Predicting drug resistance evolution: antimicrobial peptides vs. antibiotics

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Abstract

Antibiotic resistance constitutes one of the most pressing public health concerns. Antimicrobial peptides are considered part of a solution to this problem, because they are new agents that add to our repertoire. Importantly, antimicrobial peptides differ fundamentally from antibiotics in their pharmacodynamic characteristics. Here we implement these differences within a theoretical framework to predict the evolution of resistance against antimicrobial peptides and compare it to antibiotic resistance. Our analysis of resistance evolution finds that pharmacodynamic differences all combine to produce a much lower probability that resistance will evolve against antimicrobial peptides. The finding can be generalized to all drugs with pharmacodynamics similar to AMPs. Pharmacodynamic concepts are familiar to most practitioners of medical microbiology, and data can be easily obtained for any drug or drug combination. Our theoretical and conceptual framework is therefore widely applicable and can help avoid resistance evolution if implemented in antibiotic stewardship schemes or the rational choice of new drug candidates.

Antibiotic resistance is prevalent [1] and evolves quickly. On average it takes two years from the introduction of a new antibiotic to the clinic until resistant strains emerge. Prudent use and the introduction and development of novel antibiotics are currently considered to be the most effective ways to tackle resistance evolution[2]. The prediction of when and how antibiotic resistance evolves and spreads is notoriously difficult, but would be extremely informative for antibiotic stewardship and the development of new drugs.

Amongst the new drugs under development are antimicrobial peptides (AMPs)[3]. One of the alleged advantages of AMPs is that bacterial resistance would evolve much more slowly than against antibiotics[4,5], a highly desirable property[6].

We have recently demonstrated that AMPs affect growing bacterial populations differently from antibiotics, i.e. they differ in their pharmacodynamics[7]. Pharmacodynamic characteristics of susceptible and resistant bacterial strains can be used to predict the evolution of resistance[8]. Such predictions are based on a concept called the 'mutant selection window' (MSW, Fig 1)[9,10]. The MSW has been successfully applied in animal models, demonstrating its value to understand resistance emergence *in vivo*[11].

The width of the mutant selection window is partly determined by the steepness of the pharmacodynamic curve (see Fig 1). Importantly the concentration range between no killing and maximal killing is much narrower for AMPs than antibiotics, resulting in a much steeper curve. The maximum killing rate of AMPs is much higher than of antibiotics, as reflected in quicker killing time[12] (see also Fig S1). Another difference relevant to the evolution of resistance is the finding that many antibiotics increase mutation rates of bacteria[13,14], but AMPs do not show such an effect[13,14].

Here we build on a theoretical framework to study pharmacodynamics[15,16]. We use an approach that explicitly models the steepness of the curve[15], which is not incorporated in many other pharmacodynamic models[17]. We use this approach as this allows to calculate the size of the mutant selection window that generalizes over all possible resistant strains. Gullberg *et al.* demonstrated[10] that resistant mutants are already under positive selection below the MIC (minimum inhibitory concentration) of the susceptible strain. We therefore use the mutant selection concentration (MSC, Fig 1A) as the lower boundary, not the MIC of the sensitive strain that was used previously[8,9]. We show, based on empirically estimated parameters that the probability of resistance evolution against AMPs, defined by pharmacodynamic properties only, is much lower than for antibiotics. We therefore provide a robust and generalizable predictive framework for studying the evolution of drug resistance. This is particularly useful to apply when new drugs are introduced, i.e. before resistance has evolved.

Results

The mutant selection window (Fig 1) shows the concentration of an antimicrobial under which susceptible strains are suppressed, but resistant strains can still grow[9]. We show that the lower bound of the mutant selection window (MSC) can be calculated based solely on the pharmacodynamics of the susceptible strains and the costs of resistance (Fig 1A, Fig 2A, equation 3). The cost is defined here as the reduction of growth rate in a drug free environment.

The pharmacodynamics of AMPs and antibiotics differ significantly[7]: the pharmacodynamic curves of AMPs are much steeper as captured by a higher Hill coefficient κ (see Fig 2A); the step from a concentration with no effect to a killing concentration is therefore much smaller. This feature is likely due to a higher number of "hits" that AMPs need to deliver to bacteria to kill them and perhaps cooperative binding of AMPs molecules to the cell membrane[18]. This results in a narrower MSW for AMPs than antibiotics The MSW opens at lower concentrations when the costs of resistance are low. Our re-analysis of data on antibiotic resistance against a variety of antibiotics in a number of different bacterial species (data from[19]) shows that the upper bound of the MSW correlates with the cost of resistance (Fig 2B). Taken together we are now in a position to estimate the size of the MSW for any drug, if estimates of pharmacodynamic parameters based on the sensitive strains, including the MIC, the maximum effect and the steepness of the pharmacodynamics curve are available (Fig 1A, Fig 2C).

