist in the infected macrophage compartment denoted by I. The model considers two bacterial phenotypes: drug-sensitive B_s (I_s), and drug-resistant B_r (I_r) while the total density is given by $B = B_s + B_r$ (and $I = I_s + I_r$). The baseline dynamics of uninfected macrophages M are modeled via a logistic growth with parameters r and K, where K denotes the maximum number of healthy macrophages in the absence of infection, and r their replenishment rate. The infection of healthy macrophages by extracellular bacteria is modelled via mass-action kinetics at infection rate β. After engulfment by phagocytes, intracellular bacterial growth is described through the parameter N, the burst size. Infected macrophages I_s and I_r can burst through necrosis at rate δ and they can die via bacteria-induced apoptosis at rate a. In the extracellular environment, free bacteria are cleared at rate c.

Phenotypic diversity between the two bacterial sub-populations is expressed in a different burst-size $N(1-\gamma)$ for infected macrophages with resistant bacteria I_r, representing an intracellular fitness cost of resistance $0 < \gamma < 1$. The second assumption on the resistant phenotype is that of high level resistance (HLR), meaning the antibiotic, when applied, has no effect on extracellular bacteria B_r. We examine infections that start as fully sensitive ($B_r(0) = 0$), but allow resistance to arise from mutation during intracellular replication. The HLR mutation probability per drug-sensitive cell per division is denoted by m. Backward mutation is assumed negligible.

Antibiotic treatment gets triggered when the total extracellular bacterial density B reaches the level Ω, i.e., the symptom threshold, and is administered for τ days of duration with antibiotic efficacy defined by the killing rate A_m. Ignoring pharmacodynamics, A_m in our formulation, represents effectively the average net rate of antibiotic-induced extracellular bacterial killing at the infection site, per unit of time. We assume an extinction threshold B_{ext} when either extracellular or intracellular bacterial compartment falls below this level. For resistance emergence, we also adopt an "emergence threshold", to prevent spurious generation from unrealistically low numbers of cells ($B_{emergence} = B_{ext}$).

For bacterial infections of humans it is generally poorly understood which types of immunity – innate or adaptive – are most important in control of infections. Here we do not make an explicit distinction between innate and adaptive immunity, and rather implement immune response kinetics generically via two mathematical features: antigen-dependent stimulation and negative feedback on infected macrophages. The major assumption we make in modeling immune response is that immune response gets triggered after extracellular bacteria reach some density, defined by the half-saturation constant k. When bacterial density is high, immune response magnitude increases at maximal rate σ until infection is cleared. This immune response eliminates the infected macrophages at rate ν. The initial immune response level is

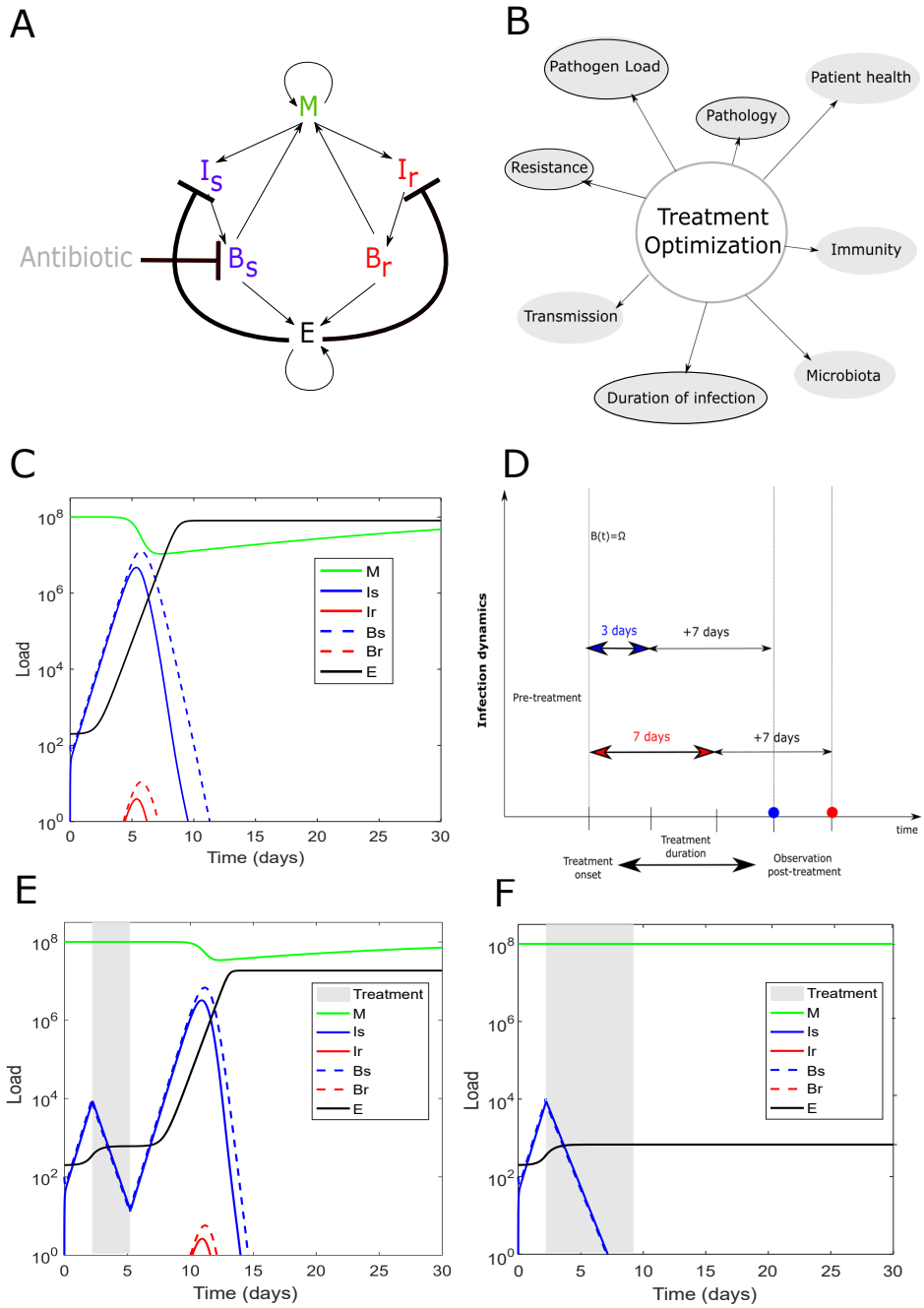

Figure 1: Basic framework and examples of simulations. Panel A shows schematic representation of a generic mathematical model of intracellular bacterial infection (see Eqns. (1)–(6)). Panel B illustrates different optimization target criteria for antibiotic treatment. In panel C we simulate the dynamics of a bacterial infection (in accord with Eqns. (1)–(6) using parameters from Table 1) in the absence of treatment. In panel D we show the schematic of timeline of a treatment which is started when bacterial density reaches Ω, and which is continued for 3 or 7 days with outcome being measured 7 days after the treatment stop. In panels E-F we illustrate the infection dynamics for short (panel E) or long (panel F) treatment with antibiotics. In these simulations $\Omega = 10^4$, corresponding to the treatment starting at 2 days post infection, and $A_m = 11/\text{day}$, corresponding to a reduction in the half-life of extracellular bacteria by $T_{1/2} = \ln(2)/A_m = 1.5$ h. Pathology dynamics associated with panels E-F are shown in Figure S1.

given by $E(0) = E_0$. Immune competence can thus be expressed in terms of four parameters: higher initial level E_0, higher activation rate σ, lower antigen threshold k required for half-maximal stimulation, or higher killing rate v of infected macrophages. The dynamics are captured by the following system of ordinary differential equations:

$$\frac{\mathrm{d}M}{\mathrm{d}t} = rM\left(1 - \frac{M}{K}\right) - \beta MB, \tag{1}$$

$$\frac{\mathrm{d}I_s}{\mathrm{d}t} = \beta MB_s - I_s(\delta + a + vE), \tag{2}$$

$$\frac{\mathrm{d}I_r}{\mathrm{d}t} = \beta MB_r - I_r(\delta + a + vE), \tag{3}$$

$$\frac{\mathrm{d}B_s}{\mathrm{d}t} = (1 - m)NI_s\delta - \beta MB_s - (c + A_m)B_s, \tag{4}$$

$$\frac{\mathrm{d}B_r}{\mathrm{d}t} = \delta N[(1 - \gamma)I_r + mI_s] - \beta MB_r - cB_r, \tag{5}$$

$$\frac{\mathrm{d}E}{\mathrm{d}t} = \sigma E\frac{B}{B + k}. \tag{6}$$

As conceptually highlighted by [38], optimizing antibiotic treatment is likely to involve multiple different aims, including reducing bacterial load, minimizing antibiotic resistance, reducing the duration of infection (Figure 1B). In our analyses we use several instantaneous and cumulative measures. Instantaneous measures include the total density of bacteria $B(t)$, density of drug-resistant bacteria $B_r(t)$, or overall pathology $H(t)$. Pathology translates from infection variables to host 'health' and typically results from a combination of pathogen-intrinsic and host-intrinsic factors, likely to vary across hosts [39]. We adopt one simple approach in which pathology (between 0 and 1) is defined as the deviation of variables, e.g, uninfected macrophages $M(t)$, bacterial load $B(t)$, or adaptive immunity levels $E(t)$) from their initial values. The instant pathology resulting from bacterial growth is denoted by $H^B(t)$, the one resulting from immune activation by $H^E(t)$, and the one resulting from healthy macrophage depletion $H^M(t)$. An additional parameter, g, scales the importance of changes in each of these infection variables (deviations from 'homeostasis') for host health; the higher g, the less tolerant the host. Cumulative pathology is derived by integrating instant pathology over time:

$$H^B(t) = 1 - \left(\frac{B_{ext}}{B(t)}\right)^g; \quad H^B_{tot}(T) = \int_0^T H^B(t)dt, \tag{7}$$

$$H^E(t) = 1 - \left(\frac{E_0}{E(t)}\right)^g; \quad H^E_{tot}(T) = \int_0^T H^E(t)dt, \tag{8}$$

$$H^M(t) = 1 - \left(\frac{K}{M(t)}\right)^g; \quad H^M_{tot}(T) = \int_0^T H^M(t)dt. \tag{9}$$

Other cumulative infection measures include: duration of infection, which is the period from $t = 0$ up to the point where all populations of bacteria reach extinction, pathogen burden $B_{tot}(T) = \int_0^T B(t)dt$, and resistance burden $R_{tot}(T) = \int_0^T B_r(t)dt$. In contrast to instantaneous metrics, cumulative metrics capture more accurately total infection history.

