

1 **Predicting drug resistance evolution: antimicrobial peptides vs. antibiotics**

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14

15 **Abstract**

16

17 Antibiotic resistance constitutes one of the most pressing public health concerns.

18 Antimicrobial peptides are considered part of a solution to this problem, because they

19 are new agents that add to our repertoire. Importantly, antimicrobial peptides differ

20 fundamentally from antibiotics in their pharmacodynamic characteristics. Here we

21 implement these differences within a theoretical framework to predict the evolution of

22 resistance against antimicrobial peptides and compare it to antibiotic resistance. Our

23 analysis of resistance evolution finds that pharmacodynamic differences all combine

24 to produce a much lower probability that resistance will evolve against antimicrobial

25 peptides. The finding can be generalized to all drugs with pharmacodynamics similar

26 to AMPs. Pharmacodynamic concepts are familiar to most practitioners of medical

27 microbiology, and data can be easily obtained for any drug or drug combination. Our

28 theoretical and conceptual framework is therefore widely applicable and can help

29 avoid resistance evolution if implemented in antibiotic stewardship schemes or the

30 rational choice of new drug candidates.

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32

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

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

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

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

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

70

71

## 72 **Results**

73

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

80

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

95  
96 Next we wanted to explore if the differences between AMPs and antibiotics in the  
97 width of the MSW correlated with different probabilities of drug resistance evolution  
98 within a host. A further difference between AMPs and antibiotics is that antibiotics  
99 increase mutagenesis but AMPs do not<sup>13,14</sup>. We incorporated this difference in  
100 addition to the difference in the steepness of the pharmacodynamics relationship into  
101 a stochastic model describing bacterial replication and evolution under selection  
102 pressure from AMPs. We consider two cases here: (a) do resistant mutants emerge  
103 and (b) do resistant mutants drive the susceptible strains to extinction?

104

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

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

116 We have shown before<sup>7</sup> that combinations of AMPs have higher  $\kappa$  and lower MICs  
117 than individual AMPs. This also results in differences in resistance selection and the  
118 extinction of susceptible strains, consistent with the results above.

119

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

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

136

137

## 138 **Discussion**

139

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

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

154 time becomes shorter with higher population sizes, these characteristics may have less  
155 significance for clinical infections <sup>21</sup>.

156

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

166

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

179

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

191

192

### 193 **Materials and Methods**

194 To inform the parameterization of the predictive models, we used two main sources.  
195 The pharmacodynamic parameters are from our own study determining  
196 pharmacodynamics for AMPs and antibiotics under standardized conditions<sup>7</sup>. The  
197 estimates of mutation rates again are from our own comparative study on mutation  
198 rates under AMP and AB treatment<sup>13</sup>.

199

#### 200 *Calculation of the size of the mutant selection window*

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

$$203 \quad size_{MSW} = \frac{MIC_R}{MSC}. \quad (1)$$

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:

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

211  
212 (<sup>7,15,16</sup>). 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  $\psi(a \rightarrow 0) = \psi_{min}$ . 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 <sup>32</sup>). The Hill coefficient  $\kappa$  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  $c = 1 - \psi_{max} / \psi_{min}$  (Fig 1A, 2A). The pharamcodynamic function  
221 can be described 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  $MIC_R \gg MIC_S$  and  
226  $\kappa_R \gg \kappa_S$ . The analytic solution of the  $MSC$  is

227 
$$MSC = MIC_s \left( \frac{c\psi_{min,S}}{\psi_{max,S}(c-1) + \psi_{min,S}} \right)^{1/\kappa_s} . \quad (3)$$

228

229 *Model of evolution and prediction of resistance*

230 To study resistance evolution we used a mathematical model that incorporates  
231 pharmacodynamics (PD) and pharmacokinetics (PK) and captures population  
232 dynamics of bacterial populations under treatment with antimicrobial drugs<sup>15</sup>. We ran  
233 stochastic simulations and calculated the probability of resistance emergence, the  
234 probability of the resistant strain, the time to resistance emergence and the risk of  
235 resistance (the resistance hazard<sup>20</sup>).