Next we wanted to explore if the differences between AMPs and antibiotics in the width of the MSW correlated with different probabilities of drug resistance evolution within a host. A further difference between AMPs and antibiotics is that antibiotics increase mutagenesis but AMPs do not[13,14]. We incorporated this difference in addition to the difference in the steepness of the pharmacodynamics relationship into a stochastic model describing bacterial replication and evolution under selection pressure from AMPs. We consider two cases here: (a) do resistant mutants emerge and (b) do resistant mutants drive the susceptible strains to extinction?

We find that resistance emerges with a much higher probability for the parameter settings of antibiotics (top row Fig 3B) than for AMPs in our simulations (bottom row Fig 3B, Fig 3A). All intermediate cases, where we simulated changes in one or two of the parameters kappa, mutation rate and maximum effect, also reduce the probability of resistance emergence compared to 'pure' antibiotics.

We also find that resistant mutants are much more likely to drive the susceptible bacterial populations to extinction under antibiotic than under AMP treatment (Fig 3 B). Again, this result also holds when we study intermediate cases (Fig S4). In summary our results show that the application of drugs with low κ, mutation elevation and low maximum effect, i.e. characteristics found in most common antibiotics, inherently bears a high risk of causing the evolution of resistance.

We have shown before[7] that combinations of AMPs have higher κ and lower MICs than individual AMPs. This also results in differences in resistance selection and the extinction of susceptible strains, consistent with the results above.

Day *et al* [20] provided an approach to calculate a resistance hazard: a measure that combines the time of resistance emergence and its selection within a host. We calculated similar resistance hazard for AMPs in comparison to antibiotics. The simulation results show (Fig 3C) that the hazard is much higher and the concentration range much wider under antibiotic treatment than under AMP treatment. Also, when resistance evolves, it emerges earlier in the antibiotic scenario than in the AMP scenario at low concentrations (Fig 3D). Time of emergence is mostly affected by κ and mutation rate: higher κ and mutation rate confer delayed resistance emergency (Fig S5).

Increasing κ and/or the maximum effect are hence desirable for any drug as well as advantageous to hosts managing their microbiota using AMPs. Our model therefore provides useful information for the development of new antimicrobial drugs: higher κ and maximum effect will impose much weaker selection on the bacteria to evolve resistance, i.e. will be less likely to cause the evolution of resistance. Currently mostly AMPs display these properties, but it is likely that new antibiotics that target the cell membrane or wall display similar pharmacodynamics.

Discussion

For the purpose of our approach, we employed theory that is blind to the molecular mechanism of killing. Instead we focused on differences between AMPs and antibiotics that seem to be rather generalizable: pharmacodynamics and mutagenesis. Our model predictions clearly show that AMPs, or in fact any antimicrobial drug with similar pharmacodynamics, are much less likely to select drug-resistant mutants than antibiotics because of the smaller size of the MSW.

The smaller MSW under AMPs is a direct consequence of the fact that their pharmacodynamics functions are steeper[7]. It is important to note that this relationship hinges on the realization that the window opens at the concentration at which the resistant strains have a higher growth rate than the sensitive strain, well below the MIC of the sensitive strain[10]. Thus, a high Hill coefficient (κ) would constitute a promising characteristic of new antimicrobials. The other characteristics in which AMPs differ from antibiotics – the mutagenesis and the maximum effect – affect mostly the time until resistance emerges, but not the size of the MSW. Because this

time becomes shorter with higher population sizes, these characteristics may have less significance for clinical infections [21].

One recommendation derived from our modeling approach is that drugs that show pharmacodynamics resembling AMPs should be good candidates for slowing the evolution of resistance. Interestingly, combinations of AMPs result in increased κ, which our model predicts to bear lower risks of evolution of resistance. It is often argued that combination therapy reduces resistance evolution (but also see [22]), as it is supposedly more difficult to evolve resistance against more than one mechanism at a time. Our approach indicates that combination therapy might even prove effective if there are mutations that confer complete cross-resistance to the drugs in the combination.

It has been proposed that bacterial resistance evolution against AMPs is highly unlikely [4,5]. Yet, *in vitro* experimental evolution has demonstrated that resistance to AMPs can arise [23–25] and AMP-resistance mechanisms have been characterized [26]. Against antibiotics, resistance can increase the MIC by 2-3 orders of magnitude in a relatively small founder population, a range that has not been observed for AMPs. Though AMPs provide promising leads for drug development [3], their conserved killing mechanisms also argue for caution. In their paper 'arming the enemy', Bell et al. [27] discussed the high likelihood of cross-resistance against, for example, human AMPs. This problem has hardly been studied. Our analysis suggests how one could reap the benefits of AMPs without arming the enemy: we should rely on agents with AMP-like pharmacodynamics. This in principle can be done without using AMPs themselves.