2.2. Dynamics of the mathematical model

Because our mathematical model does not allow bacterial escape from immunity, with non-decreasing immune response (Eqn. (6)), the model yields by default a typical acute self-limiting dynamics in the absence of antibiotics, with some role played by target-cell limitation, extracellular bacterial death and

Parameter	Interpretation	Default	Range
r	Growth rate of uninfected macrophages	0.09/day	
K	Carrying capacity of macrophages	10^8	
β	Infection rate of macrophages by bacteria	1.2×10^{-7}/day	
δ	Necrosis rate of infected macrophages	0.2/day	
a	Apoptosis rate of infected macrophages	12/day	
N	Burst size of infected macrophages with drug-sensitive bacteria	100	
γ	Fitness cost of high-level resistance, HLR (reduction factor for burst size of B_r)	0.1	
c	Death/removal rate of bacteria in the extracellular environment (e.g. by neutrophils)	2/day	
σ	Maximal growth rate of the adaptive immune response	2/day	
k	Half-saturation constant for antigen-dependent immunity	10^4	$10^3 - 10^4$
m	Mutation probability leading to HLR per bacterial cell	10^{-7}	
A_m	Antibiotic-induced killing rate on drug-sensitive bacteria	10/day	1 – 20/day
v	Killing rate effected by adaptive immunity on infected macrophages	1×10^{-5}/day	
B_{ext}	Pathogen extinction threshold	0.1	
$B_{emergence}$	Resistance emergence threshold	0.1	
B_0	Initial inoculum	100	
E_0	Initial immunity	200	
g	Tolerance factor for pathology induction from infection variables	0.1	
Ω	Bacterial density at which treatment starts	10^5	$10^3 - 10^6$

Table 1: Parameters of the mathematical model on the intracellular bacterial infection. Mathematical model is given in Eqns. (1)–(6). We show the default set of parameters used in simulations as well as the range of parameters varied in some of our analyses. Most of these parameters have been chosen to give a reasonable dynamics for the bacteria and immune response (e.g., rapid bacterial and immune response growth). Yet, it should be noted that few if any of these parameters have been accurately measured for bacterial infections of humans. Our analytical and numerical analyses (see Supplementary material Text S1 and [40]) should provide insights on generic extrapolation of infection phenomena beyond the specific numerical values assumed here.

restricted intracellular persistence (Figure 1C and see Supplementary Text S1). In such a system, addition of antibiotics helps to limit infection-induced damage, lowering infection levels, and controlling its progression. The duration of treatment and the efficacy of antibiotics (defined by the kill rate A_m) determine the probability that infection is controlled (Figure 1E&F). Indeed, if the antibiotic treatment starts early, is short (3 days), and the antibiotic kill rate is moderate, by the end of treatment bacteria may not be eliminated (Figure 1E). Moreover, because of the intrinsic dependence of the immune response expansion kinetics on bacterial density, immune response can stop expanding during treatment, thus, allowing for post-treatment relapse. In contrast, under the same conditions, longer, 7-day treatment results in a better outcome of bacterial clearance (Figure 1F). In this specific example, other measures of pathology show a consistent pattern which suggests benefits of the longer treatment (Figure S1).

2.3. Long treatment is beneficial under some circumstances

To further understand conditions under which longer treatment may be beneficial we performed simulations in which we varied the duration of treatment (3 vs. 7 days), the kill rate of the antibiotic ($A_m = 10$/day vs. $A_m = 15$/day), and the threshold at which treatment is started ($\Omega = 10^3$ vs. $\Omega = 10^6$, Figure 2). In this example, antibiotic kill rates were chosen to ensure bacterial decline during therapy ($A_m > 7$/day, see Figure S3). When comparing 3- vs. 7-day treatment, we observe that the difference in

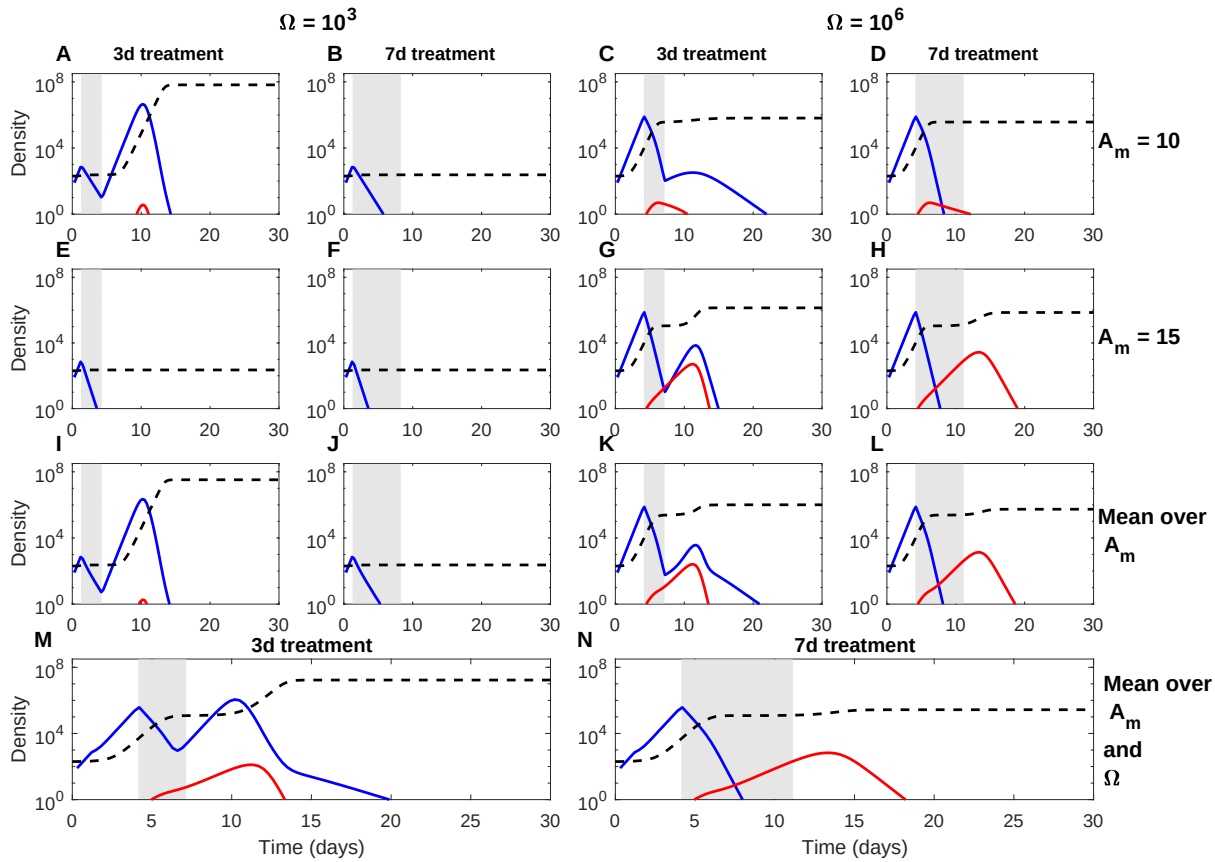

Figure 2: Advantage of short (3 days) or long (7 days) treatment depends on the antibiotic efficacy (defined by the killing rate A_m) and the bacterial load at which treatment is started (defined by Ω). We simulated dynamics of intracellular bacterial infection (see Eqns. (1)–(6)) assuming that infection starts with antibiotic-susceptible bacteria and varied the duration of the antibiotic treatment (3 or 7 days, denoted by gray rectangulars), bacterial density at which treatment is initiated ($\Omega = 10^3$ or $\Omega = 10^6$), and the antibiotic-induced kill rate of extracellular bacteria ($A_m = 10$/day or $A_m = 15$/day). In all plots, the solid blue line is for susceptible bacteria (B_s), the solid red line is for resistant bacteria (B_r), and the black dashed line is for kinetics of immunity (E). Panels **A-H** show the dynamics for the default set of parameters given in Table 1 and with specific values for Ω and A_m ($\Omega = 10^3$ for panels **A**, **B**, **E**, **F**, and $\Omega = 10^6$ for panels **C**, **D**, **G**, **H**; $A_m = 10$/day for panels **A-D** and $A_m = 15$/day for panels **E-H**). In addition, we calculated the average dynamics of the cell populations by summing up population densities for two different values of A_m (panels **I-L**). Specifically, cell dynamics in panel **I** was found by averaging bacterial and immune response densities shown in panels **A** and **E**. Finally, panels **M-N** show the typical bacterial dynamics averaged over both A_m and Ω for the 3-day and 7-day duration treatment (performed in the same way as described for panels **I-L**).

efficacy between the two durations is bigger if the treatment starts early ($\Omega = 10^3$), with longer-duration treatment being more efficient in achieving clearance. On the other hand, if treatment starts later, at

higher bacterial loads and consequently higher expected levels of immune response, the two treatments perform similarly, the main qualitative difference being that longer treatment selects more resistance. By prolonging the competitive suppression of drug-sensitive bacteria, longer treatment favours more the ascent of the less-fit drug-resistant bacteria, and the effect is exacerbated at higher antibiotic kill rates.

2.4. Short vs. long duration superiority varies with instantaneous metrics

To understand more systematically the impact of the duration of treatment on the likelihood of bacterial clearance, we performed another set of simulations in which we varied antibiotic kill rate A_m more widely (in $[1, 20]$/day, where 30% of the interval is in the non-inhibitory range, and 70% in the inhibitory one), and determined whether treatment resulted in bacterial clearance. We defined treatment success as $B(t) < B_0$ at observation point post-treatment, corresponding to bacterial clearance, a condition intended to mimic clinical trials.

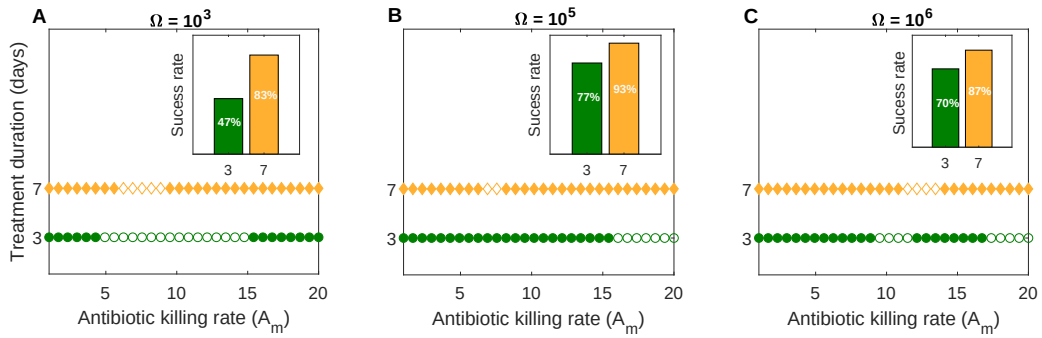

Figure 3: Relative effectiveness of longer treatments depends on antibiotic kill rate and symptoms/treatment threshold. We simulated dynamics of intracellular bacterial infection using a mathematical model (given in Eqns. (1)–(6)) assuming that infection starts with antibiotic-susceptible bacteria and varied the duration of the antibiotic treatment (3 or 7 days), bacterial density at which treatment is initiated ($\Omega = 10^3$, panel **A**; $\Omega = 10^5$, panel **B**; $\Omega = 10^6$, **C**), and the antibiotic-induced kill rate A_m from 1 to 20 per day. Treatment was considered to be successful if 7 days post-treatment bacterial density $B(t)$ was lower than its initial value ($B(t) < B_0$), otherwise, treatment was considered as a failure. Successful or failed treatments are denoted by filled or empty markers, respectively. Inserts in every panel indicate the overall success rate for treatments when averaging treatments over different values of the antibiotic kill rate.