236

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

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

245 
$$\begin{aligned} \frac{dS}{dt} &= r_s(1-\mu)S \left( 1 - \frac{S+R}{K} \right) - [d_s(a,t) + d_n]S \\ \frac{dR}{dt} &= r_r R \left( 1 - \frac{S+R}{K} \right) + r_s \mu S \left( 1 - \frac{S+R}{K} \right) - [d_r(a,t) + d_n]R, \end{aligned}$$

246 (4)

247

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

251 
$$d(a, t) = \frac{(\psi_{max} - \psi_{min})(a(t) / MIC)^{\kappa}}{(a(t) / MIC)^{\kappa} - \psi_{min} / \psi_{max}} \quad (5)$$

252

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

254 
$$a(t) = \sum_n \frac{Dk_a}{k_a - k_e} (e^{-k_e[t-(n-1)\tau]} - e^{-k_a[t-(n-1)\tau]}), \quad n = 1, 2, 3K$$

255 (6)

256

257 Here,  $k_a$  is the absorption rate, and  $k_e$  is the decay rate.  $D$  is the dose given each time,  
258  $n$  is the number of doses,  $\tau$  is the dose frequency. We use the average concentration in  
259 the course of treatment to represent the dose level of treatments. Then we calculate  
260 the average concentration,

261 
$$\bar{a} = \frac{1}{t} \int a(t) dt \quad (7)$$

262

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

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

$$279 \quad H(a, t) = \frac{1}{Kt} \int S(a, t) p_{S \rightarrow R}(a) \psi_R(a) dt, \quad (7)$$

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

285

### 286 *Implementation*

287 The analysis was performed in R (v. 3.1.3&v. 3.2.2)<sup>34</sup> using RSTUDIO (v.  
288 0.98.1103&0.99.903)<sup>35</sup>. The code is available upon request.

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389 results. GY was primarily responsible for the predictive modelling, DYB for the  
390 PDwork. All authors contributed to the writing of the paper. JR wrote the first draft,  
391 RRR led the mathematical work.

392

393 **Competing interests:** None of the authors has competing interests.

394

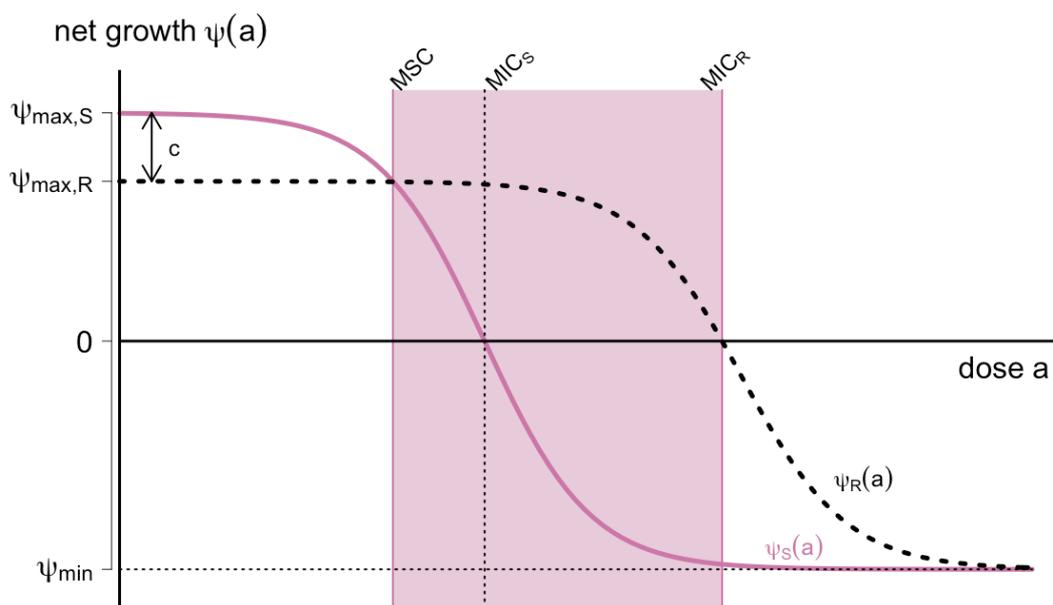
395 **Data and materials availability:** The model will be made available as a remarkup  
396 document for use.