Pharmacodynamic estimates can be easily and routinely obtained. This can also be achieved for drug combinations[7]. A report by the *Leopoldina,* the German National Academy of Sciences, recently recommended to use new drugs only in combination to avoid fast resistance evolution[28]. The scientific support for this notion is limited and controversial[22,29,30]. In clinical situations pharmacodynamic approaches can provide a first informed guess. Also, the risk of resistance evolution based on the pharmacodynamics of drug candidates will be a useful additional criterion to develop new drugs. We would also like to note that the concept of the mutant selection window has been applied to understand antiviral resistance evolution[31], and hence our approach has the potential to inform antiviral resistance research and ultimately treatment as well.

Materials and Methods

To inform the parameterization of the predictive models, we used two main sources. The pharmacodynamic parameters are from our own study determining pharmacodynamics for AMPs and antibiotics under standardized conditions[7]. The estimates of mutation rates again are from our own comparative study on mutation rates under AMP and AB treatment[13] .

Calculation of the size of the mutant selection window

The size of the mutant selection window (MSW) depends on the lower and upper bound of the MSW and is calculated as

$$size_{MSW} = \frac{MIC_R}{MSC}. \qquad (1)$$

204 The lower bound of the MSW is the concentration for which the net growth rate of the
205 resistant strain is equal to the net growth rate sensitive strain and is called the minimal
206 selective concentration (MSC). The upper bound of the MSW is the MIC of the
207 resistant strain (MIC_R) (Fig 1 A) .
208 To analytically describe the MSW, we use the pharmacodynamic (PD) function $\psi(a)$,
209 which mathematical describes the net growth rate with a Hill function:

$$\psi(a) = \psi_{max} - d(a) = \psi_{max} - \frac{(\psi_{max} - \psi_{min})(a/MIC)^{\kappa}}{(a/MIC)^{\kappa} - \psi_{min}/\psi_{max}} \quad (2)$$

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211
212 ([7,15,16]). Here, a is the antimicrobial drug concentration, $\psi(a = 0) = \psi_{max}$, $d(a)$ is the
213 effect of the antimicrobial with the dose a, and $\psi(a \to 0) = \psi_{min}$. Therefore, the
214 maximal effect E_{max} is $E_{max} = \psi_{max} - \psi_{min}$. The parameter MIC denotes the
215 concentration that results in zero net growth (this definition differs from the "official"
216 MIC definition by Mouton et al [32]). The Hill coefficient κ describes the steepness of
217 the curve; functions with higher kappa describe steeper curves (Fig 2A). For
218 illustration of the pharamcodynamic parameters see Fig S3). Cost of resistance c is
219 included as a reduction of the maximum growth rate of the resistant strain in absence
220 of antimicrobials with $c = 1\text{-}\psi_{max}/\psi_{min}$ (Fig 1A, 2A). The pharamcodynamic function
221 can be descirbed for both a drug susceptible strain S and a drug-resistant strain R, with
222 $\psi_S(a)$ *and* $\psi_R(a)$, respectively.The MSC is calculated as $\psi_S(a) = \psi_R(a)$. We assume
223 that the net growth rate of the resistant strain below the MSC is approximately at the
224 same level as without antimicrobials and therefore set $\psi_R(a) \approx \psi_{max,S}(1-c) = \psi_{R,approx}$,
225 for $0 < a < MSC$ (illustrated in Fig 2A). This is valid because $MIC_R >> MIC_S$ and
226 $\kappa_R >\approx \kappa_S$. The analytic solution of the MSC is

$$MSC = MIC_s \left(\frac{c\psi_{min,S}}{\psi_{max,S}(c-1)+\psi_{min,S}} \right)^{1/\kappa_s}. \quad (3)$$

Model of evolution and prediction of resistance

To study resistance evolution we used a mathematical model that incorporates pharmacodynamics (PD) and pharmacokinetics (PK) and captures population dynamics of bacterial populations under treatment with antimicrobial drugs[15]. We ran stochastic simulations and calculated the probability of resistance emergence, the probability of the resistant strain, the time to resistance emergence and the risk of resistance (the resistance hazard[20]).

To simulate treatment, we consider a patient harboring 10^6 susceptible bacteria. Bacterial mutation rates are assumed to depend on the antimicrobial used for treatment (antibiotics or AMPs). When a resistant strain arises it is assumed to have an MIC ten-fold that of susceptible wild-type strain. For simplicity, we only consider one type of mutant. Antimicrobials are administered every day (see Supplement for pharmacokinetics), and treatment lasts one week.