We observed variation in efficacy between the two treatment durations being dependent on the kill rate and the timing of treatment relative to the natural infection course (Figure 3). Overall, 3-day duration treatment led to less infection resolution in early treatment onsets when compared to the 7 day duration treatment. For early treatment, there is 47% success rate with 3-day treatment duration, and 83% with 7-day treatment duration. However, when we increase the treatment onset, infection resolution increases in both cases, producing more similar success rate in intermediate to late treatment onsets. Moving to intermediate $\Omega = 10^5$, there is an improvement in effectiveness for both treatments: short treatment achieves a 77% of clearance rate, while long treatment achieves 93% success. When treatment is applied at a later stage of infection ($\Omega = 10^6$), 3-day treatment duration becomes less successful than before, (70% of cases), but so does also long treatment: it only works in 87% of antibiotic killing rates. This supports that later-applied treatment induces more complications, mainly because of resistance selection.

This increase and then decrease in efficacy with treatment timing, can be explained with the kinetic balance between two processes: on one hand, at later stages of infection there is more immunity, contributing to the overall infection clearance, thus boosting the success of any treatment (increasing success pattern), but at the same with higher Ω, there is more opportunity for drug-resistant bacteria to have

emerged by the time treatment begins. Thus, especially at such high bacterial loads, if resistance selection is a much stronger force counteracting any benefits of higher immunity, it can diminish the net rate of treatment success (decreasing success pattern). One can see the failures on the left (Figure 3A) as treatment interfering with immunity, where possible relapses are dominated by drug-sensitive bacteria. In contrast, the failures on the right (Figure 3C) can be attributed to resistance emergence and selection, where possible relapses post-treatment, this time with drug-resistant bacteria, prevent microbiologic resolution of infection (see Supplementary Figure S4-Figure S5).

2.5. Patterns of instantaneous comparison depend on total amount of drug and may mask rate of change

In our analysis we assumed that the duration of treatment and the antibiotic kill rate can be varied independently. An alternative approach may be to assume that the overall amount of killing induced by the drug is conserved; this could roughly correspond to limiting the total amount of the drug given to a patient. Making the total kill rate constant leads to 3-day treatment being more 'aggressive' with a higher kill rate and 7-day treatment being relatively milder with a lower kill rate. In that case (see Figure S7) the two treatment durations have similar success rate for low Ω but the superiority of '7-day and mild' treatment increases with Ω, instead of decreasing as in Figure 3, indicating inappropriateness of "too high kill rates for too long" at later stages of infection.

Another point to keep in mind is that the instantaneous metric of success describing whether bacterial density is above a certain level (e.g. B_0) may not be 100% indicative of the state of infection: it does not tell us about bacterial rate of change at that point; indeed, bacteria could be decreasing or increasing. Thus, apparent successful resolution with a 7-day duration measured according to this criterion (e.g. $A_m \approx 11$ in Figure 3C), could hide underneath a population of bacteria persisting or growing at low level, while apparent unsuccessful resolution with a 3-day treatment, might in fact indicate an infection on its way to clearance. This is what our simulations confirm (see Figure S6), pointing to certain extreme scenarios of resistance selection in the late- and long- treatment combination. Such scenarios highlight a dichotomy between 'acute growth' and 'long low persistence' as two modes of resistance selection through antibiotic intervention. Short treatments will tend to select for the first mode. In contrast, longer treatments may yield in some cases indefinite persistence of resistant bacteria at levels 'invisible' to the host immune response.

2.6. Short vs. long duration superiority varies with cumulative metrics

While instantaneous measures may be strong determinants of health, they reveal little about the past trajectory of an infection, and it is expected that cumulative infection history may be equally or more important for health. In addition, cumulative bacterial burden is typically a direct indicator of transmission potential to other hosts. Next, we zoom deeper into such infection outcomes, and examine quantitatively the comparison between 3-day and 7-day treatment, accounting for infection history. We compare infection duration, bacterial burden, resistance selection, and pathology. Along a spectrum of low-to-aggressive kill rates, we notice that there is great variability in quantitative infection measures for short and long duration treatment (Figure 4).

Statistical comparison of 3-day and 7-day treatment outcomes (see Table S1), based on infection duration, total bacterial burden, and resistance burden, over antibiotic killing rates $A_m \in [1, 20]$, reveals that 3-day treatment duration is superior when antibiotics are applied at intermediate bacterial densities ($\Omega = 10^5$), leading to lower duration of infection and resistance selection (Figure 4 B,H). In contrast, 7-day duration wins when treatment begins at early stages of infection ($\Omega = 10^3$), or when considering pathogen burden at intermediate onset (Figure 4A,D,G,E). At late treatment onset, (when late is too late) longer treatment leads to lower infection duration (Figure 4C), but treatment duration does not produce

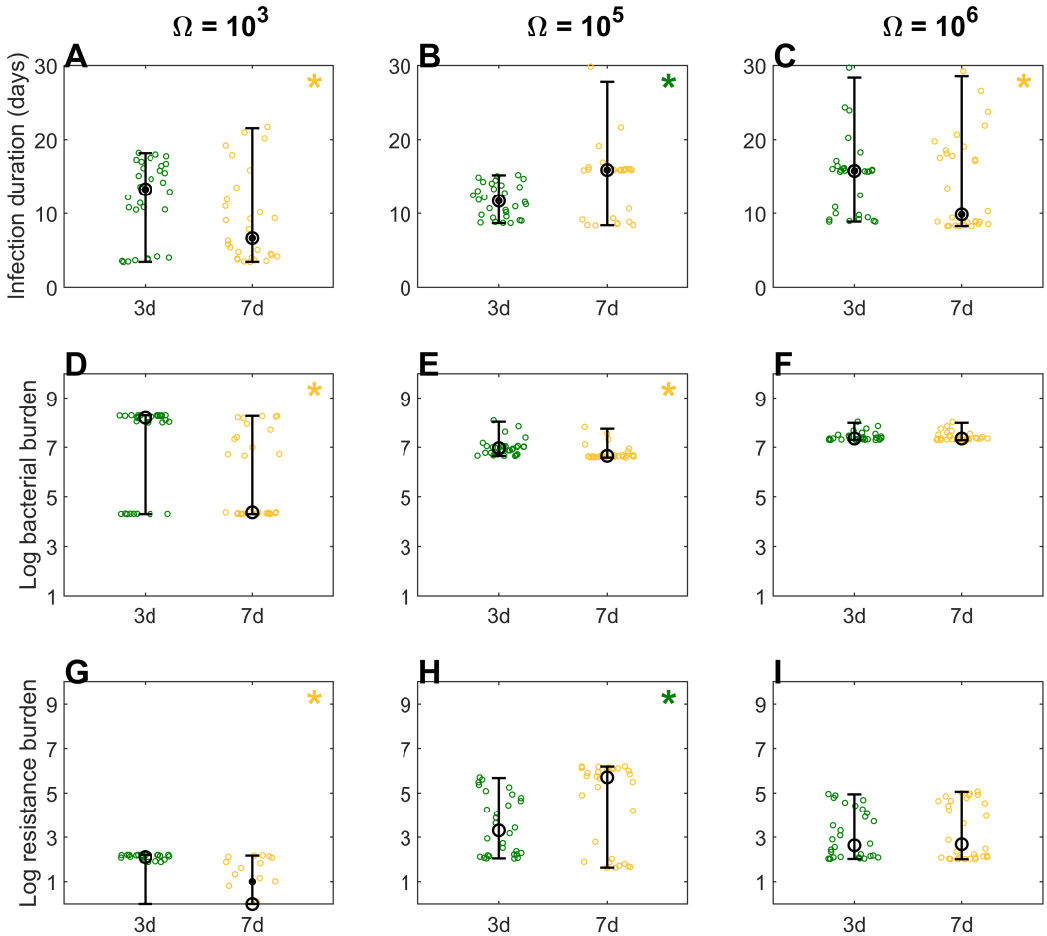

Figure 4: Benefits of short or long treatment vary with different infection burden measures. We simulated dynamics of intracellular bacterial infection using a mathematical model (given in Eqns. (1)–(6)) assuming that infection starts with antibiotic-susceptible bacteria and varied the duration of the antibiotic treatment (3 or 7 days), bacterial density at which treatment is initiated ($\Omega = 10^3$, panels **A, D, G**; $\Omega = 10^5$, panels **B, E, H**; $\Omega = 10^6$, panels **C, F, I**) for multiple values of the antibiotic-induced kill rate A_m varied from 1 to 20 per day. As the outcome of the treatment we estimated the duration of infection (panels **A-C**), cumulative bacterial burden at 7 days after treatment end (panels **D-F**), and the cumulative resistance burden at 7 days after treatment end (panels **G-I**). The black circles denote the median value from each subset of simulations and the vertical bars comprise the 95% quantile of all simulations across all A_m values. The ($*$) marker in each subplot (top-right) refers to statistically significant differences ($p < 0.05$, evaluated using the Mann-Whitney U test), and the color indicates the direction of the difference, namely the treatment duration yielding a lower value of the outcome, thus being more favourable for infection control.

significant differences in the other two outcomes, suggesting non-inferiority of 3-day treatment when considering total bacterial burden and resistance selection (Figure 4F,I).

This analysis indicates that the benefits of the short versus long treatment depend on metrics used to define successful treatment. For example in intermediate onsets $\Omega = 10^5$, when comparing overall bacterial burdens, longer treatment is superior ((Figure 4E), while when comparing resistance selection, shorter treatment is better (Figure 4H). Such a variability further suggests difficulty at generating a general rule of thumb on optimality of antibiotic treatment.

Extending our *infection-to-health* analysis, we then check how cumulative pathology (Eqn. (7)-Eqn. (9), where $g < 1$) varies along antibiotic kill rates and timing of treatment Ω, and how the regimes of 3-day and 7-day duration perform. We plot our three metrics of cumulative pathology at seven days post-treatment (7 d.p.t) for the short and long treatment as a function of different kill rates (Figure 5), zooming on an axis of treatment (dosing or antibiotics kill efficacy) that was collapsed in previous analyses (Figure 4). Recall that because we perform the comparison relative to the time that has passed since treatment end, in absolute terms, the point we are considering for 3-day treatment measures is 4-days earlier than the point in time for 7-day treatment. This implies that when cumulative bacterial pathology is higher in a host that underwent 7-day treatment rather than 3-day treatment at 7 d.p.t., it is really bad, the patient displays more prolonged complications due to this treatment. Similarly, if the 3- and 7-day measures correspond, this means that this patient has had the same benefit from either treatment, but in the case of 3-day treatment, this benefit arrived 4-days earlier.

The first result to observe across all cumulative pathology measures is that the relative effectiveness of the two treatment durations depends on the bactericidal effect of the antibiotic, i.e. among the 3-day and 7-day treatment there is no universal winner, re-affirming that even in clinical trials treatment duration comparison may not be decoupled from the particular dosing and antibiotic efficacy selected.

The second observation, focusing on the top row of Figure 4, is that when treatment starts early in the infection course, 7-day treatment is generally more effective than 3-day treatment in lowering pathology, except for a small range of doses around the minimal inhibitory concentration ($A_m \approx 6/\text{day}$), where the situation is reversed. As antibiotic killing rates increase further, the two treatment durations lead to the same pathology. When treatment starts at a point over infection when bacteria have reached higher levels within the host ($\Omega = 10^5$), 3-day treatment duration is typically non-inferior or superior to 7-day treatment in terms of pathology, but only short antibiotic treatment with low bactericidal rates (low A_m) can produce substantial reductions in pathology relative to no-treatment. The same pattern is preserved for treatment starting even later, only that in this case there are some kill rates where longer duration treatment may be more favourable. Typically kill rates in the sub-inhibitory and minimal inhibitory regime are more successful in reducing pathology relative to the no-treatment scenario.