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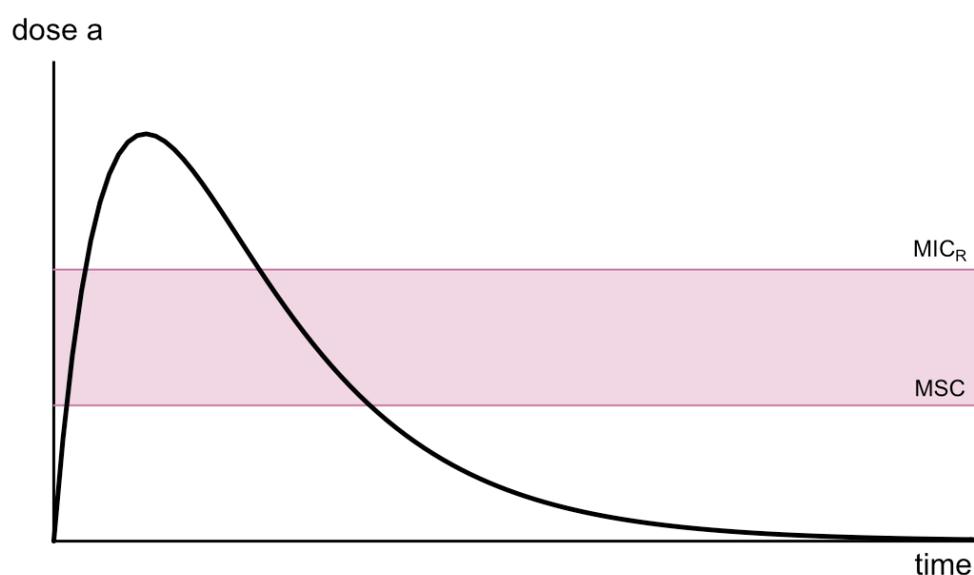
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403 **Fig 1. The revised mutant selection window and pharmacodynamic parameters.**

404 **(a)** The mutant selection window (MSW) is defined as the antimicrobial concentration  
405 range in which resistant mutants are selected<sup>9</sup>. Following<sup>10</sup>, 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 (<sup>15</sup>, see Materials and Methods and Fig S3 for details). In short, the function consists

409 of the four pharmacodynamic parameters: net growth in absence of  
410 antimicrobials  $\psi_{max}$ , net growth in the presence of a dose of antimicrobials, which  
411 effects the growth maximal,  $\psi_{min}$ , the *MIC* and the parameter  $\kappa$ , which describes the  
412 steepness of the pharmacodynamic curve. Here, the two pharmacodynamics functions  
413  $\psi_S(a)$  (continuous pink line) and  $\psi_R(a)$  (dotted black line) describe the net growth  
414 of the *S* and *R*, respectively, in relation to the drug concentration  $a$ . Cost of resistance  
415  $c$  is included as a reduction of the maximum growth rate of the resistant strain  
416  $\psi_{max,R}$ , with  $c = 1 - \psi_{max,R}/\psi_{max,S}$ . Note that with this definition, cost of  
417 resistance is expressed as reduction in net growth rate in absence of antimicrobials ( $a$   
418  $= 0$ ). The lower bound of the MSW is the concentration for which the net growth rate  
419 of the resistant strain is equal to the net growth rate of the sensitive strain and is called  
420 the minimal selective concentration (MSC) (see Materials and Methods for analytic  
421 solution). The upper bound is given by the MIC of the resistant strain  $MIC_R$ . We  
422 calculate the size of the MSW as :  $size(MSW) = \frac{MIC_R}{MSC}$ . **(b)** The boundaries of the  
423 MSW applied to the pharmacokinetics of the system.