The population dynamics of the susceptible and resistant strains is captured in the following system of differential equations:

$$\begin{aligned} \frac{dS}{dt} &= r_s(1-\mu)S\left(1-\frac{S+R}{K}\right)-[d_s(a,t)+d_n]S \\ \frac{dR}{dt} &= r_r R\left(1-\frac{S+R}{K}\right)+r_s\mu S\left(1-\frac{S+R}{K}\right)-[d_r(a,t)+d_n]R, \end{aligned} \quad (4)$$

Where S represents the wild-type strain and R represents the resistant strain. r is the replication rate, μ is the mutation rate. $d(a,t)$ is the killing rate of antibiotics or AMPs, which is in essence described in equation 2, but is now time dependent, with

$$d(a,t) = \frac{(\psi_{max} - \psi_{min})(a(t)/MIC)^{\kappa}}{(a(t)/MIC)^{\kappa} - \psi_{min}/\psi_{max}} \quad (5)$$

since we include time dependent pharmacokinetic function $a(t)$ (Fig S2):

$$a(t) = \sum_{n} \frac{Dk_a}{k_a - k_e}\left(e^{-k_e[t-(n-1)\tau]} - e^{-k_a[t-(n-1)\tau]}\right), \quad n = 1, 2, 3\text{K} \quad (6)$$

Here, k_a *is* the absorption rate, and k_e is the decay rate. D is the dose given each time, n is the number of doses, τ is the dose frequency. We use the average concentration in the course of treatment to represent the dose level of treatments. Then we calculate the average concentration,

$$\bar{a} = \frac{1}{t}\int a(t)dt \quad (7)$$

We implemented the model in Equation 3 stochastically using the *Gillespie algorithm* [33], which particularly allowed us to monitor how frequently mutants arise. Parameters were selected based on empirical data as stated above. The net growth rate of wild-type in the absence of antimicrobials was set as 1. Mutants suffer fixed or resistant-level related costs (see Fig 2). κ of AMPs and antibiotics were set as 5 and 1.5, respectively [7]. ψ_{min} for AMPs is fixed as -50 hour^{-1}; and for antibiotics is fixed as -5 hour^{-1}. Mutation rates in AMPs are 10 times lower than in antibiotics [13]. All the parameters and their values are listed in Table S1. All the pharmacokinetic parameters are the same in different simulations (see Fig S2). For each set of parameters, cohorts of five hundred patients were simulated. Successful treatment is defined as complete clearance of both sensitive and resistant strains at the end of the one-week treatment. For each cohort of patients, we calculate the probability of treatment success as the proportion of individuals in whom treatment was successful. In each individual, we

score the time of emergence of resistance strains, and estimate the resistance hazard based on the average probability of treatment success and the population size of bacteria over time. The hazard function can be written as,

$$H(a,t) = \frac{1}{Kt}\int S(a,t)p_{S\to R}(a)\psi_R(a)dt, \qquad (7)$$

where K is the capacity, S denotes population size of sensitive strain and $p_{S\to R}$ is probability of a treatment developing resistance, which is calculated from the results of simulations, ψ_R is the growth rate of resistant strain. Our hazard function calculates the average proportion of resistant population under certain treatment dose and duration.

Implementation

The analysis was performed in R (v. 3.1.3&v. 3.2.2) [34] using RSTUDIO (v. 0.98.1103&0.99.903) [35]. The code is available upon request.

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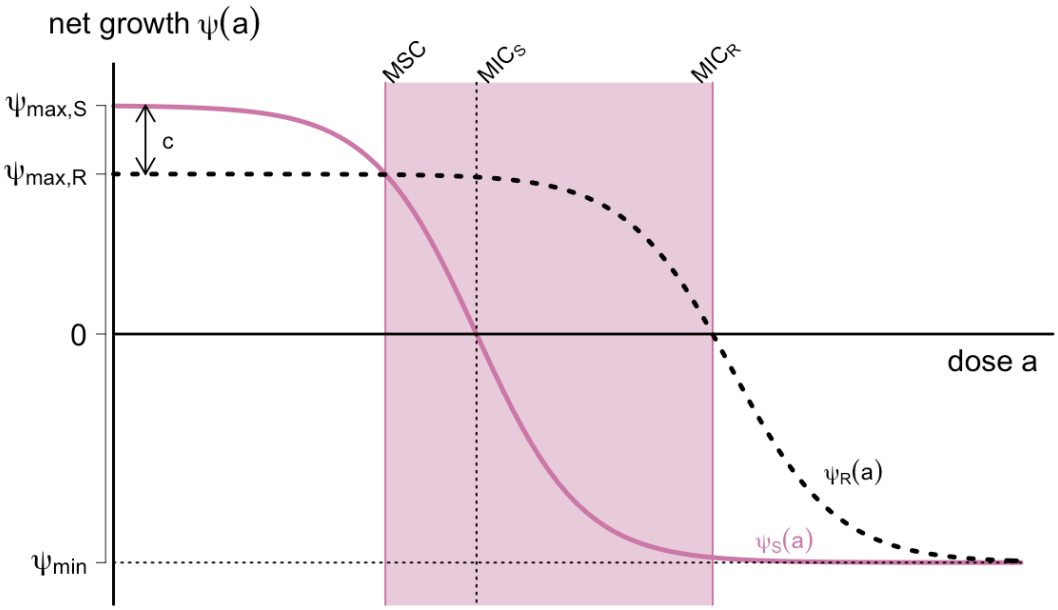
Competing interests: None of the authors has competing interests.