Notice however, that by comparing Figure 5 A, B, and C, we can see that the greatest marginal gain in pathology reduction is obtained with high antibiotic kill rates applied early, independently of the duration of infection (Figure 5A). The main message of these results is that to maximally reduce bacteria-induced pathology, if treatment starts early, high antibiotic killing rates should be used, while if treatment starts later, lower antibiotic killing rates should be used. In these ($\Omega - A_m$) combinations, 3-day and 7-day treatment perform equally well, therefore 3-day should be sufficient.

When pathology is due to immune activation (Figure 5 D-E-F), more immune pathology means higher level immunity. Only for early treatment ($\Omega = 10^3$), the 7-day duration might outperform 3-day duration when combined with high antibiotic killing rates, because generally 3-day antibiotic treatment produces greater reduction in immunity-associated pathology. Recall that in absolute terms, we are comparing measures 7-days after respectively a 3-day and 7-day antibiotics course has been finished. Thus, the result that the shorter therapy yields overall similar pathology, hence similar overall immunization, to natural infection (green line overlapping with dashed line across Figure 5 D-E-F) but at the benefit of much lower bacteria-pathology (see top row of Figure 5), points to its great advantage, relative to the longer therapy that even yields pathology levels beyond what's expected from natural infection.

When pathology results from depletion of healthy macrophages, generally either duration treatment produces big benefits relative to no treatment, and it seems that the 3-day treatment performs similarly to 7-day alternative, except for early onset and at low antibiotic killing rates (Figure 5 G). Only in that case, 7-day treatment could be favoured as a means to preserve as many healthy macrophages as possible.

Overall, cumulative pathology comparisons indicate that the 3-day treatment seems to be almost al-

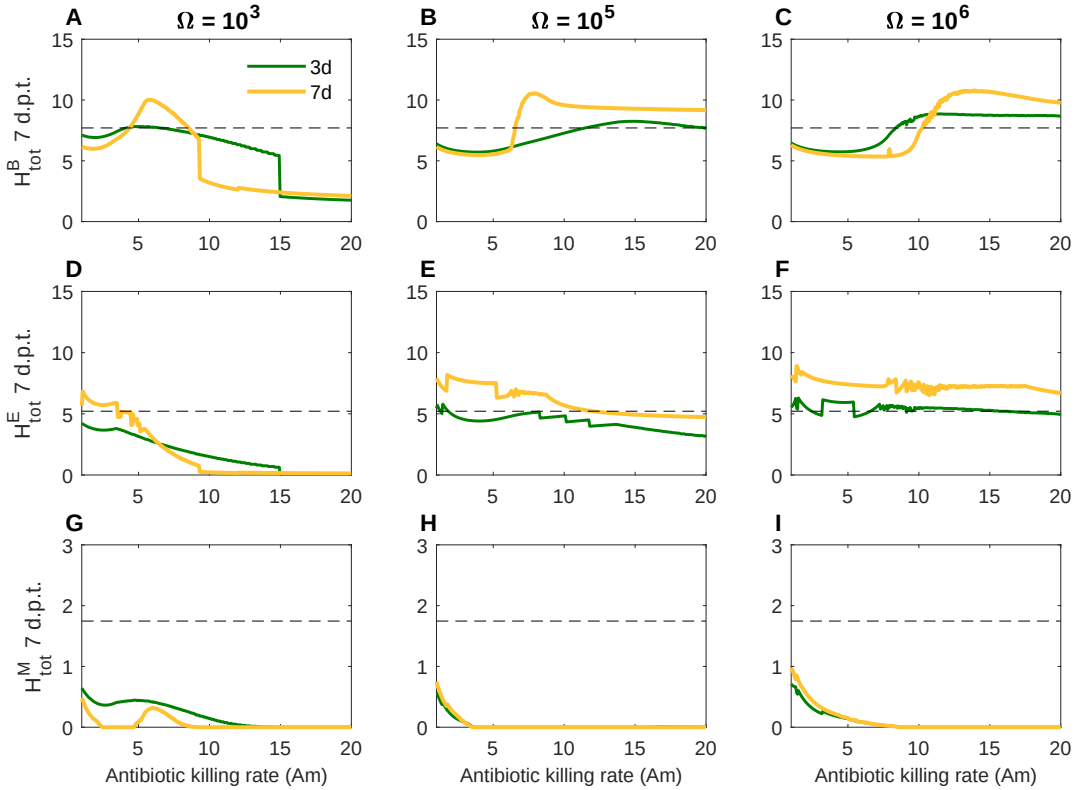

Figure 5: Benefits of short or long treatment vary with different cumulative measures of pathology. We simulated the dynamics of intracellular bacterial infection using a mathematical model (given in Eqns. (1)–(6)) as before, and measured the outcome of the treatment by cumulative pathology due to bacterial growth (Eqn. (7), panels **A-C**), due to immune response (Eqn. (8), panels **D-F**), or depletion of target cells (Eqn. (9), panels **G-I**). Measurement of the pathology was done for 7 days after stop of treatment. The dashed line represents the cumulative pathology in the case of no-treatment ($A_m = 0$) calculated at the final time point of infection (extinction). The columns correspond to different times of treatment initiation: when bacterial density is low $\Omega = 10^3$, intermediate $\Omega = 10^5$, and high $\Omega = 10^6$.

ways non-inferior or better than the 7-day duration. This applies more when treatment starts upon higher bacterial densities, and is mainly due to the fact that short treatment in this case restricts opportunities for bacterial growth, while not significantly impairing the ongoing critical activation of the host immune response. It is only by allowing for immune action, that selection of resistant bacteria can be prevented, and possible relapses avoided or minimized.

By comparing instantaneous (Figure 3) and cumulative infection measures (Figure 5) 7-days post-treatment, at the same kill rate (A_m) and treatment onset Ω, we can also see that the treatment that minimizes instantaneous bacterial levels, may not be the same treatment that minimizes the cumulative burden. For example, antibiotic treatment at the kill rate $A_m = 6$/day, if applied early, and if maintained for 7 days, will seem better in clearing infection (than 3-day treatment) when patients are checked for presence of bacteria 7-days post treatment (Figure 3A). However, if patient health is based on cumulative infection history, the same 7-day treatment patients will be scored as doing worse than 3-day treatment ones (Figure 5A). Thus, whether it's instantaneous or cumulative infection variables that matter more for health, remains unclear, but as seen above, it is an important factor in treatment efficacy comparison.

2.7. Further complexity in predicting optimal treatment

In the final set of analyses we explored optimality of treatment for general combinations of dose, duration and timing (Figure 6). Interestingly but somewhat expected at this stage, the results of this optimal treatment search in a space of possible treatments reveal different optima for different infection characteristics, selected as target. Furthermore, as repeatedly suggested from our earlier results, the optimal dose-duration combination is intrinsically linked to treatment timing Ω (Figure 6).

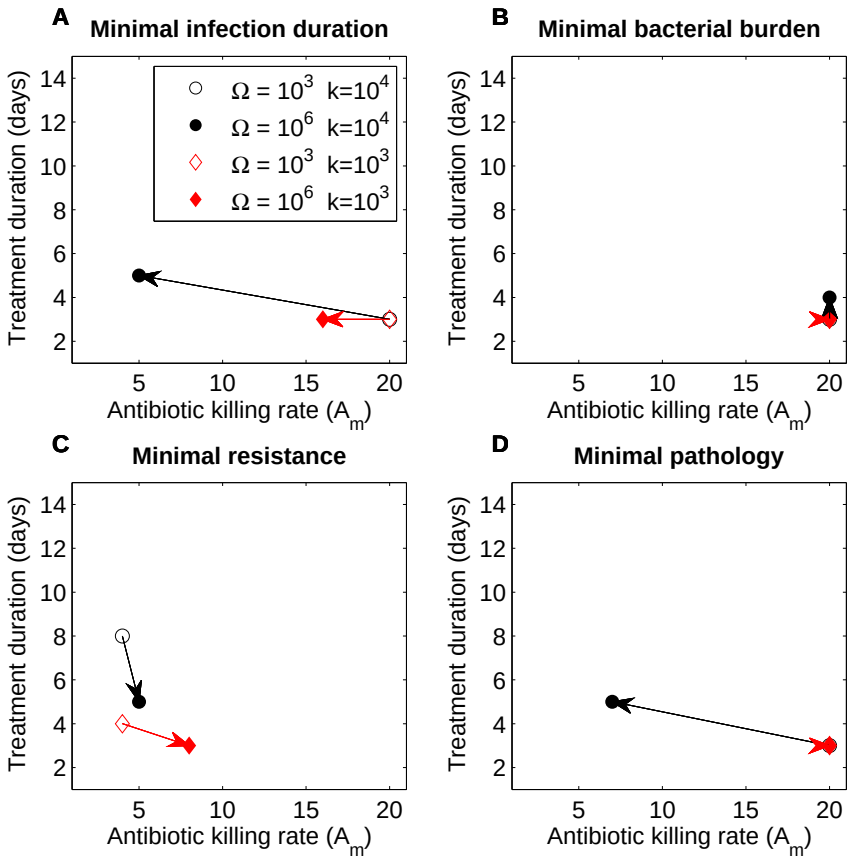

Figure 6: Optimal treatment varies by target criterion and host immune traits. We simulated different treatments in all combinations (A_m-duration) shown in the grid. We searched for those treatments that minimize different infection criteria over a 30-day period. Among equivalent optima, we selected those that minimize the total amount of drug ($A_m\times$ duration of treatment). Scatter points depict optimal treatments resulting in different scenarios, that minimize certain criteria, and do so by using the smallest possible amount of drug (dose × duration). Filled and empty markers refer to different treatment timing, while the colors indicate different levels of host immunity. **A.** Treatment that minimizes infection duration. **B.** Treatment that minimizes bacterial burden. **C.** Treatment that minimizes resistance burden. **D.** Treatment that minimizes bacteria-induced pathology. In red we depict a more immune-competent host, where activation of effector cells in response to bacteria happens faster, and arrows indicate direction for later treatment (i.e., from lower to higher Ω).

Here, for our default parameters, we find that early in infection, short and most aggressive treatment (high kill rate) is optimal, leading basically to the fastest possible clearance, but later in infection, short and milder treatment (lower kill rate) works better (Figure 6A). Notice however, that for bacterial burden minimization (Figure 6B), the best treatment does not seem to depend on timing, it is very short treatment (3 days) combined with the highest possible kill rate. For resistance minimization (Figure 6C), we obtain

optima in the other end of the spectrum: the longest needed treatment duration (8 days) if treatment is applied early ($\Omega = 10^3$) with a relatively small dose needed ($A_m = 4$), but if treatment onset corresponds to later stages of infection ($\Omega = 10^6$), to limit resistance selection, the treatment needed is only 5 days with only slightly higher dose ($A_m = 5$). Notice that the early interventions, always produce higher marginal reductions in infection target criteria than later-onset treatments (Table S2,Figure S8) but require more aggressive kill rates. Thus, although we observe that the optimum shifts with treatment timing and with the criterion to be optimized (Figure 6), in terms of optimal treatment duration, relatively short treatments ($\leq$ 5 days) seem to be generally favoured .

