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Suppressing evolution of antibiotic resistance through environmental switching

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Abstract
Ecology and evolution under changing environments are important in many subfields of biology with implications for medicine. Here, we explore an example: the consequences of fluctuating environments on the emergence of antibiotic resistance, which is an immense and growing problem. Typically, high doses of antibiotics are employed to eliminate the infection quickly and minimize the time under which resistance may emerge. However, this strategy may not be optimal. Since competition can reduce fitness and resistance typically has a reproductive cost, resistant mutants' fitness can depend on their environment. Here we show conditions under which environmental varying fitness can be exploited to prevent the emergence of resistance. We develop a stochastic Lotka-Volterra model of a microbial system with competing phenotypes: a wild strain susceptible to the antibiotic, and a mutant strain that is resistant. We investigate the impact of various pulsed applications of antibiotics on population suppression. Leveraging competition, we show how a strategy of environmental switching can suppress the infection while avoiding resistant mutants. We discuss limitations of the procedure depending on the microbe and pharmacodynamics and methods to ameliorate them.

Keywords Antibiotic resistance · Changing environments · Competition · Lotka-Volterra · Microbial ecology · Pulsed antibiotic treatment

Introduction

Populations will face a variety of environmental fluctuations of both biotic and abiotic nature. Since phenotypes typically have different reproductive success in differing environments, the dynamics of these fluctuations can be crucial in determining phenotypic composition. Here, we consider the effects of varying environments on the emergence and maintenance of antibiotic resistance.

The rise of microbial resistance is a looming catastrophe, and prudential use of antimicrobials is a fundamental means to prevent it (Laxminarayan et al. 2013). Such strategies to limit the chance of resistance can be made at all levels of disease dynamics, from population-level protocols to individual patient therapies. Studies of antibiotic resistance in vivo, in hospitals, and in the community at large using mathematical models can help address the pharmacodynamics, pharmacokinetics, and epidemiology of resistance (Lipsitch and Levin 1997; Bonhoeffer et al. 1997; Austin and Anderson 1999; Czock and Keller 2007; Gloede et al. 2009; Greulich et al. 2017; Nielsen and Friberg 2013). Such models have found use in effectively modeling real-world experimental data (Tam et al. 2007; Schmidt et al. 2009; Bhagunde et al. 2011; Nielsen et al. 2011). In particular, modeling has been used in identifying dosing regimens that suppress the emergence of resistance (Tam et al. 2005, 2008).

There are several mechanisms by which bacteria can be resistant to antibiotics (Poole 2002), an example of which is overexpression of the efflux pump (Borges-Walmsley et al. 2003; Webber and Piddock 2003; Sun et al. 2014), which bacteria use to expel antibiotics. Typically, such resistant mechanisms have a fitness cost, which can result in trade-offs between resistance and growth (Martínez and Baquero 2002; Ender et al. 2004; Wang-Kan et al. 2017; Basra et al. 2018). Example costs of resistance include less

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energy for other cellular processes, and impaired motility. When the antibiotic is present, the resistant mechanism pays for its cost by providing a fitness advantage relative to the susceptible strain. However, when the antibiotic is not present, the resistant mechanism incurs a fitness disadvantage. Resistance, therefore, can be reversed under evolutionary forces by altering the environment (Andersson and Hughes 2010). As such, a pulsed protocol, where the antibiotic is periodically applied so that the environment switches from antibiotic to antibiotic-free regimes, may be able to eliminate the bacteria. However, there is a risk that resistant mutants evolve to reduce the fitness cost of resistance rather than lose the resistance mechanism, whereby they could be competitive whether the antibiotic is present or not (Olivares Pacheco et al. 2017). Further, mutations in regulatory genes can produce phenotypes of irreversible resistance (Van Bambeke et al. 2000). These risks can cripple pulsed protocols aimed at controlling the infection while preventing resistance. Preventing a sustained presence of resistance is therefore a high priority.

Here, we develop a mathematical model of pulsed protocols of antibiotic and antibiotic-free regimes, switching rapidly from one environment to the other, to control a bacterial population. We consider concentration-independent (i.e., time-dependent) bactericides such as β-lactams (e.g., penicillins and cephalosporins), which require high maintained concentrations to be effective (AliAbadi and Lees 2000). We assume that there is a maximum benefit to the concentration amount (due either to the pharmacodynamics of the bactericidal mechanism or tolerance of the patient to the antimicrobial). Therefore, we fix the dose concentration when applied and find the proper periods for each regime that prevent the emergence of resistance while eliminating the infection.

Previous theoretical studies have shown that pulsed protocols of antibiotics can eliminate bacteria (Kussell et al. 2005; Cogan 2006; Cogan et al. 2012; Acar and Cogan 2019). However, these studies feature a "persistent phenotype," that neither grows nor dies under application of the antimicrobial agent (Balaban et al. 2004; Zhang et al. 2012). Bacteria may transition between the persister type and wild type, depending on the environmental conditions. The number of persisters remain at low levels and act as a staging ground for the bacteria to repopulate after the antimicrobial is removed. Pulsed protocols of antibiotics, however, can disrupt this process and lead to the elimination of the bacteria. Experimental studies have shown that pulsed protocols can be effective in controlling such a system (Sharma et al. 2015).

Although pulsed protocols can eliminate non-persister and persister colonies, they have more difficulty in eliminating colonies with resistant phenotypes that can grow when antibiotics are present. However, these protocols have been shown to be effective in containing an infection both theoretically and experimentally (Baker et al. 2018; Hansen et al. 2020). In such cases, antimicrobials can act as ecological disturbances and can be approximately as effective as a constant application of the antimicrobial in controlling the bacterial load while also diminishing the probability of the emergence of resistance (Baker et al. 2018). With short durations of high concentrations of drugs, the period under which resistance is selected for can be minimized.

The above studies have explored pulsed protocols in different ways: controlling persisters, and controlling emergence of resistance. The resistant strain we consider here does grow in the presence of antibiotics, and thus are not persisters. Our scenario is thus more similar to, and an extension of, Baker et al. (2018). Our main contribution is to show how leveraging competition can not only suppress the emergence of resistance as in Baker et al. (2018), but also reduce the overall bacterial load. Additionally, we explore the impact of other important mechanisms on pulsed protocols including the evolvability of the bacteria and the lethality of the antibiotic. We compare these results to a protocol of constant application. Though pulsed protocols can, on average, outperform a constant application, constant applications are more likely to completely eliminate the bacteria. However, they are also more likely to result in an uncontrolled population of resistant mutants. Thus, with pulsed protocols we aim to mitigate the emergence of resistance and reduce the risk of the evolutionary escape from the antibiotic.