Data and materials availability: The model will be made available as a remarkup document for use.



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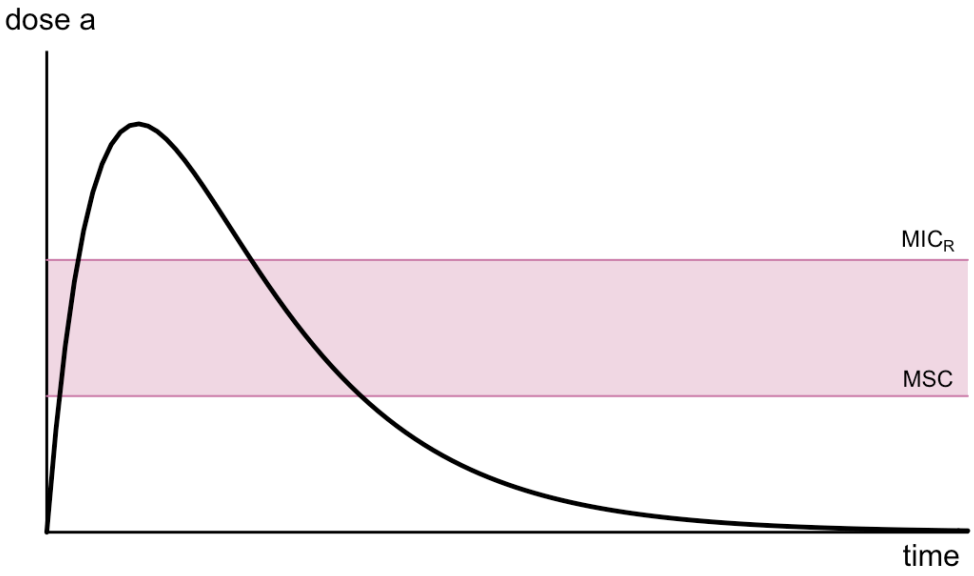
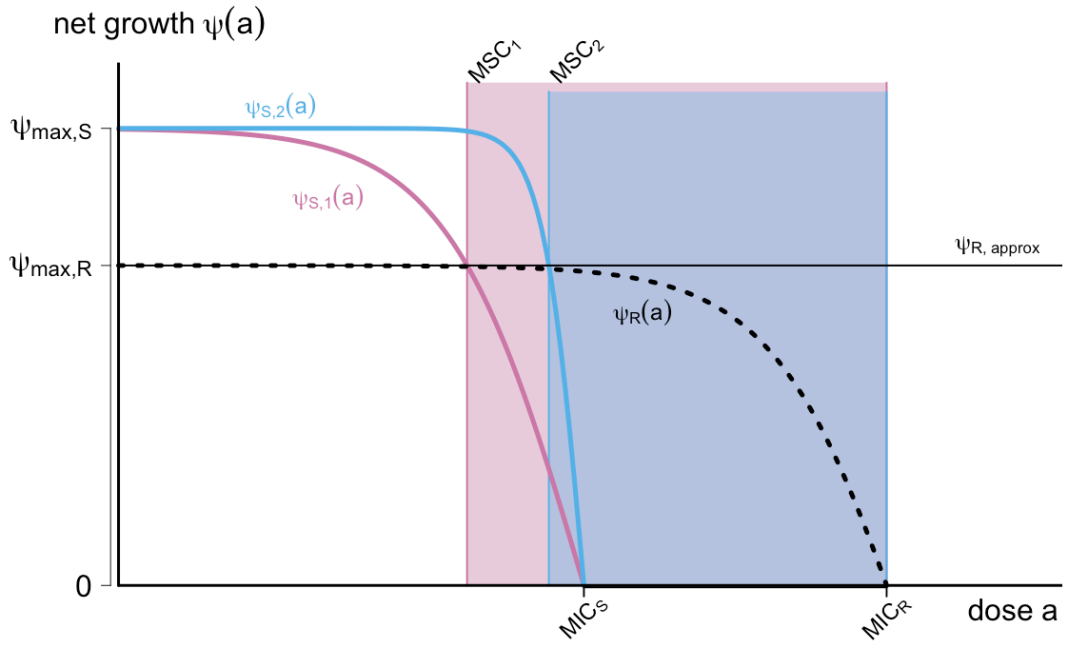


402
403 **Fig 1. The revised mutant selection window and pharmacodynamic parameters.**