The optimal treatment may also depend on the underlying host immune competence, for example the host-intrinsic parameter k for immune stimulation, where smaller k, e.g. $k = 10^3$, represents swifter host responses, thus, a higher immune ability to respond to infection. The analysis (see Table S2, Figure S8) shows that when treatment onset is early ($\Omega = 10^3$) optimal dose-duration is conserved for all criteria (empty black and red markers overlap in Figure 6), except for resistance selection (Figure 6C), whose minimum now requires half the treatment duration (4 days), compared to the higher-k scenario. Recall that more immunity alters the competitive advantage between the drug-sensitive and resistant bacteria. For infection duration minimization, less killing is needed compared to early treatment, but same duration. For minimizing bacterial burden and its associated pathology, the optimal dose-duration combination does not vary with treatment timing, or on host immune activation, pointing to aggressive kill rates and minimal duration as best universal strategy, the only impediment being toxicity on the patient and collateral adverse effects from such high antibiotic dosing. In contrast, for resistance burden, later timing, requires higher dose and shorter duration, in similar optimization trend as in the high-k scenario.

Overall, the optimality landscapes for different criteria vary and the preferred treatment given a target (e.g. minimum) may not be robust. This result realistically highlights that it may be difficult to pinpoint which treatment parameters should be used in a given patient, without prior knowledge of the underlying immune competence, or the location of this patient along his individual infection course [41]. While calling for more empirical attention on dynamic infection processes to verify the cross-talk between pathogen, host and intervention, our theoretical model findings also suggest that under appropriate dosing, short courses of antibiotics could be effective for several infection criteria.

3. Discussion

Antibiotics are key to modern medicine and preserving their effectiveness is a global priority. Higher antibiotic exposure leading to higher levels of antibiotic resistance has been shown from the individual to the population level [42, 43]. Avoiding antibiotic overuse remains an essential step in addressing the antibiotic resistance challenge [44, 45]. In this study, we focused on a comparison between short and long antibiotic treatment, taking into account several infection target criteria. We used a mathematical model for a generic intracellular bacterial infection, where the action of antibiotics, on extracellular bacteria, is combined with that of the immune response of the host, acting on infected phagocytes. We found several parameter regimes and target criteria by which 3-day treatment is non-inferior and even superior to 7-day treatment, favouring a reduced use of antibiotics to achieve similar clinical outcomes. However, we also observe that optimal dose-duration combination varies widely, depending on the time point of treatment administration, host immunity characteristics, and the infection criterion to optimize.

The key in understanding how treatment duration influences bacterial dynamics is how bacterial load impacts generation of the immune response. We assumed that immune response expansion is directly driven by the amount of bacteria and that immune response does not contract during the timescale of infection. Therefore, any treatment that does not result in clearance of the infection may slow down immune response dynamics which then leads to relapse following treatment end. How the dynamics

of immune response, both innate and adaptive, depends on the presence of the infection in humans is not understood and may well depend on the type of infection [46]. In particular, yellow fever virus-specific CD8 T cell response continues to expand long after the infection disappears from the blood [47]. Better understanding mechanisms regulating abundance of pathogen-specific immune responses in tissues in human infections is likely to constrain mathematical models and will help to make more robust predictions.

The importance of treatment timing

Typically the timing of treatment relative to the natural infection course is not known, and while some models assume it happens when the bacteria reach their peak [27] or implement treatment throughout the infection simulation [24], they neglect potential underlying feedbacks between pre- during- and post-treatment processes. The reality is that we do not know when an infection triggers severe symptoms that lead the host to seek treatment, and whether this is early during the infection course, or later on, may depend on host-specific tolerance factors. Treatment onset may be caused by bacterial density reaching a certain critical level or by the associated immune response leading to super-critical inflammation. In either case, it is a parameter that is likely to be pathogen- and host-dependent, and thus important to study as a potential modulator of treatment effects [19]. Although the temporal dimension of key events in host-pathogen interaction has received a lot of attention in studies of pathogen virulence [48], including in nested models that bridge within- to between-host phenomena [49], the temporal dimension of therapeutic intervention relative to natural infection course (age of infection) has received surprisingly little attention in models of antibiotic treatment.

Physiological factors driving patient symptoms and treatment onset remain unclear. Thus, it is essential that this aspect is studied more in theoretical models of intervention, and in clinical trials, for example following longitudinally immune biomarkers over infection. Only by accounting for host defense mechanisms and their effective contribution over infection, in deeper integrative frameworks, can we understand the right amount of antibiotics needed to treat. Individuals are expected to vary in their symptom threshold, as evidenced by differences in microbiologic confirmation at baseline across patients with the same symptoms [21], but they can also vary in other traits of immune response - bacteria interaction.

The role of host immune defenses

A properly functioning immune system is essential for controlling bacterial growth and virulence within host. The reach of immunity can be vast, including factors that restrict rate of entry of bacteria into host cells (β in our model), those that limit intracellular replication (N in our model), those that increase elimination of infected cells (a), and the adaptive immune response activation kinetics; all present in our model, explicitly or implicitly. In our exploration of optimal treatment as a function of host parameters we limited to only one parameter - the half-saturation constant for effector cell activation k, and we found that variation in k is sufficient to imply a different optimal treatment in the antibiotic kill rate-duration space. In reality, within-host variability in bacterial killing rates by the immune system can also be an important factor generating heterogeneity, from the local lesion scale up to the clinical outcome of a bacterial infection [50].

Overall, our study highlights the challenge of designing general principles in a multitude of host-pathogen scenarios. Combinatorial diversity of many attributes of host susceptibility upon infection, including age, immunity, history, inoculum size and others, have been recognized as constituting a major challenge to prediction of host-microbe interactions at the individual level [51]. In our study of antibiotic treatment effects in a single host, we find that the benefits of short vs. long treatment depend on multiple details of the infection, highlighting inevitable uncertainties in decision-making of antibiotic prescriptions. At the epidemiological level, it is also being recognized that ranking antibiotic treatment protocols

is highly dependent on methodological factors, e.g. the criterion of choice for comparison [52]. This difficulty in drawing general principles is similar to the one already observed in studies of the evolution of virulence, where early work has argued that a single factor such as the route of transmission determines virulence of pathogens [53, 54], while later studies, however, showed that a single factor is unlikely to determine optimal virulence of very diverse pathogens [55].

The difficulty of reaching generalized conclusions on optimality of antibiotic treatment duration might force one to attempt to logically constrain mathematical models so that a robust conclusion can be reached. For example, by assuming that immune system-mediated killing of bacteria is always high, treatment does not have to be long to be efficient since strong immunity can easily finish the job of eliminating bacteria if antibiotic does not [29]. However, what makes immune response "strong", how quickly it becomes "strong", how long it is "strong" for, and how the strength varies between individuals remains unknown, limiting the applicability of such a generic argument for the actual bacterial infections of humans. Better understanding of optimal duration of treatment is unlikely to come just from thought experiments performed with mathematical models, but from solid quantitative experimental data, which can constrain model behavior and may allow to discriminate between alternatives [56].

Similarly, efficacy comparisons of treatment with bacteriostatic or bactericidal effects, must factor in host immunity contribution, as already noted [29]. The question of whether and why bacteriostatic drugs might be superior to bactericidal drugs [57] links to the same discussion how host immunity and antibiotics dynamically interact. The answer will depend on specific underlying kinetics of host immune defenses as a function of pathogen density (whether constant, low, high, or tightly coupled), and even on the possible direct immunomodulatory effects of some drugs [58, 59], which here we did not consider.

Limitations and outlook

There is a number of potential limitations of our modeling analysis, but these are also open frontiers for further study. The model only captures the basic population feedbacks between growth, death and immune interaction processes of bacteria, and considers a well-mixed system. When considering particular scenarios, new model elements may need to be included, such as those involving cell-cell transmission, activated and non-activated macrophages, fitness differences between sensitive and resistant bacteria in other traits, e.g. extracellular death rate, entry into host cells, or differential susceptibility to the immune response. Another aspect we did not elaborate is the differential investment of bacteria in the intracellular vs. extra-cellular lifestyle over infection (but see [40] for an exploration of parameter dependencies driving such fitness effects). This would place different pressures for immune control over the intra-cellular residing microbes, and antibiotics-mediated control on the free bacteria outside cells. Further increasing model complexity is unlikely to make predictions on optimal treatment easier to reach, but it may reveal interesting phenomena for synergistic feedbacks between treatment and immunity in specific cases.

The model is broadly deterministic with a quasi-stochastic component in mimicking bacterial extinction and emergence of resistant bacteria. Naturally, implementing a fully-stochastic formulation at low population numbers may affect model outcomes. We expect that adding stochasticity to the comparison between 3-day and 7-day treatment, will tend to increase efficacy of short and moderate therapies, because it will generate more possibilities for infections to spontaneously die out [60]. The definitions of pathology we adopt are also very simple and consider separate cases of single variables being important for infection-induced damage. The reality is much more complex and true pathology in an infection is a combination of multiple interrelated host variables deviating from homeostasis [61], as well as the integral of such deviation through time. However, our aim was to illustrate that even with such simple criteria, optimal treatment duration is far from robust. More formal multi-objective treatment optimization could be attempted in the future to highlight explicit trade-offs between different target measures.

Perhaps the strongest limitation of the model is the lack of exact parameter estimates from which we

can draw final quantitative conclusions. We did not choose to focus on any specific host-pathogen system, leaving the model general for intracellular acute bacterial infections such as those caused by *Listeria monocytogenes*, *Salmonella*, *Shigella*, or *E. coli*. Naturally in defined experimental systems, or particular human infection data, this model could be empirically constrained. Certainly, the steps forward in infection control and antibiotic resistance management will require on one hand, more quantitative data on specific infections of humans (e.g. [62, 63]) with some degree of host stratification, and on the other hand, more integrative frameworks that adopt an ecosystem approach to disease [64]. In ecology, the distinction between pulse (relatively short) and press (relatively longer) perturbations has long been recognized and studied in relation to ecosystem species composition and interactions [65]. In infection systems where pathogenic microbe interactions intertwine with host immunity feedbacks and the dynamics of natural commensal species, the full scale of intervention effects, spanning timing, intensity and duration variability, remains yet to be unravelled.

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Author contributions

E.G. and V.V.G conceived and designed the study and supervised the findings. F.P carried out numerical simulations and contributed to the interpretation of results. V.V.G provided critical feedback during all phases of the work. E.G. wrote the manuscript with input from all the co-authors. E.G. supervised the technical and analytical details of the project. All authors have given final approval of the version submitted.