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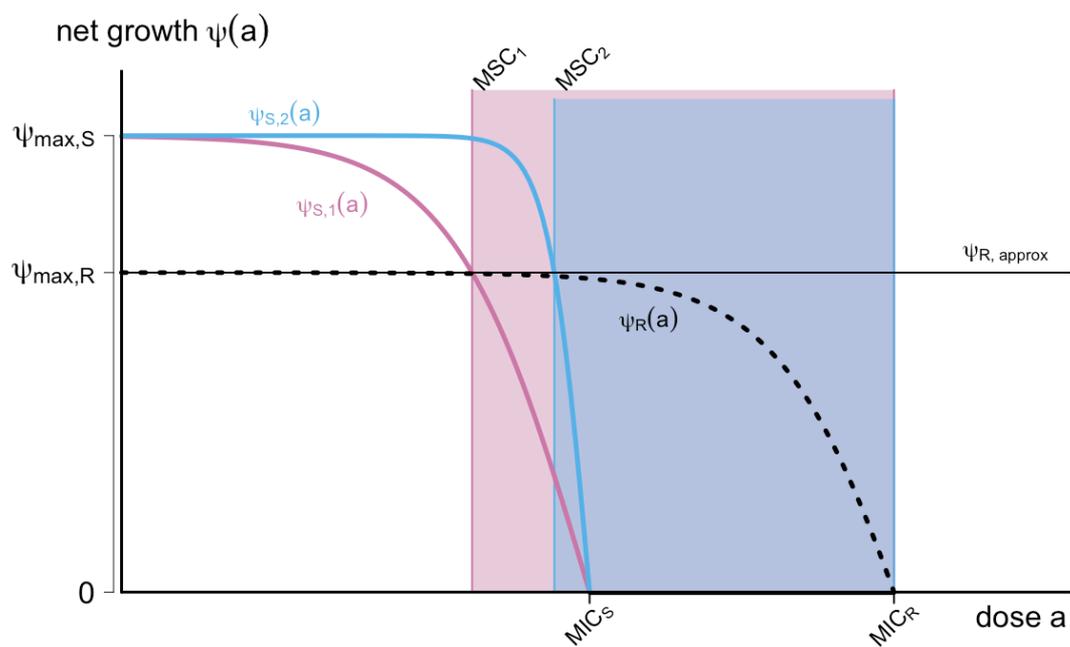
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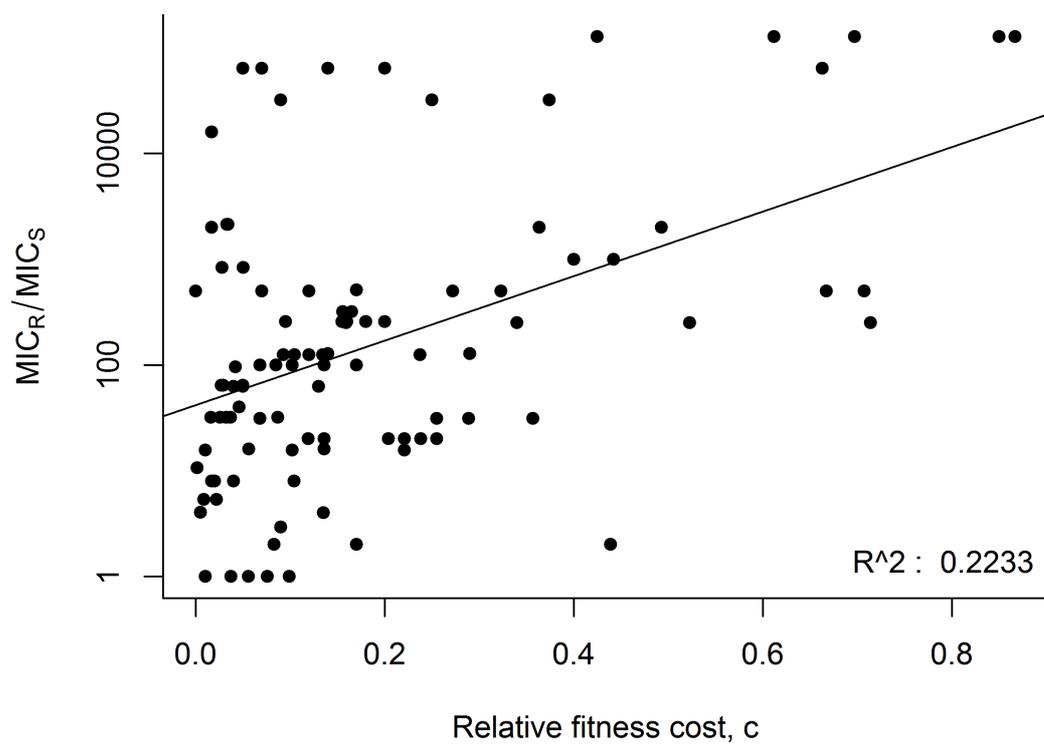
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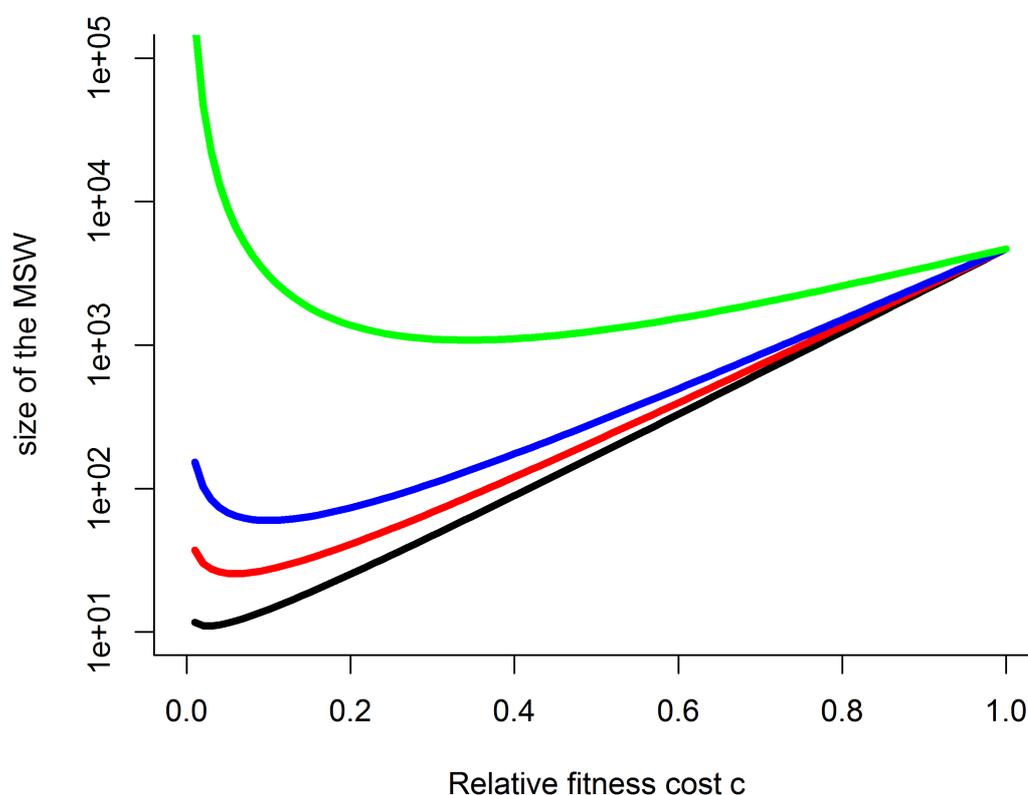
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**Fig 2. The mutant selection window for arbitrary mutant strains.** The two