404 **(a)** The mutant selection window (MSW) is defined as the antimicrobial concentration
405 range in which resistant mutants are selected [9]. Following [10], we determine the MSW
406 using net growth curves of a susceptible strain *S* and a resistant strain *R*.
407 Mathematically, net growth is described with the pharmacodynamic function $\psi(a)$
408 ([15], see Materials and Methods and Fig S3 for details). In short, the function consists

of the four pharamcodynamic parameters: net growth in absence of antibicrobials ψ_{max}, net growth in the presence of a dose of antimicrobials, which effects the growth maximal, ψ_{min}, the *MIC* and the parameter κ, which describes the steepness of the pharamcodynamic curve. Here, the two pharmacodynamics functions $\psi_S(a)$ (continuous pink line) and $\psi_R(a)$ (dotted black line) describe the net growth of the *S* and *R*, respectively, in relation to the drug concentration a. Cost of resistance c is included as a reduction of the maximum growth rate of the resistant strain $\psi_{max,R}$, with $c = 1 - \psi_{max,R}/\psi_{max,S}$. Note that with this definition, cost of resistance is expressed as reduction in net growth rate in absence of antimicrobials (a = 0). The lower bound of the MSW is the concentration for which the net growth rate of the resistant strain is equal to the net growth rate of the sensitive strain and is called the minimal selective concentration (MSC) (see Materials and Methods for analytic solution). The upper bound is given by the MIC of the resistant strain MIC_R. We calculate the size of the MSW as : $size(MSW) = \frac{MIC_R}{MSC}$. **(b)** The boundaries of the MSW applied to the pharmacokinetics of the system.



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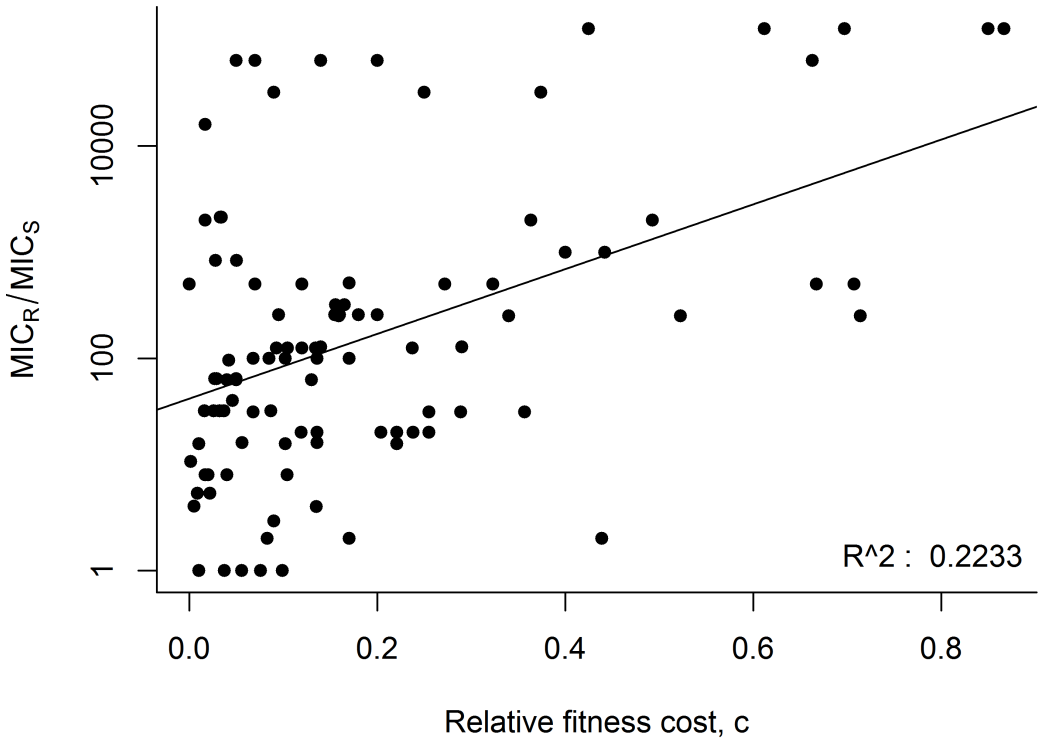