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Mathematical modeling suggests that benefits of short or long antibiotic treatment depend on details of infection:

Supplemental Information

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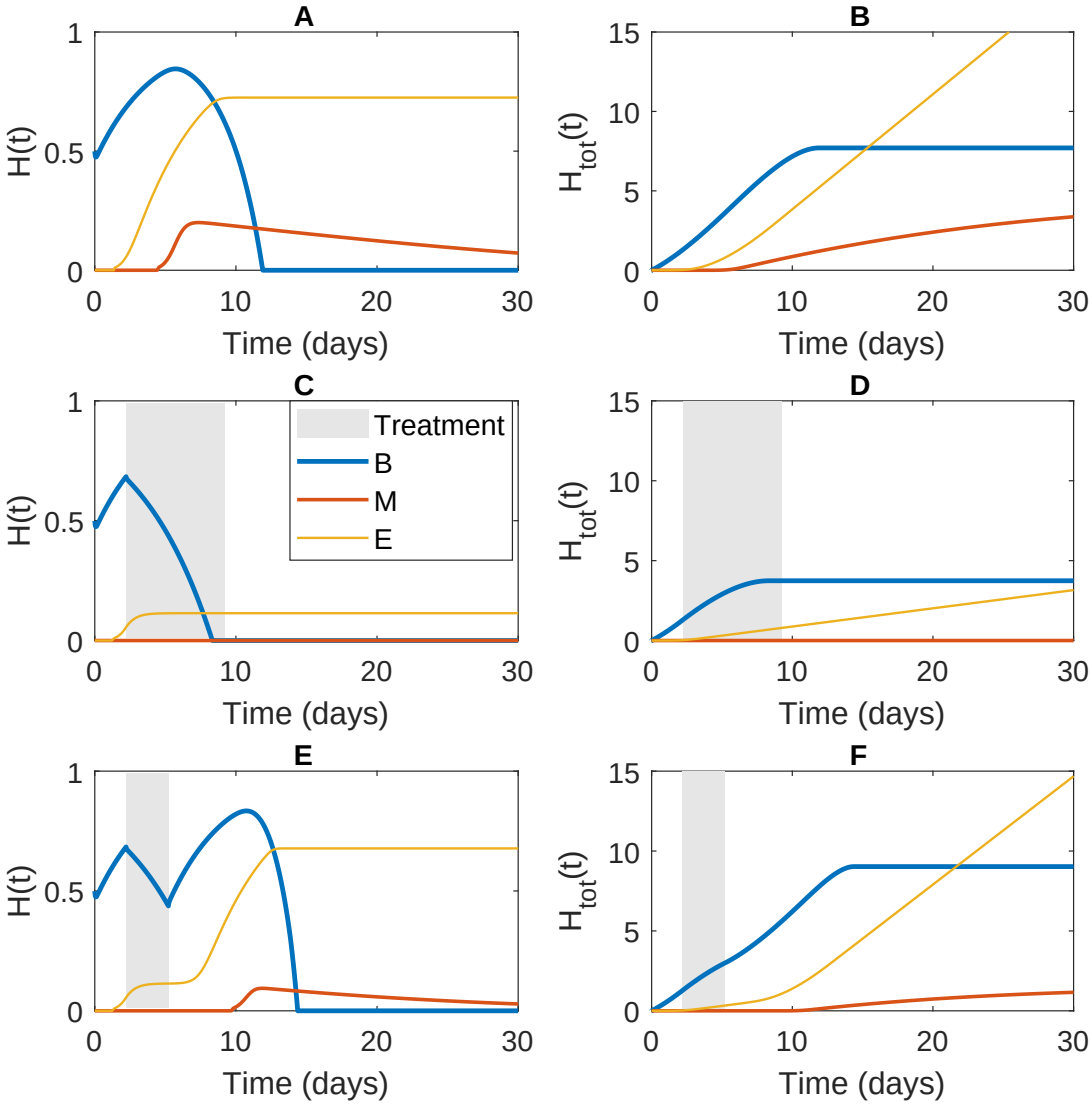


Supplemental Figure S1: Pathology dynamics during an acute infection, for cases when it is due to bacterial growth (B), immunity activation (E) and macrophage depletion (M) in the host. A) Instantaneous pathology without treatment. B) Cumulative pathology (integrated over time) without treatment. C) Instantaneous pathology with 7-day treatment. D) Cumulative pathology with 7-day treatment. E) Instantaneous pathology with 3-day treatment. D) Cumulative pathology with 3-day treatment. Both durations are simulated at killing rate $A_m = 11$. These dynamics of pathology are generated from the dynamics of infection represented in the Figure 1 in C, E and F in the main text.

Text S1. Three general infection outcomes are possible with our model

Infection dynamics in our model result from a complex nonlinear interplay of three basic processes: i) resource-consumer dynamics between bacteria and macrophages, ii) predator-prey dynamics between immunity and bacteria, where stimulation comes from the extracellular compartment and suppression effects are exerted on infected macrophages, and iii) elimination of extracellular bacteria from antibiotics. Despite the intrinsic coupling between all three and the high number of parameters involved, model analysis reveals some clear patterns.

Dynamics without immune action

In the absence of immunity ($E = 0$), three basic regimes of infection are possible: clearance, persistence with coexistence (B_s, B_r) and persistence of resistant bacteria only. The parameter combinations distinguishing these regimes involve critical conditions between the rate of resource consumption on one hand (infection of macrophages and bacterial growth), and bacterial decay dynamics and target cell replenishment on the other. The transition from a stable clearance steady state to persistence is driven by the pathogen fitness criterion:

$$\frac{K\beta}{c}\left(\frac{N\delta(1-\gamma)}{a+\delta} - 1\right) > 1, \tag{S.1}$$

where a sub-condition is $N > \frac{a+\delta}{\delta(1-\gamma)}$, illustrated in Figure S2, requiring a high-enough intra-cellular replication inside macrophages, relative to apoptosis of infected cells. Condition S.1 is similar to the reproduction number threshold $R_0 > 1$ in epidemic models.

The three regimes are illustrated in Figure S2 A&B, as a function of resistance fitness cost γ and burst size N. The border between mixed persistence and resistant-only infection is driven by $\gamma = m$ critical line. When the mutation rate is higher than the fitness cost of HLR, persistence will be possible only for the resistant bacteria. In the more realistic regime $\gamma > m$, we will expect to see coexistence within host between drug-sensitive and drug-resistant bacteria. In this case, in the absence of antibiotics ($A_m = 0$) and of immune kinetics ($E = 0$), given criterion S.1, the equilibrium is given by:

$$M^* = \frac{c(a+\delta)}{\beta[\delta N(1-m) - \beta(a+\delta)]} \tag{S.2}$$

$$I_s^* = \frac{cr(\gamma-m)[(a+\delta)(c+\beta K) - \beta\delta NK(1-m)]}{\gamma K\beta^2[\delta N(1-m) - \beta(a+\delta)]^2} \tag{S.3}$$

$$I_r^* = \frac{crm[(a+\delta)(c+\beta K) - \beta\delta NK(1-m)]}{\gamma K\beta^2[\delta N(1-m) - \beta(a+\delta)]^2} \tag{S.4}$$

$$B_s^* = \frac{r(\gamma-m)[(a+\delta)(c+\beta K) - \beta\delta NK(1-m)]}{\gamma K\beta^2[\delta N(1-m) - \beta(a+\delta)]} \tag{S.5}$$

$$B_r^* = \frac{rm[(a+\delta)(c+\beta K) - \beta\delta NK(1-m)]}{\gamma K\beta^2[\delta N(1-m) - \beta(a+\delta)]} \tag{S.6}$$

where the ratio between drug-sensitive and drug-resistant (less fit) bacteria in a persisting microbial population is:

$$\frac{B_s^*}{B_r^*} = \frac{I_s^*}{I_r^*} = \frac{\gamma}{m} - 1, \tag{S.7}$$

reflecting a 'mutation-selection' equilibrium. The fitter, drug-sensitive bacteria will dominate whenever the fitness cost of their drug-resistant competitors is high enough, relative to their generation rate through mutation, namely when $\gamma > 2m$. As can be seen from the analytical formulations above, the infection

persistence potential depends on multiple parameters: for example it increases with burst size N and is reduced with increasing mortality of infected macrophages, a (Figure S2 C).

Despite this potential for chronicity, as persistence is typically achieved through oscillatory behaviour around the equilibrium, even though analysis predicts persistence for given parameters, numerically due to the extinction threshold imposed, we might observe clearance after the first peak. Thus, in Figure S2, the white region denoting clearance is effectively higher in simulations.

Dynamics with immune action (our baseline model of Acute Dynamics)

As soon as the equation for immune action is active $E > 0, dE/dt > 0$, as we assume in our model, the only regime possible becomes clearance (Figure S2 D). This is due to the monotonically- increasing immune feedback on infection, resulting in either a self-limiting acute dynamics or direct clearance, depending on initial conditions. Again a plays an important role here in the magnitude of the bacterial peak. In such dynamics, the final level of adaptive immune response, E^*, necessarily satisfies:

$$E^* \geq E_{crit} = \frac{1}{v}\left[\frac{\delta N(1-\gamma)}{\frac{c}{K\beta}+1} - (a+\delta)\right], \tag{S.8}$$

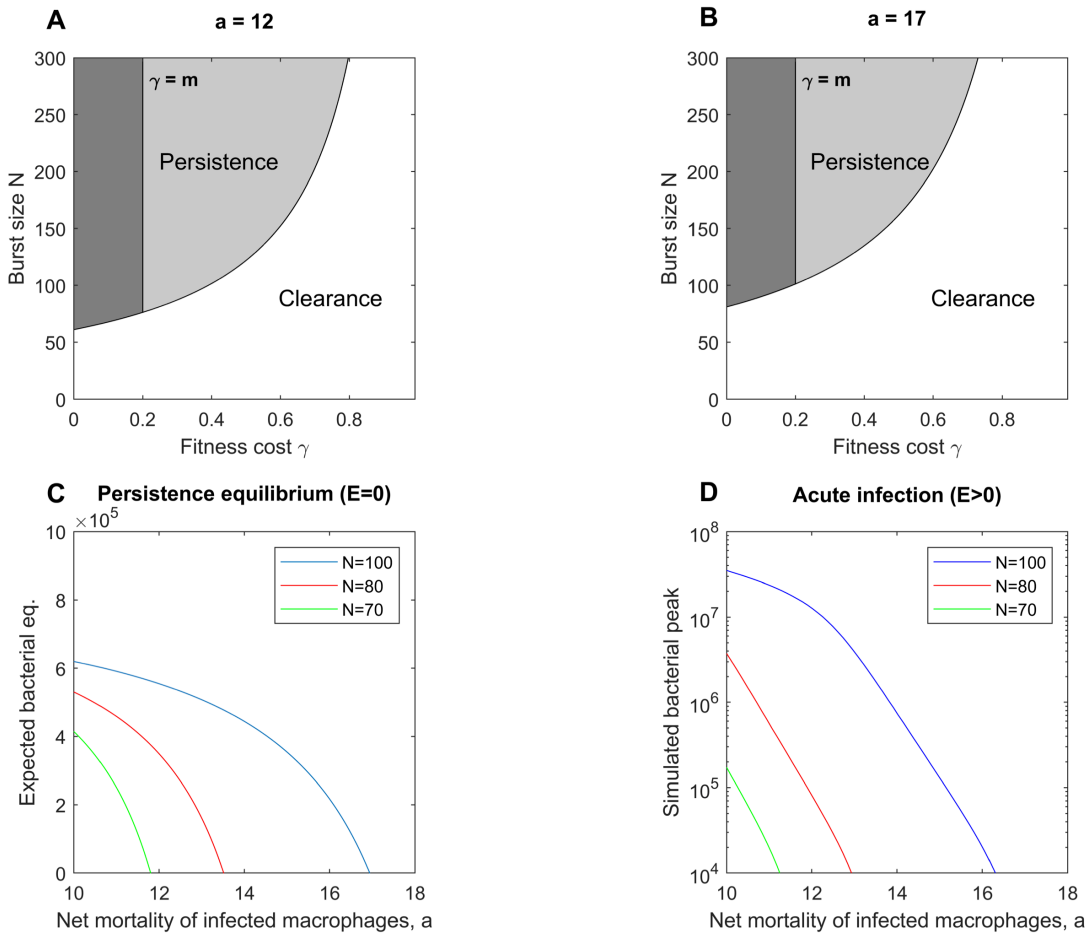
a critical condition to ensure stable clearance. Notice that if $E_0 > E_{crit}$, the initial immunity present in the host upon infection will be high-enough to drive any initial inoculum towards clearance.