The key mechanism of our models in suppressing the population is competition between the two phenotypes. Two common models of microbial competition are resource-competition models (Baker et al. 2018) and the competitive Lotka-Volterra equation (Stein et al. 2013; Gonze et al. 2018). The latter of which we employ here. Competition can be low when the total size of the population is small (e.g., the population is well below the carrying capacity or there is a high amount of resource relative to the number of bacteria). In such a case, both phenotypes can grow. Yet, we can still suppress the number of resistant bacteria and the average bacterial load over time. We explore the impact of various parameters and pulsed protocol durations on the average bacterial load over time. Our models also features stochasticity, which we develop in a stochastic kinetic framework (Wilkinson 2011). We show that only when selection against the resistant type is high when the antibiotic is not applied can pulsed protocols effectively control the population.

Methods

Stochastic birth-death processes are widely used in biological modeling (Novozhilov et al. 2006), and, in particular, stochastic modeling of the Lotka-Volterra system (Huang

et al. 2015). Our stochastic model features processes of birth, death, competition, and mutation, as detailed in Box 1. These processes operate on two phenotypes X and Y, which represent a wild-type strain, which is susceptible to the antibiotic, and a mutant strain, which is resistant, respectively.

Consider first the dynamics of the birth and death processes without the presence of antibiotics, i.e., in the antibiotic-free regime. Reaction set 1 represents these processes, where b and d are the birth and death rates, respectively, for the wild-type strain with $b > d > 0$. We assume that the death rate for the resistant strain is also d. However, resistance frequently comes at a fitness cost: a reduced growth rate relative to the wild type (Nagaev et al. 2001; Gagneux et al. 2006; Nilsson et al. 2006; Sandegren et al. 2008). Thus, we assume that the birth rate is reduced by a cost $b > c > 0$ for resistance. The birth rate of the resistant strain is thus $b - c$ (costs applied to birth rather than death rates have also been applied similarly in ecological games (Hauert et al. 2008)).

In the presence of the antibiotic, the above processes still occur, but with an additional set of reactions involving the antibiotic. Since we are considering a concentration-independent or time-dependent antibiotic, we will assume that the amount of antibiotic, $\bar{A}$, remains unchanged while we are in the antibiotic regime. At the maximum dose $\bar{A} = 1$, normalized. The antibiotic is bactericidal and kills both types of bacteria. Reaction set 3 represents death from the antibiotic with rates α and α' for the susceptible and resistant strains, respectively. Note that for X to be susceptible and Y to be resistant, we must have $\alpha > b - d > c + \alpha'$.

Competition dominates microbial interactions (Foster and Bell 2012) whereby bacteria compete over nutrients or space (Ghoul and Mitri 2016). Bacteria also engage in direct antagonism such as by the production of bacteriocin to inhibit the growth of competitors (Bucci et al. 2011). Frequently in Lotka-Volterra systems, intra-specific competition is assumed to be greater than inter-specific. As such, the rate of death would be greater for competition between bacteria of the same type than those that are different. However, since the wild-type and mutant strains are so heavily related, their niches heavily overlap and they would both require similar nutrients. Therefore, competition would be expected to be high (Coyte et al. 2015). Further, since the population is well-mixed, we would expect the interactions to be frequency dependent and not biased toward intra-specific competition. Reaction set 2 details the processes by which the bacteria die from competition with others. Death occurs due to a bacterium losing access to nutrients or space; the loser receives less or none and thus has some chance of dying. Let γ be the rate at which two bacteria compete over a critical resource in a well-mixed population. When bacteria of the same type meet and compete, the chance of either one dying would be the same. However, the cost of antibiotic resistance results in a loss of competitive ability (Letten et al. 2021), and thus a wild-type is more likely to survive competition with a mutant, and the mutant is less likely to survive in such an encounter. We use a Moran-like process (Moran et al. 1962) in determining which bacterium wins and which loses when two meet and compete. The rate at which a focal bacterium survives is its fitness divided by the fitness of its opponent, i.e., the relative fitness of the focal bacterium. Fitness then is a proxy for the competitiveness of each type. Such a process is common in the theoretical literature in modeling competition (Taylor et al. 2004; Imhof and Nowak 2006; Fudenberg et al. 2006) and particularly local competition in spatial settings (Roca et al. 2009). Fitnesses in our model are the growth rates of each type: $b - d$ and $b - c - d$ for wild-type and mutant strains, respectively. If the focal type and its competitor are the same type, then the relative fitness is equal to one and therefore death occurs at rate γ. For the wild-type vs the mutant, relative fitness is $\kappa \propto (b - d)/(b - c - d)$ for the wild-type vs the mutant, and thus $1/\kappa$ for the mutant vs the wild-type. Note that we do not set the relative fitness $\kappa = (b - d)/(b - c - d)$, but rather proportional to it. Since, competition may scale nonlinearly with respect to relative fitness.

Bacteria of one type can produce mutants of the other type at rate μ, which occurs in both regimes (Reaction set 4). However, under the stress of the antibiotic, resistant mutants will arise from wild-type bacteria at a higher rate $\mu' > \mu$. Mutations are an important mechanism leading to antibiotic resistance (Martinez and Baquero 2000; Woodford and Ellington 2007), including single-point mutations (Andersson and Hughes 2011; Meka et al. 2004a, 2004b). Additionally, resistance from single mutational events tend to be costly (Melnyk et al. 2015). For example, consider the loss of flagellar function in *Pseudomonas aeruginosa*, which alone can give a fitness advantage in the presence of an antibiotic, and is costly relative to the wild type when no antibiotic is present (Rundell et al. 2020). Single mutations can also lead to overproduction of efflux pumps, which can provide resistance (Lister et al. 2009). Then, in an antibiotic-free environment, mutations can restore impaired function, reverting the bacterium to the wild type. And, competition from any remaining wild-type bacteria can out-compete the resistant mutants.

The environmental switching is controlled by a choice of the "on" and "off" durations of the drug. We assume 100% bioavailability of the drug at application, e.g., intravenous application. Thus, when the antibiotic is "turned on," its effects are immediate. Further, when it is "turned off," the dissipation of the antibiotic, i.e., the rate at which it breaks into ineffective material, is metabolized, etc. is rapid, which is common for concentration-independent and time-dependent antibiotics. In the antibiotic regime, we apply the maximum effective dose.

Thus, the set of pulsed protocols we consider are sequences of durations of the regimes, where $\bar{A} = 1$ when the antibiotic is "on" and $\bar{A} = 0$ when it is "off".