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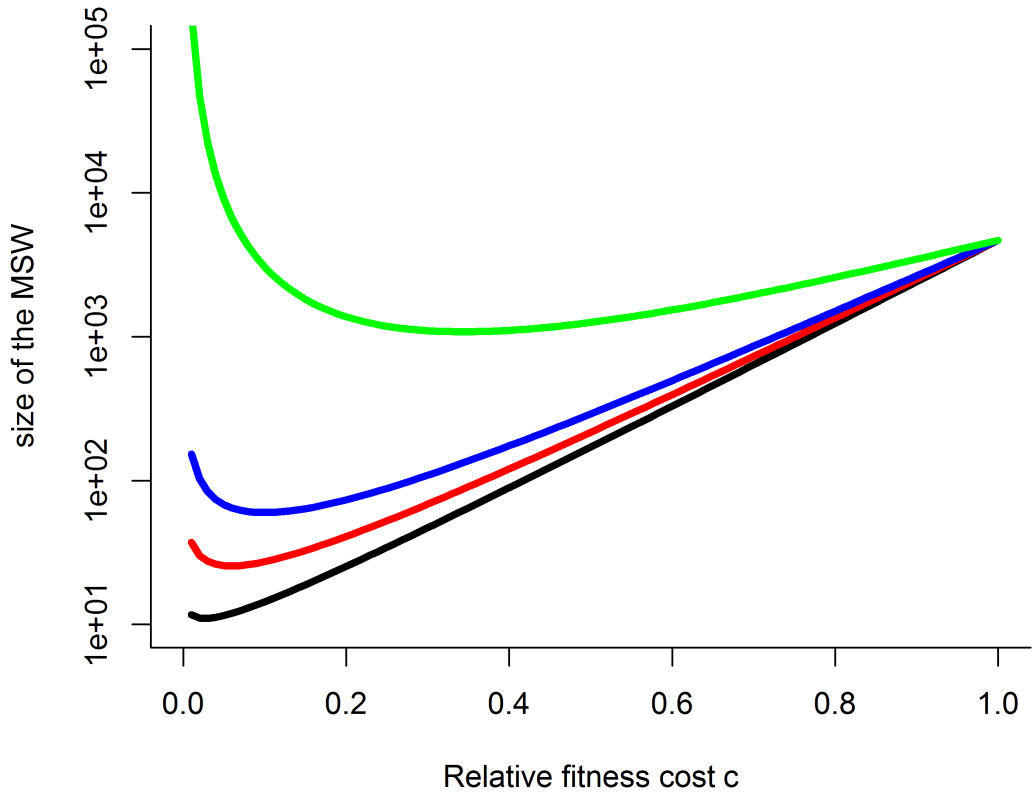
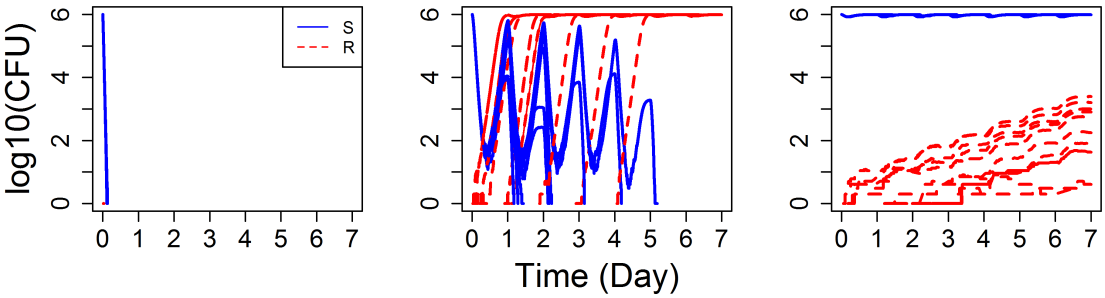


Fig 2. The mutant selection window for arbitrary mutant strains. The two boundaries of the MSW, MSC and MIC_R, are influenced differently by the pharmacodynamic parameters of the sensitive strain S and the resistant strain R. **(a)** The lower boundary of the MSW (MSC) depends primarily on the pharmacodynamic parameters of the sensitive strain, assuming that the net growth rate of the resistant strain below the MSC is approximately at the same level as without antimicrobials: $\psi_R(a) \approx \psi_{max,S}(1-c) = \psi_{R,approx}$, for $0 < a < MSC$ (ψ_R: dotted black line; $\psi_{R,approx}$:continuous black line) (see Materials and Methods for details). The effect of each of the four pharamcodynamic parameters and of the cost of resistance on the MSC is depicted in Fig S1. We plotted the pharmacodynamic function $\psi_S(a)$ of two sensitive strains with varying κ values: $\psi_{S,1}(a)$ representative for Abs with a small κ ($\kappa = 1.5$, pink) and $\psi_{S,2}(a)$ representative for AMPs with a large κ ($\kappa = 5$, blue).

Increasing the κ value results in increasing the MSC (MSC_1 (pink) <MSC_2(blue)). **(b)** The upper boundary of the MSW is per definition the MIC_R, which is linked to its fitness cost (data from [19]), i.e. the upper boundary MIC_R increases with costs c $log_{10}\left(\frac{MIC_R}{MIC_S}\right) = 3.05c + 1.62$ ($R^2 = 0.22$) **(c)** The relationship between cost of resistance, other pharmacodynamic parameters, and the size of the MSW is complex. Here we show that both boundaries of the MSW, MSC and MIC_R, are influenced by costs and resulting, the lowest MSW window size is achieved at intermediate cost of resistance. Parameters used are: $\psi_{max,S} = 1$, $\psi_{min,S} = -1$, $\kappa_S = 5.5$ (black), $\kappa_S = 2.5$ (red), $\kappa_S = 1.5$ (blue), $\kappa_S = 0.5$ (green), $MIC_S = 10$, and $log_{10}\left(\frac{MIC_R}{MIC_S}\right) = 3.05c + 1.62$.