In contrast, if we are in the intermediate case where immune response level is fixed above 0, and remains constant (e.g. $0 < E \equiv const < E_{crit}$), the dynamics would result in similar 3-profile model outcomes as with $E = 0$, but here associating the additional level of immunity to an equivalent increase in the value of the parameter a. Sub-optimally locked levels of the immune response naturally open the way for more chronic infection scenarios within host, as may be the case in immune-compromised individuals.

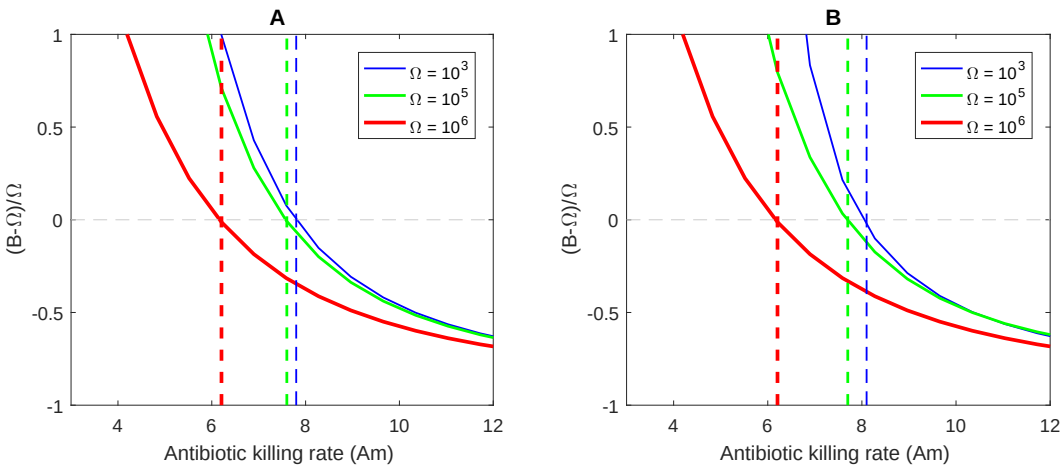
In the bulk of our study, where in addition, we investigate transient antibiotic treatment effects ($A_m > 0$), we do not proceed with the analytical approach, because the formulae become cumbersome. By focusing on parameters that satisfy criterion S.1 and simulations with strictly growing immune action, we address numerically qualitative descriptions of infection behaviour, manifested over shorter and clinically-relevant time-scales.

Text S2. Short-vs- long duration comparison keeping treatment intensity fixed

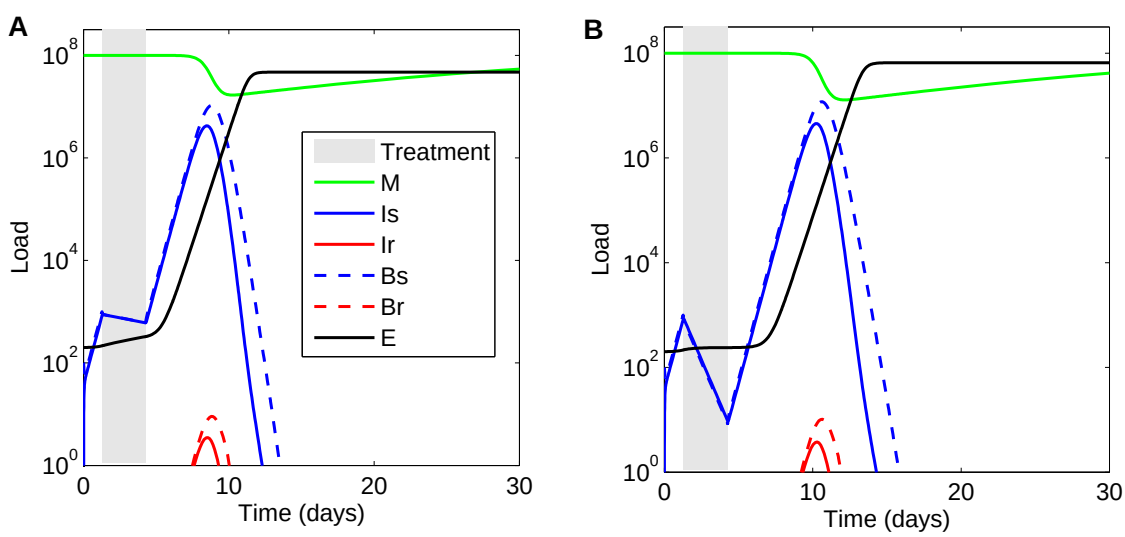
To explore this case, we chose overall intensities in the range $[7, 84]$, corresponding to kill rates $A_m \in [1, 12]$ for 7-day treatment, and killing rates per unit of time of $A_m \in [7/3, 84/3]$ (higher) in 3-day treatments. Treatment comparison under overall intensity constraints shows that *3-day and aggressive* treatment leads to infection resolution in 50-60% of the cases (less sensitive to treatment timing), while *7-day and mild* treatment is generally more successful, and its success increases with later treatment onset tending to 90% success rate (Figure S7). This is different from the case where the comparison between short and long treatment is based on fixing the kill rate. Indeed, this second type of comparison between 3-day and 7-day treatment confirms that at later stages of infection when the risk of resistance selection is high, and when there is more immunity expected to contribute to infection clearance, it is better to distribute the same amount of antibiotic mildly and over longer time.



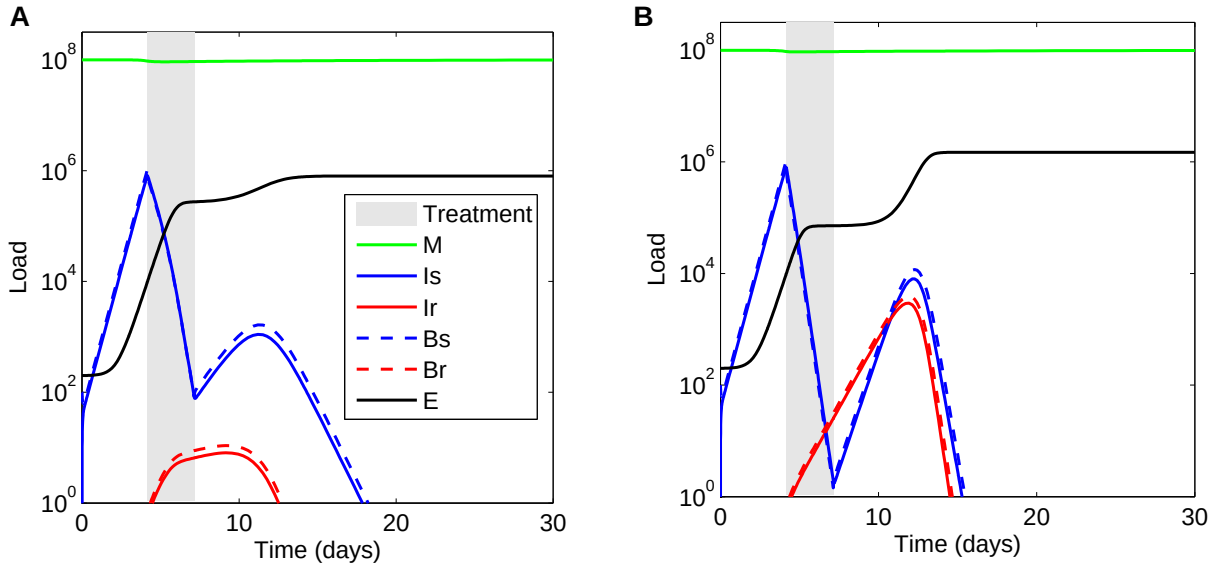
Supplemental Figure S2: Different regimes of infection and the role of infected cell mortality. A. Three scenarios possible for $E = 0$. The clearance-persistence line depends on the critical parameter combination $N > \frac{a+\delta}{\delta(1-\gamma)}$. The dark gray region depicts resistant-only persistence, while the light gray region depicts coexistence. B. As infected macrophage mortality a increases, the region of infection persistence decreases. C. Analytical equilibrium under no-immune action, as a function of a. D. Bacterial peak from simulated dynamics under immune action (when $E > 0, dE/dt > 0$), as a function of a.



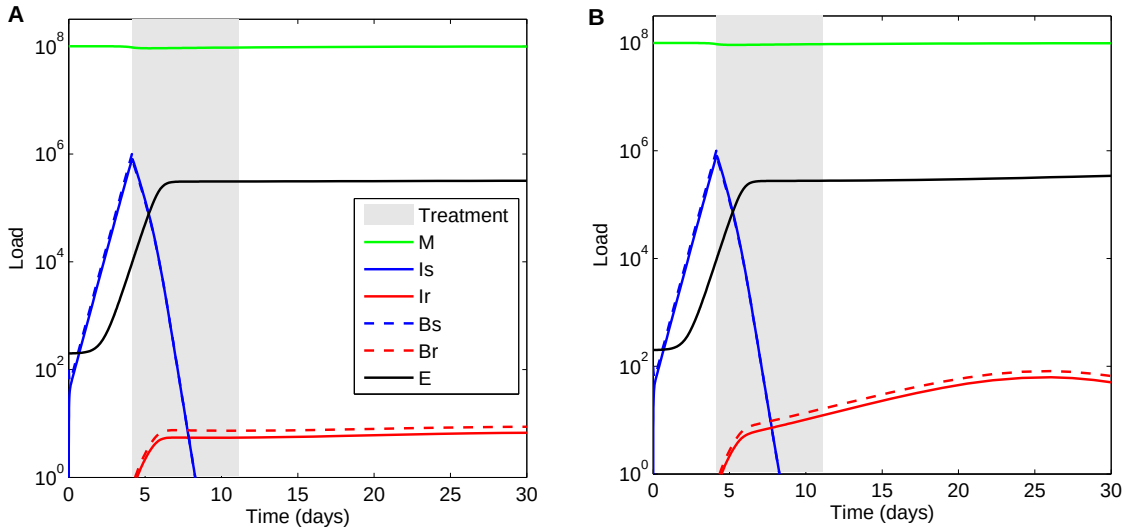
Supplemental Figure S3: Minimum effective kill rate for the infection. We compare $B(t)$ at the end of treatment with the $B(t) = \Omega$ at the beginning of treatment. A) For a 3 day treatment duration. B) For a 7 day treatment duration. In A), the minimum effective kill rate for $\Omega = 10^3$ is 7.8; for $\Omega = 10^5$ it is 7.6 and for $\Omega = 10^6$ it is 6.2. In B), the minimum effective kill rate for $\Omega = 10^3$ the minimum effective kill rate is 8.1; for $\Omega = 10^5$ it is 7.7 and for $\Omega = 10^6$ it is 6.2. These results show that most of our kill rates in the simulations $A_m \in [1, 20]$ are in effective killing (supra-inhibitory) regime, and only about 30% of the killing rates are in the sub-inhibitory regime. The duration of treatment does not seem to affect the minimum effective kill rate. Analytically, in terms of model parameters, this minimum effective kill rate can be approximated by: $A_{crit} \approx N\delta - \beta K - c$, assuming a 1:1 ratio of infected macrophages and extra-cellular bacteria (I/B), typically more accurate in the later stages of infection.