We conducted numerical simulations of the model to test the effects of various protocols. We average realizations for each parameter combination. Using Julia's DifferentialEquations and Catalyst packages (Rackauckas and Nie 2017), we simulate the dynamics as a stochastic differential equation (chemical Langevin equation); the equations of which are explicitly displayed in the SI Appendix. Table 1 lists the default parameter values used with rates per hour. We vary these values to explore nearby parameter space. We assume that a new generation occurs after $1/(b-d) = 1$ hour. We estimate that the relative fitness of the resistant strain in the antibiotic-free environment is $(b-c-d)/(b-d) \approx 0.8 \implies c = 0.2$ per hour, which is within experimentally evaluated values (Melnyk et al. 2015). Our default value for the mutation rate is $\mu = 10^{-9}$. Under stress from the antibiotic, the mutation rate can be larger, up to ten times the non-stressed rate (Kuban et al. 2004). Thus, we consider $\mu' = 10\mu$. We assume that resistant bacteria die from the antibiotic at one tenth the rate susceptibles do (Coates et al. 2018). As such, we fix the death rate of mutants via the antibiotic to $\alpha' = 0.1\alpha$. The initial condition is a population of 100% susceptible bacteria, $X_0 = 10^9$. We explore a variety of competition parameters κ. We consider fixed "on/off" durations, where we repeat switching until the population is extinct or 14 days have elapsed.

Box 1: Stochastic Lotka-Volterra processes

Birth/death: $X \xrightarrow{b} 2X \quad Y \xrightarrow{b-c} 2Y \quad X \xrightarrow{d} \emptyset \quad Y \xrightarrow{d} \emptyset$ (1)

Competition: $2X \xrightarrow{\gamma} X \quad 2Y \xrightarrow{\gamma} Y \quad XY \xrightarrow{\gamma/\kappa} Y \quad XY \xrightarrow{\gamma\kappa} X$ (2)

Death via antibiotic: $\bar{A}X \xrightarrow{\alpha} \emptyset \quad \bar{A}Y \xrightarrow{\alpha'} \emptyset$ (3)

Mutation: $X \xrightarrow{\mu(1-\bar{A})+\mu'\bar{A}} Y \quad Y \xrightarrow{\mu} X$ (4)

Results

First consider the behavior of the system when the antibiotic is present or absent. In either case, both types will coexist at equilibrium due to mutations, though the less adapted type will remain at low frequency. In the antibiotic-free environment when the population size is low, competition will also be low, which can allow both types to grow in abundance. However, the higher the competition term κ, the smaller this region is. A large κ will cause the mutant strain to be suppressed, which increases the chance that the mutant strain will be eliminated. In the remainder of the results, we detail the effects of switching the drug "on" and "off," competition, and stochasticity.

Figure 1 depicts a representative time series for a switching protocol vs. a constant application of the antibiotic. With a sufficient competitive disadvantage for resistance, i.e., high κ, we can effectively suppress the average bacterial load over time and resistant bacteria relative to a constant application as depicted in Fig. 1b (pulsed protocol) and a (constant application). Though Fig. 1a depicts a specific instance where the mutant becomes established under the constant application protocol, resistance can be prevented by rapid elimination of the population. Since the mutations and fluctuations in abundances are stochastic, it is possible that we are fortunate and constant application drives the population extinct before resistance emerges. Thus, to better understand the effectiveness of therapies we must evaluate the statistics of the bacterial load as a function of system parameters. We will show that constant application of an antibiotic tends to lead to either extreme: elimination of the entire population, or the establishment of a majority resistant population.

Averaged over 200 realizations, we calculate average bacterial load over time for pulsed protocols with various "on" and "off" durations and compare these to the bacterial load for constant application of the antibiotics. We plot these results in heat maps, where the color indicates the long-term bacterial load relative to the outcome from constant antibiotic application. Red indicates that the pulsed therapy

Table 1 Summary definitions of parameters

Parameter and default value	Definition
$b = 1.4$ per hour	Birth rate
$c = 0.2$ per hour	Cost for resistance
$d = 0.4$ per hour	Death rate
$\alpha = 1.6$ per hour	Death rate of the wild-type via antibiotic
$\alpha' = 0.1\alpha = 0.16$ per hour	Death rate of the mutant via antibiotic
$\gamma = 10^{-10}$ per cell per hour	Competition rate
$\kappa > 1$	Death rate of mutants from competition with the wild-type
$\mu = 10^{-9}$ per hour	Mutation rate
$\mu' = 10\mu = 10^{-8}$ per hour	Stress-induced mutation rate of susceptible to resistant bacteria