437 boundaries of the MSW, MSC and  $MIC_R$ , are influenced differently by the

438 pharmacodynamic parameters of the sensitive strain S and the resistant strain R. **(a)**

439 The lower boundary of the MSW (MSC) depends primarily on the pharmacodynamic

440 parameters of the sensitive strain, assuming that the net growth rate of the resistant

441 strain below the MSC is approximately at the same level as without antimicrobials:

442  $\psi_R(a) \approx \psi_{max,S}(1 - c) = \psi_{R,approx}$ , for  $0 < a < MSC$  ( $\psi_R$ : dotted black line;

443  $\psi_{R,approx}$ : continuous black line) (see Materials and Methods for details). The effect

444 of each of the four pharmacodynamic parameters and of the cost of resistance on the

445 MSC is depicted in Fig S1. We plotted the pharmacodynamic function  $\psi_S(a)$  of two

446 sensitive strains with varying  $\kappa$  values:  $\psi_{S,1}(a)$  representative for Abs with a small  $\kappa$

447 ( $\kappa = 1.5$ , pink) and  $\psi_{S,2}(a)$  representative for AMPs with a large  $\kappa$  ( $\kappa = 5$ , blue).

448 Increasing the  $\kappa$  value results in increasing the MSC ( $MSC_1$  (pink)  $<$   $MSC_2$  (blue)). **(b)**

449 The upper boundary of the MSW is per definition the  $MIC_R$ , which is linked to its