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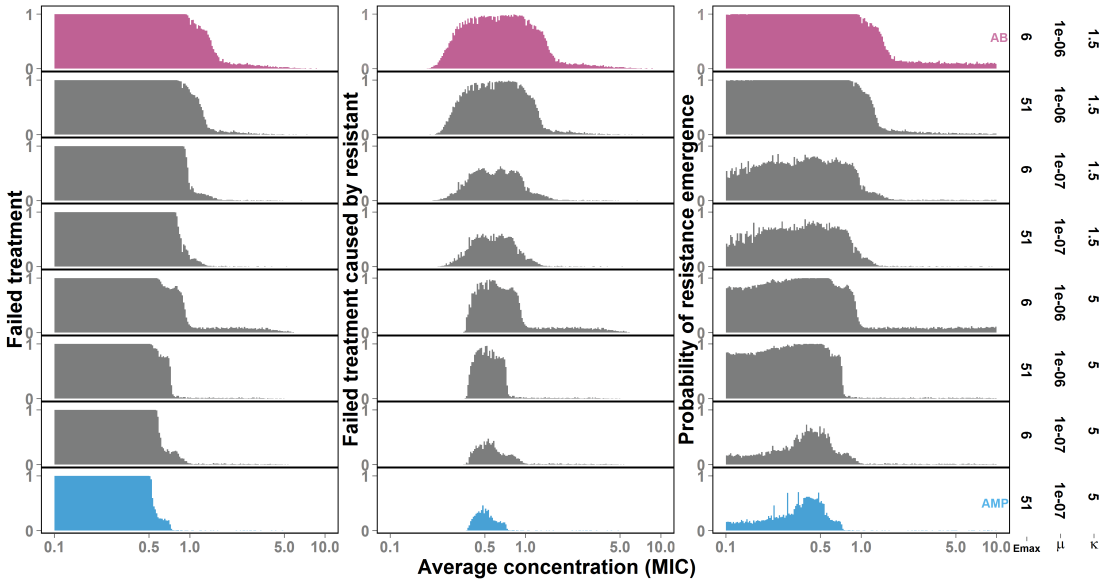

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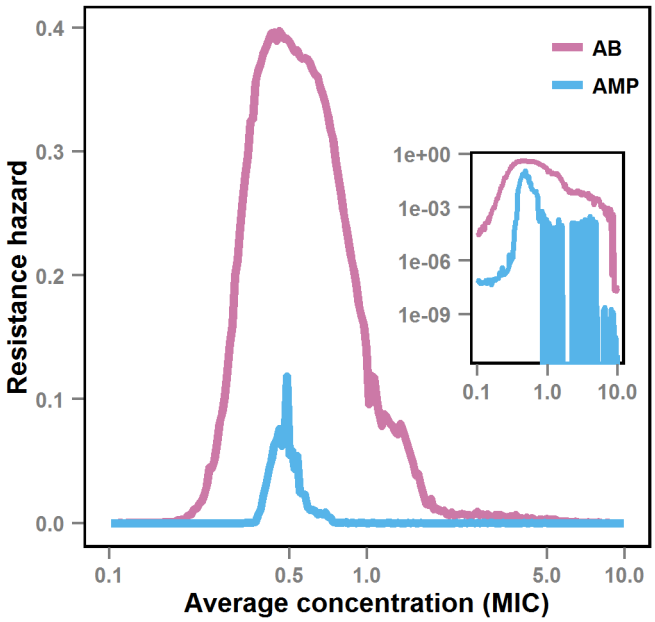

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475 **Fig 3. Evolution of drug resistance determined by pharmacodynamics.**

476 (a) At high dose antimicrobials achieve maximal effects and rapidly kill most of the

477 population, preventing resistance evolution (left). At medium dose, the sensitive strain

will not be eliminated immediately, and resistant mutants emerge (central). At low dose, the sensitive strain will not be removed, the mutants emerge as well, but will not quickly reach equilibrium due to substantial fitness costs (right, resistant: pink, susceptible: blue), **(b)** Simulations comparing the range from 'pure' antimicrobials peptides (AMP) to 'pure' antibiotics (AB) by altering μ, ψ_{min} and κ. We find that the probabilities of treatment failure (left), of failure caused by resistant strains (middle) and of resistance emergence are always higher under the AB-scenario than the AMP-scenario. A successful treatment requires less AMP than AB. **(c)** Following [20] we calculate the resistance hazard as the time-averaged proportion of mutants in a patient under a particular treatment dose. We find that AMPs are much less likely to select for resistance across concentrations than antibiotics (Inset graph: bacterial counts corresponding to the hazards). **(d)** Time to resistance is much longer under AMP than AB treatment. The parameters are: $\psi_{max,S} = 1$, $\psi_{max,R} = 0.9$, $\kappa_{AB} = 1.5$, $\kappa_{AMP} = 5$, $\psi_{min,AB} = -5$, $\psi_{min,AMP} = -50$, $MIC_S = 10$, $MIC_R = 100$,. $\mu_{AB} = 10^{-6}$, $\mu_{AMP} = 10^{-7}$, $k_a = 0.5$, $k_e = 0.2$, $d_n = 0.01$, $\tau = 1/24$.