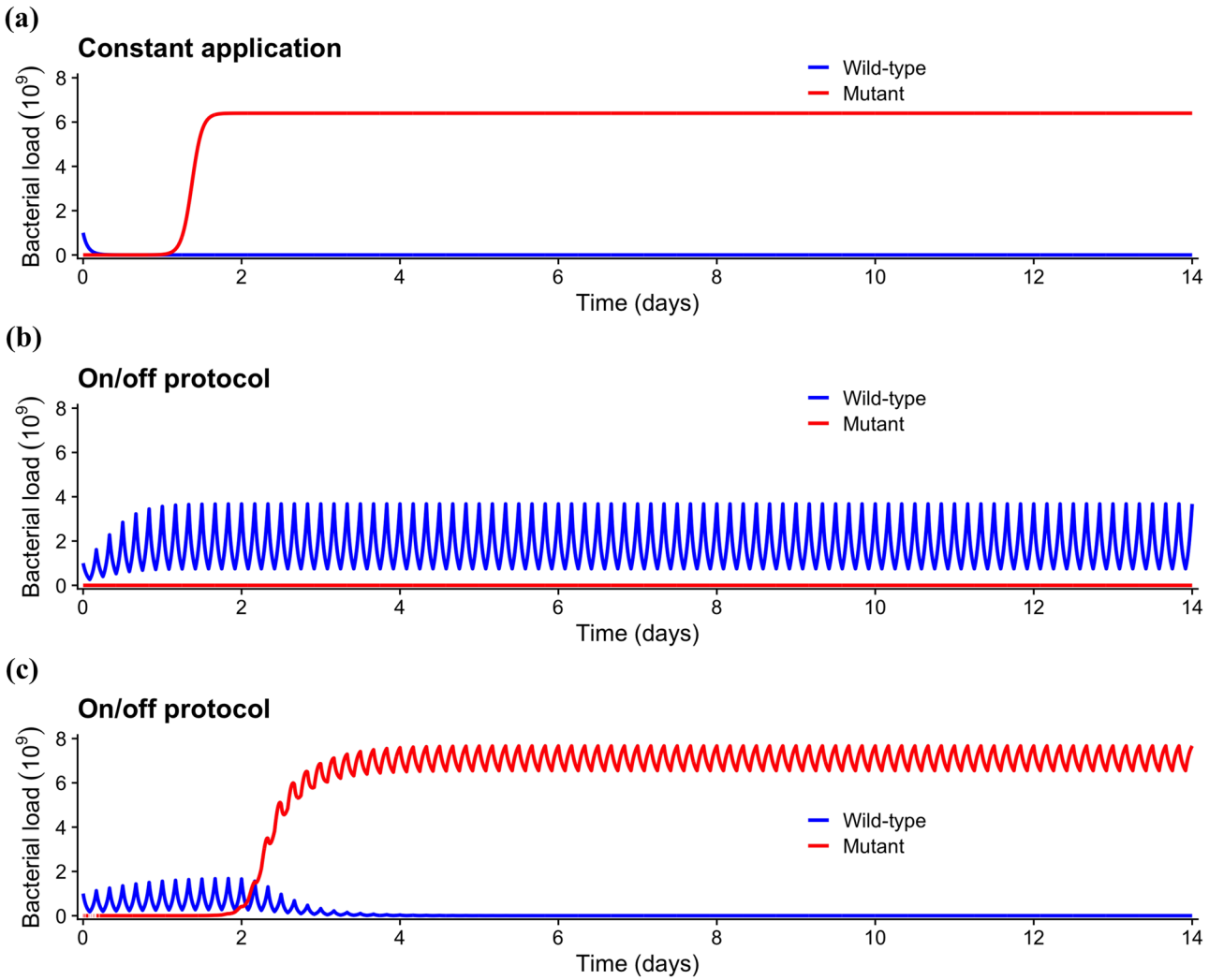

Fig. 1 Representative time series for resistance emerging under constant application, panel a, and results for a pulsed protocol ("on" and "off" for 2hrs each), panels b-d. Blue are wild strain and red resistant. Constant applications can lead to mutant take over (**a**) whereas pulsed protocols can suppress the bacterial load (**b**). $\alpha = 1.6$ and $\kappa = 4$ in both panels **a** and **b**. However, if we increase the antibiotic kill rate to $\alpha = 1.8$ while keeping the other parameters unchanged, the pulsed protocol fails (**c**). $X_0 = 10^9$ wild-type strain bacteria, and the remaining parameters, if not mentioned here, are from Table 1

is on average worse than constant application, yellow is on average equal, and blue indicates that it is on average better. We observe that pulsed protocols along a diagonal do best. One reason for this is that the switching times explored here are much less than the time to reach carrying capacity in either regime. For example, even a day-period protocol will not reach carrying capacity (the expected time to reach carrying capacity is between one and two days). In such a case, the population can swing from predominantly one type to the other (see the SI Appendix for an example time series). However, this behavior can still be beneficial, since each application of the antibiotic is another chance of eliminating the population, since switching environments drives the dominant type down potentially to extinction before the other type can become established. Another pertinent fact is that the total dose of antibiotic over the whole treatment period differs between the protocols. In particular, the region above the diagonal has lower total doses than the region below it. Nonetheless, such protocols can be more effective than those with a greater total dose: Figs. 2, 3, 4, and 5. Further, increasing the "on" duration from the diagonal can result in worse outcomes than decreasing it. In addition to plotting heat maps, we plot the average bacterial load over time for constant and pulsed protocols for various values of each parameter averaged over 200 realizations. The durations the antibiotic is "on" and "off" are each 2hrs.

Figure 2 shows that the higher the competition, the lower the diagonal (i.e., the best results come from protocols