450 fitness cost (data from <sup>19</sup>), i.e. the upper boundary  $MIC_R$  increases with costs  $c$

451  $\log_{10}\left(\frac{MIC_R}{MIC_S}\right) = 3.05c + 1.62$   $R^2 = 0.22$ ) **(c)** The relationship between cost of resistance,

452 other pharmacodynamic parameters, and the size of the MSW is complex. Here we

453 show that both boundaries of the MSW, MSC and  $MIC_R$ , are influenced by costs and

454 resulting, the lowest MSW window size is achieved at intermediate cost of resistance.

455 Parameters used are:  $\psi_{max,S} = 1$ ,  $\psi_{min,S} = -1$ ,  $\kappa_S = 5.5$  (black),  $\kappa_S = 2.5$  (red),

456  $\kappa_S = 1.5$  (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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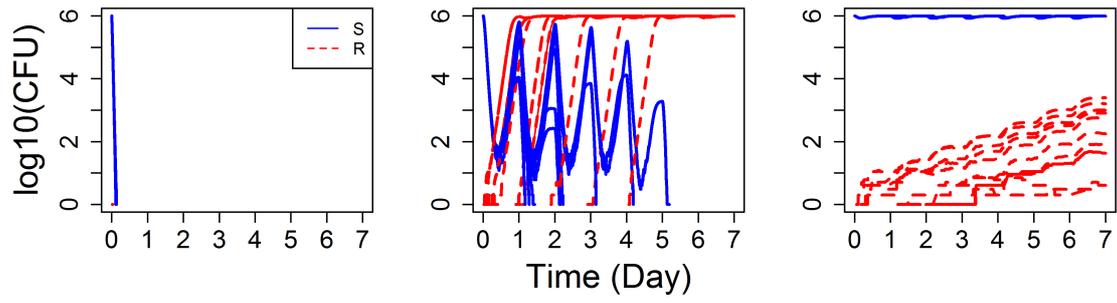
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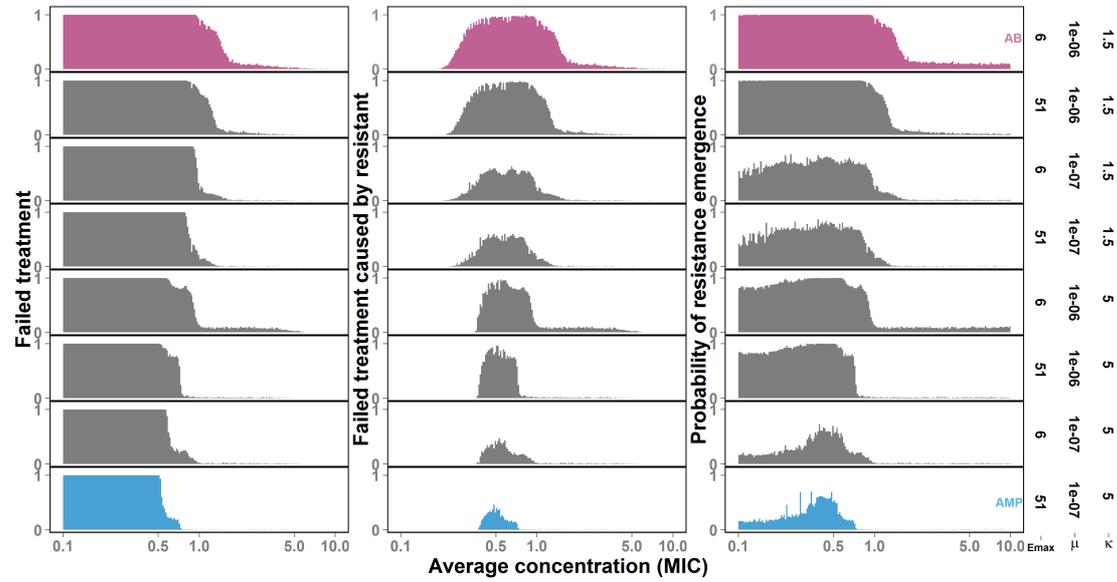
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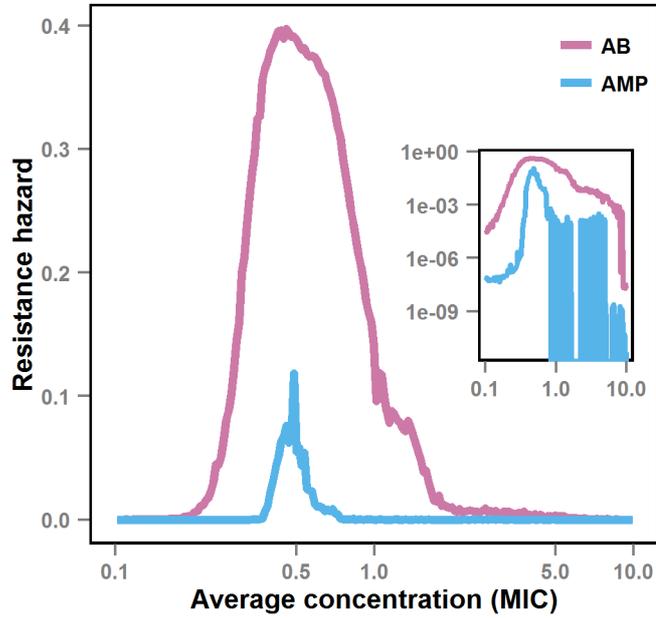
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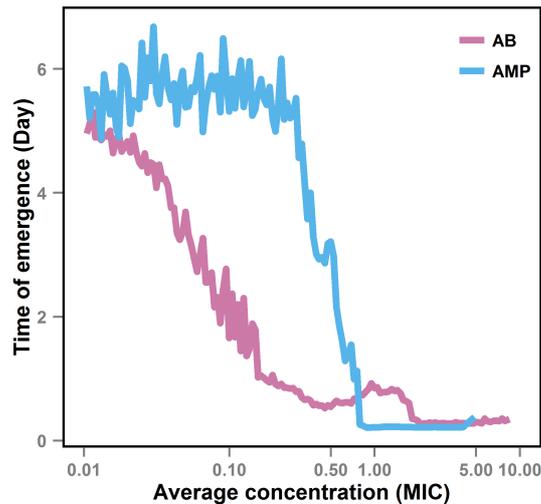
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476 **Fig 3. Evolution of drug resistance determined by pharmacodynamics.**