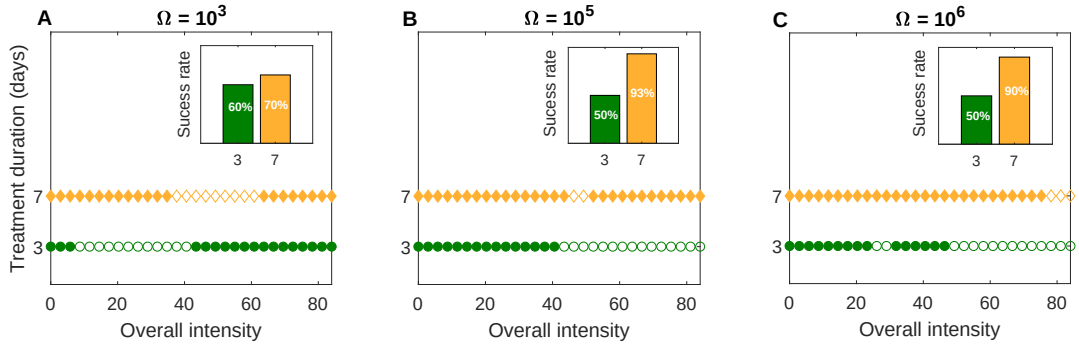
Supplemental Figure S4: Short treatment failures for early treatment in figure 3A. Illustration of infection dynamics when 3-day treatment fails if started early, $\Omega = 10^3$, A) $A_m = 6$, B) $A_m = 10$. In the failures in Figure 3A (main text) at killing rates 6 and 10, we see a large relapse post-treatment mainly made of sensitive bacteria; short ineffective treatment too early has just delayed the entire infection course, but without the risk of compromising the competition between sensitive and resistance bacteria. Resistance cannot be selected.



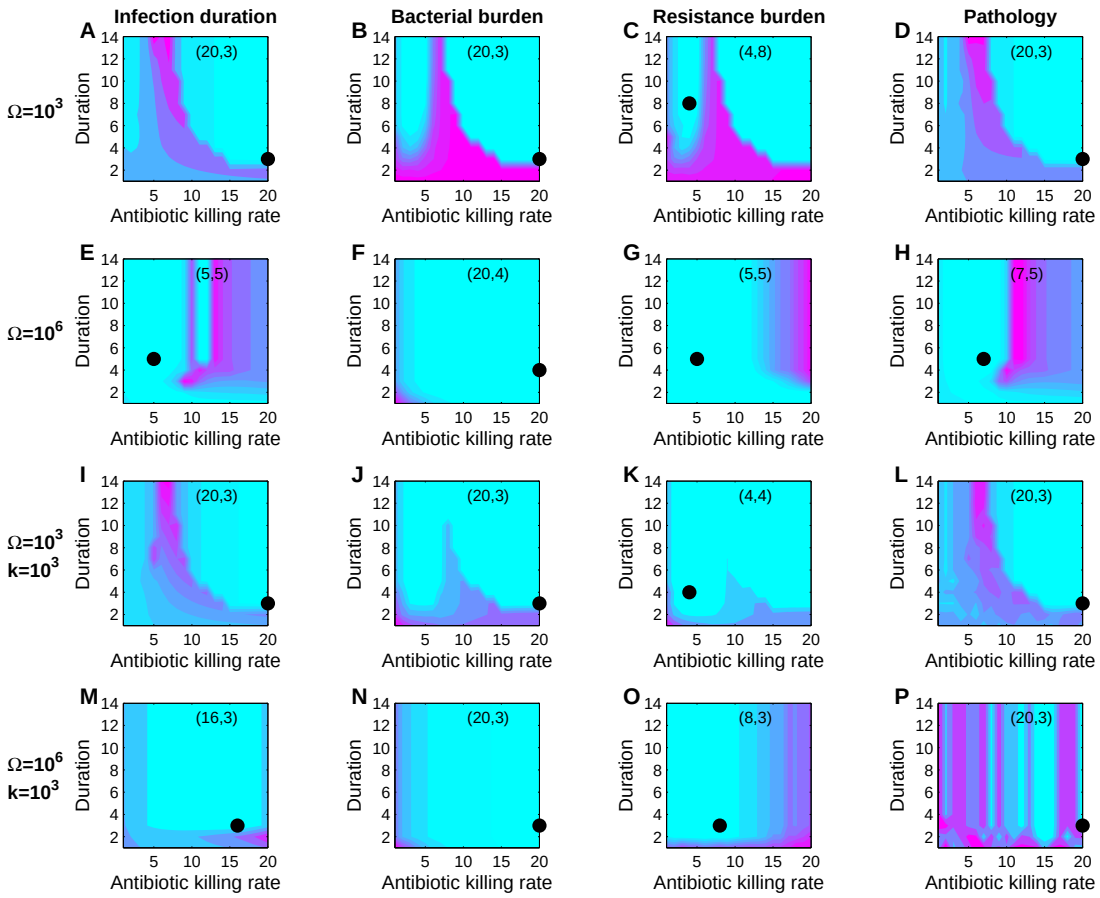
Supplemental Figure S5: Short treatment failures for late treatment in figure 3C. Illustration of infection dynamics when 3-day treatment fails if started late $\Omega = 10^6$, A) $A_m = 11$, B) $A_m = 18$. At later onsets $\Omega = 10^6$, the treatment failures seen with 3-day course of antibiotics can be of two types: around kill rate equal to 10 (relatively lower), there is possibility of a small relapse post treatment with sensitive bacteria, but the infection is on its way to clearance due to sufficient immune build-up. In contrast, at higher kill rates ($A_m = 18$), short treatment has been enough to just revert the competitive advantage in favour of drug-resistant bacteria, thus leading to a larger relapse post-treatment dominated by drug-resistant bacteria. This figure together with Figure S4 explains why increasing killing rate has a non-monotonic pattern for 3-day treatment in (Figure 3C)



Supplemental Figure S6: Long treatment resolution for late treatment in figure 3C. Illustration of infection dynamics when 7-day treatment leads to apparent microbiologic resolution ($B(t) < B_0$) if started late, $\Omega = 10^6$, A) $A_m = 10.6$, B) $A_m = 11$. Low level resistance can be selected to persist for an indefinite time after treatment. For the same dose and treatment timing, short treatment will not lead to instantaneous resolution, although infection its on its way to clearance without selecting resistance (Figure S5A). Such treatment at high kill rates can suppress the sensitive bacteria, allowing the resistant competitors to overcome their fitness disadvantage during treatment up to a level where they can persist below the immune stimulation threshold k. Thus, even though immunity has received a good initial boost from the growth of sensitive bacteria pre-treatment, its level is insufficient to kill the resistant bacteria, as they continue to persist or grow slowly, unlimited by competitors or resources, while the immune response takes a long time to catch up.



Supplemental Figure S7: Infection resolution for short and long treatments under intensity constraints. Here we simulated 'short and more aggressive' treatment versus 'longer and milder' treatment, keeping fixed the total amount of drug. Comparisons are based on bacteriologic outcome (instantaneous measure) at 7-day post-treatment for 3-day and 7-day duration at different treatment onset Ω. A) $\Omega = 10^3$, B) 10^5, C) 10^6. Orange diamonds: 7-day treatment outcome, green circles: 3-day treatment outcome. Empty markers: Infection not resolved ($B(t) > B_0$) at 7-day post treatment. Filled markers: Infection potentially resolved with ($B(t) < B_0$) at 7-day post treatment. Treatment success rate is shown in the insets, where success is averaged across intensities satisfying $B(t) < B_0$ at observation point post-treatment.



Supplemental Figure S8: Optimization details for different criteria and different scenarios in Figure 6. Here we simulated different treatment combinations, dose and duration, at different points in time over infection ($\Omega = 10^3$ early, and $\Omega = 10^6$ later onset) and in hosts with different immune competence ($k = 10^4$, slower response, $k = 10^3$, faster response) and quantified several target criteria (contourplots). Filled markers in each contourplot correspond to treatment minimizing the particular infection criterion.

Medians of distributions	Omega=10^3	Omega=10^5	Omega=10^6
Duration 3-day 7-day Mann-Whitney U test:	**A** 13.2978 6.6411 *p=2.1*10^-6*	**B** 11.7074, 15.8081 *p=4.7*10^-24*	**C** 15.8081 9.8482, *p= 6.1*10^-5*
Bacterial burden 3-day 7-day Mann-Whitney U test:	**D** 1.5481*10^08 2.3607*10^04 p=1.1*10^-19	**E** 9.1303e+06 4.4624*10^06 2.7*10^-42	**F** 2.2207*10^07 2.2184*10^07 *p=0.088*
Resistance burden 3-day 7-day Mann-Whitney U tes:t	**G** 126.9728 1 *p=8.3*10^-29*	**H** 2.1010*10^3, 5.2187*10^05 *p=6.1*10^-12*	**I** 434.0265 529.4545 *p=0.088*

Table S1. Exact results from the variability of infection summary measures obtained in simulations of Figure 4 in the main text.

Optimization Results	**Infection duration (days)**	**Bacterial burden**	**Resistance burden**	**Bacteria-induced pathology**
Slow immune response (k=10^4)				
Host: k=10^4 Omega= 10^3 (early onset)	Dose: 20 Duration: 3 *Min: 3.4056*	Dose: 20 Duration: 3 *Min: 1.992*10^04*	Dose: 4 Duration: 8 *Min: 0*	Dose: 20 Duration: 3 *Min: 3.1830*
Host: k=10^4 Omega= 10^6 (later onset)	Dose: 5 Duration: 5 *Min: 8.2490*	Dose: 20 Duration: 4 *Min: 1.923*10^07*	Dose: 5 Duration: 5 *Min: 102.5389*	Dose: 7 Duration: 5 *Min: 5.3502*
Reference (no treatment)	**11.9010**	**2.05*10^08**	**168.7554**	**7.7023**
Faster immune response (k=10^3)				
Host: k=10^3 Omega= 10^3 (early onset)	Dose: 20 Duration: 3 *Min: 3.4049*	Dose: 20 Duration: 3 *Min: 1.992*10^04*	Dose: 4 Duration: 4 *Min: 0*	Dose: 20 Duration: 3 *Min: 3.1828*
Host: k=10^3 Omega= 10^6 (later onset)	Dose: 16 Duration: 3 *Min: 6.4774*	Dose: 20 Duration: 3 *Min: 1.871*10^07*	Dose: 8 Duration: 3 *Min: 34.7090*	Dose: 20 Duration: 3 *Min: 4.0048*
Reference (no treatment)	**7.9543**	**6.11*10^07**	**43.0587**	**5.2623**

Table S2. Numerical results for the optimal treatments (Am, Duration) minimizing different infection target criteria in Figure 6 in the main text.