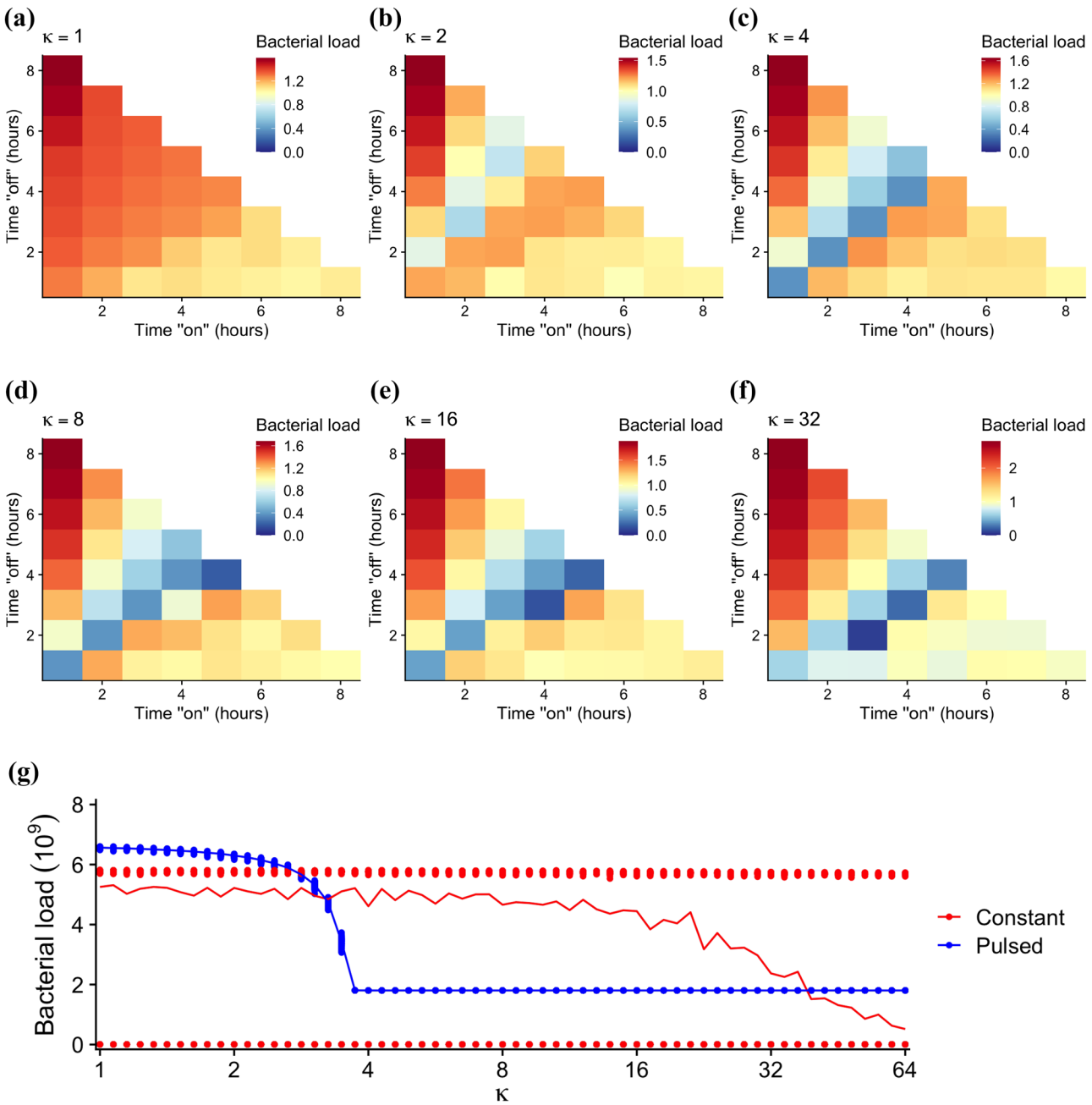

Fig. 2 Heatmaps of the average bacterial load over time from pulsed protocols relative to that of constant application of the antibiotic for $\kappa = 1, 2, 4, 8, 16, 32$ (panels **a**–**f**). Protocols that matched the average outcome of the constant application therapy are colored in yellow. Those protocols that did worse are in red, and those that did better are in blue. Panel **g** depicts the average bacterial load over time for constant and pulsed (2hrs "on" and "off" each) for various κ. The points are the results for individual realizations and the curves their average

where the duration "on" is greater than the duration "off"). The increased competition suppresses the emergence of resistance even in the antibiotic environment, and thus the duration of application can be longer. We also observe an intermediate level of competition is best for pulsed protocols relative to constant application. We can see this effect in Fig. 2g. Increasing κ decreases the average of the bacterial load of the individual realizations for the constant application as we would expect. Since, high competition between the types will suppress the emergence of resistant mutants (which is true in both environments). However, increasing competition has an initially steeper effect upon pulsed protocols before it levels off. A sufficient amount of competition is required for pulsed protocols to work. As κ is increased, the difference between the outcomes of the two protocols decreases. For high κ, constant applications

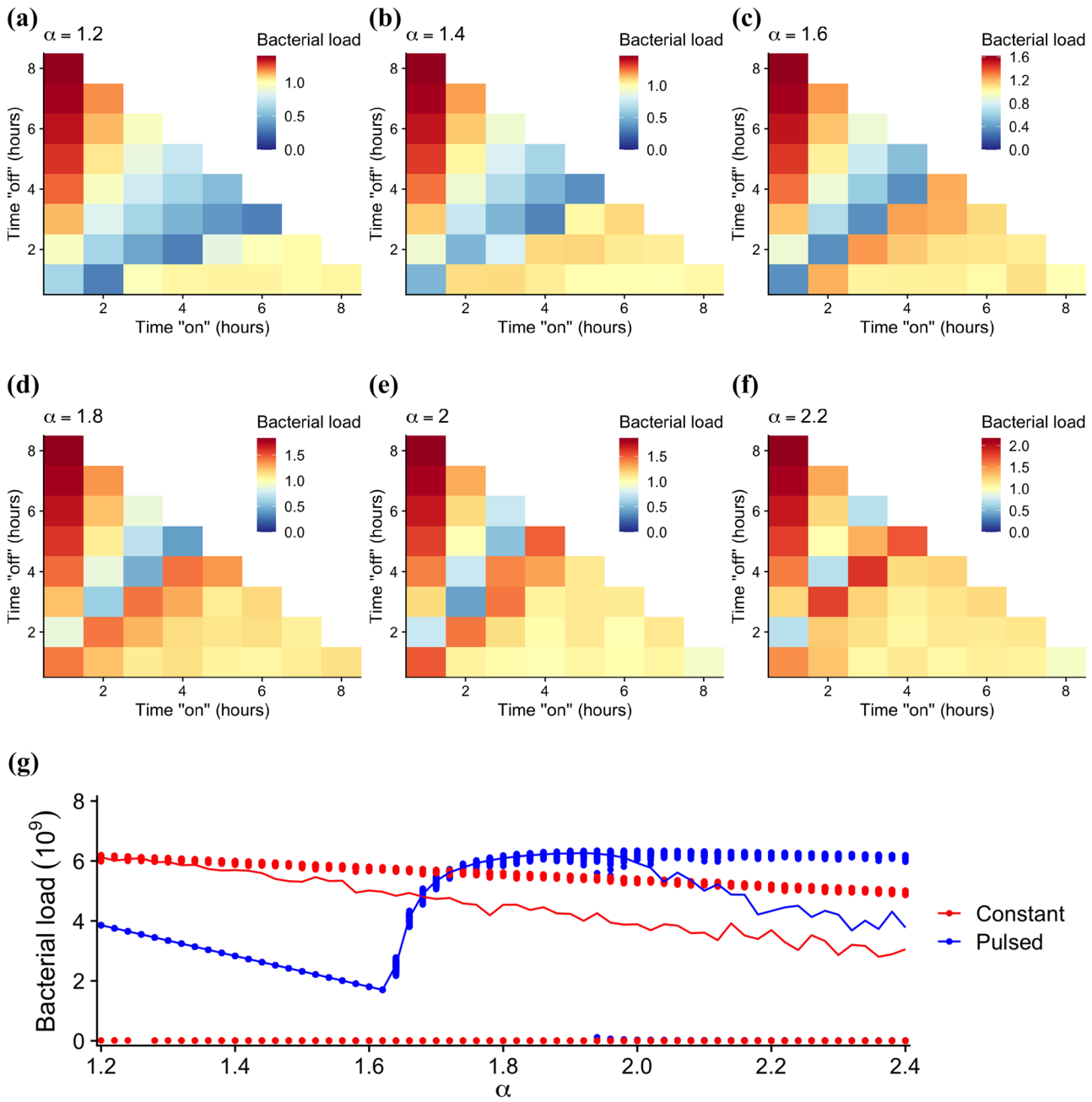

Fig. 3 Heatmaps of the average bacterial load over time from pulsed protocols relative to that of constant application of the antibiotic for $\alpha = 1.2, 1.4, 1.6, 1.8, 2, 2.2$ and $\kappa = 4$ (panels **a**–**f**). Protocols that matched the average outcome of the constant application therapy are colored in yellow. Those protocols that did worse are in red, and those that did better are in blue. Panel **g** depicts the average bacterial load over time for constant and pulsed (2hrs "on" and "off" each) for various α. The points are the results for individual realizations and the curves their average

outperform pulsed ones. We can also see this effect in the bacterial load, which changes non-monotonically with κ.