477 (a) At high dose antimicrobials achieve maximal effects and rapidly kill most of the  
478 population, preventing resistance evolution (left). At medium dose, the sensitive strain  
479 will not be eliminated immediately, and resistant mutants emerge (central). At low  
480 dose, the sensitive strain will not be removed, the mutants emerge as well, but will not  
481 quickly reach equilibrium due to substantial fitness costs (right, resistant: pink,  
482 susceptible: blue), (b) Simulations comparing the range from ‘pure’ antimicrobials  
483 peptides (AMP) to ‘pure’ antibiotics (AB) by altering  $\mu$ ,  $\psi_{min}$  and  $\kappa$ . We find that the  
484 probabilities of treatment failure (left), of failure caused by resistant strains (middle)  
485 and of resistance emergence are always higher under the AB-scenario than the AMP-  
486 scenario. A successful treatment requires less AMP than AB. (c) Following<sup>20</sup> we  
487 calculate the resistance hazard as the time-averaged proportion of mutants in a patient  
488 under a particular treatment dose. We find that AMPs are much less likely to select  
489 for resistance across concentrations than antibiotics (Inset graph: bacterial counts  
490 corresponding to the hazards). (d) Time to resistance is much longer under AMP than  
491 AB treatment. The parameters are:  $\psi_{max,S} = 1$ ,  $\psi_{max,R} = 0.9$ ,  $\kappa_{AB} = 1.5$ ,  $\kappa_{AMP} =$

492  $5, \psi_{min,AB} = -5, \psi_{min,AMP} = -50, MIC_S = 10, MIC_R = 100, \mu_{AB} = 10^{-6},$

493  $\mu_{AMP} = 10^{-7}, k_a = 0.5, k_e = 0.2, d_n = 0.01, \tau = 1/24.$

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