Another reason for the angle of the optimal diagonal of successful protocols is due to the relationships between the mean growth rates and the antibiotic kill rates. Figure 3 depicts the results for various values of α. Note that in each case we vary the death rates via antibiotics for both the wild-type and mutant type by setting $\alpha' = 0.1\alpha$. The higher the antibiotic kill rate, the shorter the duration on for the most successful protocols. Like in the case of κ, α impacts the effectiveness of pulsed protocols nonlinearly. Figure 3g depicts the mean results for various α. The higher the α, the better constant application does. However, this is not true for pulsed protocols. An intermediate value is best. This result

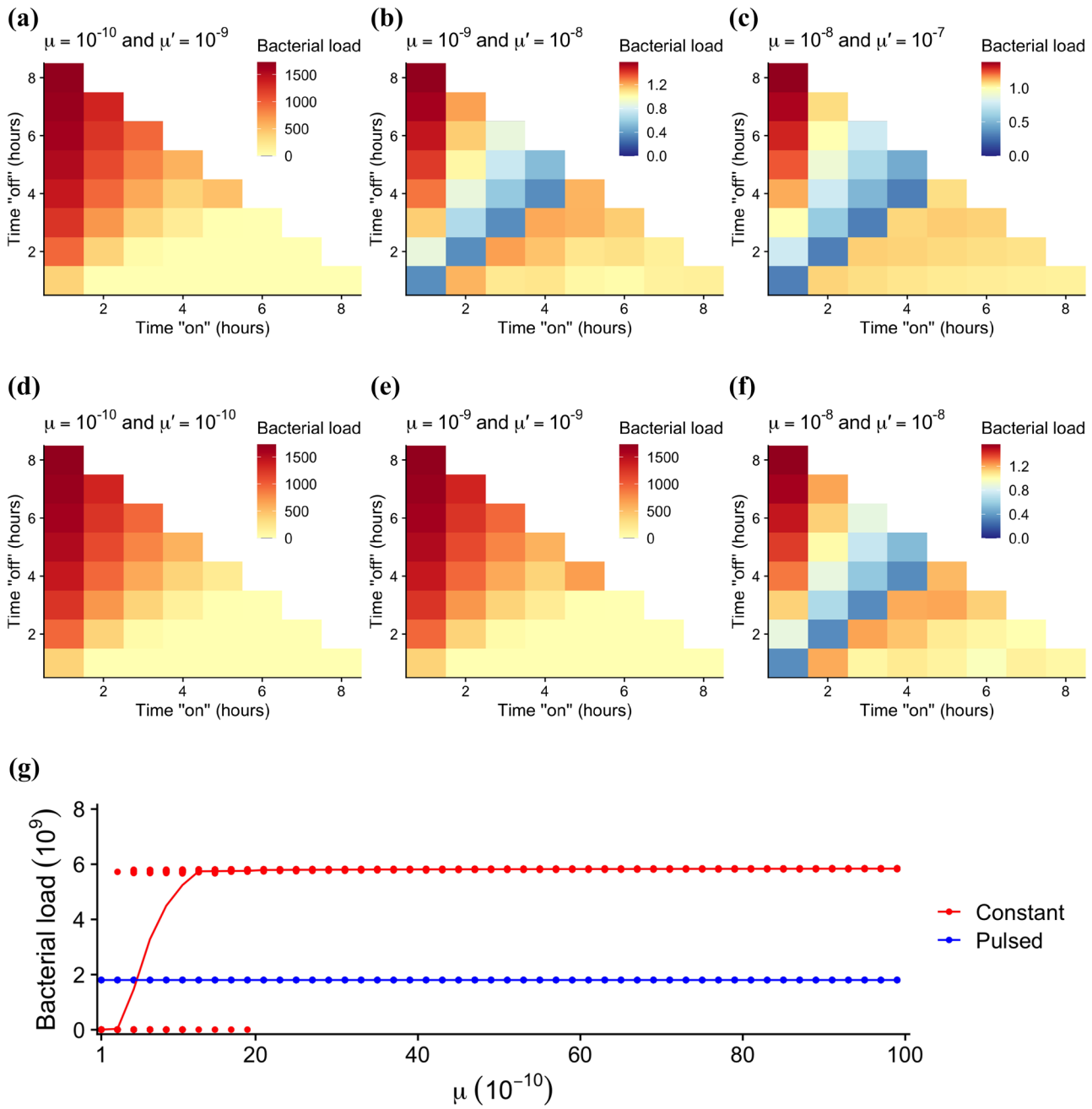

Fig. 4 Heatmaps of the average bacterial load over time from pulsed protocols relative to that of constant application of the antibiotic for $\mu = 10^{-10}, 10^{-9}, 10^{-8}$, $\mu' = 10\mu$, μ, and $\kappa = 4$ (panels **a**–**f**). Protocols that matched the average outcome of the constant application therapy are colored in yellow. Those protocols that did worse are in red, and those that did better are in blue. Panel **g** depicts the average bacterial load over time for constant and pulsed (2hrs "on" and "off" each) for various μ. The points are the results for individual realizations and the curves their average

is due to the impact of α on competitiveness. If α is too high, then the wild-type is suppressed too much, and thus cannot be used to suppress the mutant strain through competition. Figure 1c depicts a time series of the case where α is too high, resulting in failure of the pulsed protocol. Increasing competition κ, however, can mitigate this effect, shifting the minimum to the right (see the SI Appendix for an example).

To explore how robust our results are to mutation rates, we considered various values of μ and μ'. We can see the effects of various μ in the rows of Fig. 4, which show that the pulsed protocols are more effective under a higher mutation rate. The first row depicts the case where there is a stress-induced mutation rate from wild-type to mutant ($\mu' = 10\mu$). The second row depicts the results where stress does not

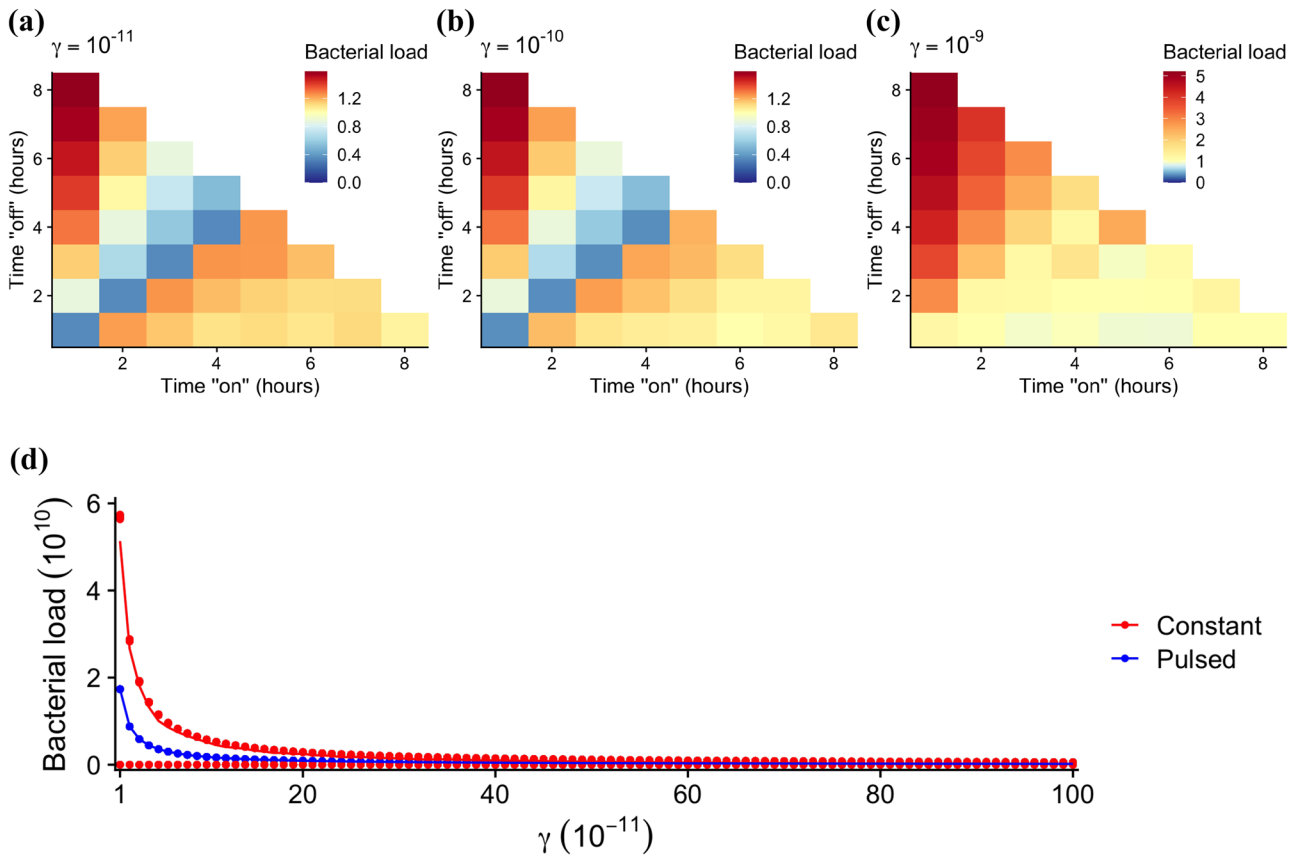

Fig. 5 Heatmaps of the average bacterial load over time from pulsed protocols relative to that of constant application of the antibiotic for $\gamma = 10^{-11}, 10^{-10}, 10^{-9}$ and $\kappa = 4$ (panels **a**-**c**). Protocols that matched the average outcome of the constant application therapy are colored in yellow. Those protocols that did worse are in red, and those that did better are in blue. Panel **d** depicts the average bacterial load over time for constant and pulsed (2hrs "on" and "off" each) for various γ. The points are the results for individual realizations and the curves their average

increase the mutation rate ($\mu' = \mu$). The stress induced mutation makes the antibiotic environment more conducive to generating resistance, and thus makes it harder to control the emergence of resistance. Figure 4g shows that the mutation rate impacts the constant application more so than the pulsed protocol. This result matches intuition; the higher the mutation rate, the less likely a constant application can eliminate the colony before a mutant arises and becomes established. In summary, the more evolvable the system, the better switching environments works.

In Fig. 5 we explored the effect of varying the contact rate γ, and observed that switching was more effective for a low γ. For a high γ, the region where both types can grow is small, which magnifies the impact of stochastic effects leading to elimination of emerging mutants. Further, same-type competitive interactions are also more intense, and thus the population is driven to extinction more quickly. Figure 5d shows that the effectiveness of both constant application and pulsed protocols increases as γ increases (and thus the carrying capacity is lower). However, the gap between the two shrinks. Thus, for a high γ system, the average of the bacterial load of the individual realizations for the constant application is similarly to the low level of the pulsed protocol.

Discussion

Mathematical modeling has been important in the fight against resistance through increasing our understanding of the dynamics and emergence of resistance (Opatowski et al. 2011). This paper contributes to this endeavor by showing that the average bacterial load over time can be reduced and the emergence of resistant mutants can be mitigated via pulsed protocols. Previous research has shown that pulsed protocols with sufficiently long periods between switching can eliminate the population while rapidly changing environments are ineffective (Marrec and Bitbol 2020). Our model would also exhibits this behavior. Long durations between pulses can (re)establish the wild-type (see the SI Appendix for a time series example). Upon application of

the antibiotic, the population would then either be eliminated or rescued by resistant mutants. However, in a clinical setting, this may not be feasible. Though quickly varying the environment can often not eliminate the population, it can suppress the bacterial load and resistance. This strategy can be thought of as "playing to not lose," which contrasts with a constant application (or long durations) where the bacteria are either eliminated or resistance flourishes (i.e., "playing to win or lose") (Fischer et al. 2015).

We found that pulsed protocols only work when competition is sufficiently high (though not too high). Else, at low population levels, resistance can be maintained. Further, the transition from an ineffectively competition level to a higher effective one occurs abruptly. We observe a rapid increase in the effectiveness of pulsed protocols as the competition level is increased from a low level ($\kappa < 4$). For higher competition levels ($\kappa \approx 4$ and greater), the effectiveness of pulsed protocols do not change. However, the effectiveness of the constant application does improve, reaching and then surpassing the pulsed protocol for high competition rates. This result is because the increased competition places sufficient pressure on resistant mutants that they only rarely become established. Previous empirical and theoretical research has also found the importance of high competition in containing an infection (Hansen et al. 2020). Further, pulsed protocols and competition can be effective in containing an infection even in a well-mixed population (Hansen et al. 2020). Spatial effects, such as those found in biofilms, could heighten the degree of competition and thus the effectiveness of the pulsed protocols. Since, spatial heterogeneity due to clumping could keep the competition level between different types high even when the population is small relative to the carrying capacity. The competition effects we consider can be interpreted crudely as arising from such effects. However, we note that the high degree of inter-specific competition that we have considered is a key assumption of our model. It is a common assumption in many theoretical papers in the literature, and a reasonable one; but we should caution that it need not hold true universally for all microbes in all environments. Accordingly, the conclusions of this paper should be accepted cautiously and in the light of this important fine print.

Our findings offer several general recommendations for pulsed drug therapies. For one, pulsed protocols are primarily effective when the emergence of resistance is likely, such as in large populations and those with high mutations (whether innately or under stress from the antibiotic). Pulsed protocols are also expected to be effective for intermediate degrees of competition and antibiotic kill rates. When competition is light or fierce or when the antibiotic is weak or very strong, constant applications of antibiotics are likely to be more effective. The optimal proportion of time for a pulsed protocol in the antibiotic-regime would, in general, be decreasing with respect to the antibiotic kill rate. Since, less time is needed to suppress the wild-type, which also reduces the likelihood of mutant expansion. This effect is why we observe the shifting of the diagonal curve up in Fig. 3. However, the greater the kill rate, the greater the chance of the constant application eliminating the bacteria before resistance can emerge. That is why in that same Figure the effectiveness of pulsed protocols decreases. Higher competition between types (κ) increases the effectiveness of suppression in both regimes. Thus, less time is needed during the antibiotic-free regime to suppress mutants (which is better to avoid mutations emerging), yet also more time can be spent in the antibiotic regime killing the wild-type. In our case, decreasing the wild-type in the antibiotic regime had the greater benefit, and so the optimal proportion of time on increased with κ as observed in Fig. 2. The improvement in suppressing resistance in the antibiotic regime can lead to constant applications outperforming pulsed applications as in Fig. 2g. This would not be the case if competition favored resistant mutants in the antibiotic regime (such as if antibiotics tended to hamper but not directly kill the wild-type bacteria). Increasing the competition through γ drives the population down in both pulsed and constant therapies: growth is inhibited, fewer bacteria are present, and mutants are less likely to arise. The results for both pulsed and constant therapies converge to the same outcome (Fig. 5d). The relationship between the times in each environment is also an important factor. To suppress resistant bacteria through competition and pulsed protocols, we must maintain the population of wild-type bacteria, and not let it become weak. We can see the implication of this in Fig. 1. Figure 1b has a relatively robust population of wild-type bacteria, while Fig. 1c has a much smaller one, which permits resistance to emerge and dominate. To minimize the emergence of resistance, we would recommend maintaining the wild-type population at the highest acceptable population size.

There are several other relevant biological and technical factors that could impact the effectiveness of pulsed protocols. For one, bactericides with significant post-antibiotic effects (PAEs), such as fluoroquinolones, may hamper our control strategy. Antimicrobials can impact the bacteria at sub-MIC levels long after they have been removed from the system (Shojaee AliAbadi and Lees 2000), and as such can select for resistant bacteria after the antibiotic is no longer applied. Additionally, sub MIC levels can lead to multidrug resistance through radical-induced mutagensis (Kohanski et al. 2010). Therefore, we must have a rapid dissipation of the antibiotic once below the MIC to prevent selection for the mutant (the range between the MIC and the point at which the susceptible strain is selected for) (Gullberg et al. 2011). PAEs are frequently caused by antibiotics that impair DNA functioning. Hence, β-lactams, which inhibit cell wall production, are a good choice for our therapies.

Antibiotics with a short half-life would also be effective with our protocols. Though not explored extensively here, we consider PAEs resulting from degrading antibiotics in the SI Appendix, where we show that increasing the half-life of the antibiotic can decrease the effectiveness of pulsed protocols. However, pulsed protocols that were ineffective can become effective due to PAEs. Essentially, the PAEs extend the period the antibiotic is "on," which can be counteracted by having a longer "off" period.

Intermediate drug concentration and monitoring of the bacterial load are other aspects that we did not consider here. The effects of concentration on the spread of the disease due to within host and between host dynamics have been found to be an important factor (Scire et al. 2019). It is not always beneficial to have a high concentration due to a U-shaped probability of resistance emerging vs. drug concentration (Day and Read, 2016). Here we only considered a specific concentration for all applications. We also restricted ourselves to the case where the system cannot be well monitored. Clearly, the best strategy is to alter the durations dependent on the state of the system, which can both maximize the duration of the antibiotic regime and prevent the emergence of resistance. The more repeated applications of the treatment, the less likely it will work. However, such observations may not be feasible, especially when the bacterial load is small and heterogeneous.

Future models could incorporate other biological factors. For example, the setting and source of resistance (source-sink dynamics) are important factors in controlling antibiotic resistance (Perron et al. 2007). The method by which resistance is spread is another important factor such as where plasmids confer resistance. In such a scenario, resistance can reemerge rapidly. Since, plasmids can remain in the population due to horizontal transfer even when the plasmid confers a cost (Lopatkin et al. 2017). Although, the transfer rate has been show to dramatically fall once the population is low (Händel et al. 2015). Compensatory mutations could also be added by which the cost to resistance could be reduced (c or κ could be reduced). However, we did explore the case where $c = 0$, and found that though pulsed protocols were less effective than when there was a cost, they could still suppress the population and resistance (see the SI Appendix for these results). Future models could also incorporate more of the complexity of interactions between the bacteria and the patients' natural flora (Wade et al. 2016; Estrela and Brown 2018).

More generally, our work fits within the theory of controlling evolving populations. Which, outside of bacteria, has been used to study cancer (Komarova 2006; Katouli and Komarova 2011; Fischer et al. 2015) and which families of chemotherapies will work best. Adaptive therapy aims to leverage evolutionary and ecological principles such as competition to treat cancer (Enriquez-Navas et al. 2015; Gatenby and Brown 2020; West et al. 2020). An adaptive therapy technique, like ours here, is to retain chemosensitive cells so that they may suppress chemoresistant ones via competition (Gatenby et al. 2009). Our results, qualitatively, may have implications for such strategies in managing cancer. More generally, our model can be viewed as control of "species" in conflict under directed actions of an external force (in our case, the application of antibiotics) or under environmental fluctuation (which could be undirected). Though our aim here has been to lower the overall bacterial load, we have shown how alternating environments can prevent one species dominating thereby sustaining coexistence. This temporal heterogeneity in competitiveness can thus act as a stabilizing mechanism that promotes diversity (as measured by the relative proportions of each type over time). This observation has broader theoretical implications to abundance and diversity of phenotypes or species competing with one another. In a switching environment, intermediate levels of interaction between different phenotypes or species can result in higher diversity, e.g., in our case, both phenotypes may coexist when the environment is varying while a constant environment leads to extinction or the resistant strain dominating (i.e less relative diversity than if both types coexist at low levels). Though our model is within a framework of bacterial competition, this phenomenon would apply to competitive Lotka-Volterra systems under environmental switching more generally. As such, we envisage further research that explores such phenomena under scenarios other than control of antibiotic resistance and through models related to our own.

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Declarations

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