Pharmacodynamic Considerations of Collateral Sensitivity in Design of Antibiotic Treatment Regimen

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ABSTRACT

Antibiotics have greatly reduced the morbidity and mortality due to infectious diseases. Although antibiotic resistance is not a new problem, its breadth now constitutes a significant threat to human health. One strategy to help combat resistance is to find novel ways to use existing drugs, even those that display high rates of resistance. For the pathogens *Escherichia coli* and *Pseudomonas aeruginosa*, pairs of antibiotics have been identified for which evolution of resistance to drug A increases sensitivity to drug B and vice versa. These research groups have proposed cycling such pairs to treat infections, similar treatment strategies are being investigated for various cancer forms as well.

While an exciting treatment prospect, no cycling experiments have yet been performed with consideration of pharmacokinetics (PK) and pharmacodynamics (PD). To test the plausibility of this scheme and search for ways to optimize it, we create a mathematical model with explicit PK-PD considerations. We study several possible treatment protocols using pairs of such antibiotics, and investigate the speed of ascent of multiply resistant mutants. Our analyses show that for low concentrations of antibiotics, treatment failure will always occur due to the rapid ascent and fixation of resistant mutants. However, at moderate to high concentrations of some types of bacteriostatic antibiotics with multiday cycling, resistance is prevented from developing and treatment succeeds. This calls for guarded optimism of such treatment protocols whose development can be directed by these types of models.

Key words: *Collateral sensitivity, Antibiotics, Treatment efficacy, in vitro pharmacodynamics, Mathematical modelling*

INTRODUCTION

The impending era of ubiquitous antibiotic resistance is engendering an urgent search for new therapeutics as well as antibiotic stewardship strategies. Clinical strains have recently been isolated that are completely pan resistant - resistant to all available antibiotics. In lieu of new drugs or vaccines, optimizing therapeutic regimen is critical to continued treatment success. Stewardship approaches incorporating such considerations would ideally reduce the risk of within-host resistance development thus minimizing endemic levels of such strains in the community.

57 The concept of collateral sensitivity (CS) was described already in the 1950s, when Bryson et al.
58 observed that an *Escherichia coli* strain became hypersensitive to polymyxin B, upon acquiring
59 chloramphenicol resistance. [1] They speculated that this collateral sensitivity could be exploited clinically.
60 Recently, research teams have identified drug pairs that exhibit mutual collateral sensitivity (MCS)
61 effects; evolution of resistance to drug A increases sensitivity to drug B and vice versa. MCS entails a
62 reciprocal positive interaction between the two drugs. For the pathogens *Escherichia coli* and
63 *Pseudomonas aeruginosa*, a number of pairs of antibiotics have been identified which exhibit MCS to
64 varying degrees. [2-5] Based on these studies, several groups have proposed antibiotic cycling of two
65 antibiotics (e.g., A -> B -> A -> B -> ..., and derivatives thereof) to exploit MCS and to treat infections. [2]
66 However, all, except one study were limited to a single exposure to each antibiotic. While recent work
67 has provided some understanding and potential mechanistic causes of such evolved sensitivity, it is
68 unclear if CS is a universally applicable treatment consideration.
69 For *P. aeruginosa* infections in prophylactically exposed cystic fibrosis, CF, patients, i) gentamicin
70 resistant strains are seen to become sensitive to penicillin due to mutation in a two component system
71 (*pmrB*), and, ii) *nalC* and *mexZ* mutations can confer aminoglycoside sensitivity in beta-lactam adapted
72 strains. [4] Data emerging from whole genome sequencing of such isolates points to the need for a
73 thorough exploration of the pharmacodynamic features of such treatment regimen where the CS
74 strategy is employed. Roemhild *et al* cycled two antibiotics at a time in a morbidostat, an in vitro
75 continuous-flow model. [6] The morbidostat maintains a population density below a desired threshold by
76 administration of bolus doses of antibiotic at specified optical densities. While a clever and useful device
77 amenable to control and automation, for the potential clinical application, it trivializes the
78 pharmacodynamics and greatly simplifies the underlying modeling. In this case, their model shows rapid
79 ascent of double mutants and experimentally, they find this in most cases. In addition to this, Nichol *et*
80 *al* recently presented a combinatorial model describing the evolutionary limitations of CS strategy
81 focusing on drugs with the same molecular target; cell wall synthesis. [7] They concluded that '*collateral*
82 *sensitivity is contingent on the repeatability of evolution'.*
83 Theoretical models can guide the testing and implementation of this cycling strategy, in light of the
84 evolutionary trends towards amplification of resistant clones during sequential monotherapies. Using
85 simulated treatment protocols, the plausibility of CS-derived treatment regimen can be evaluated *in*
86 *silico* and optimized *in vivo*. In this way, the implications for resistance development and the endemic
87 fixation of these alleles can be assessed in 'real time' before in vivo testing.
88

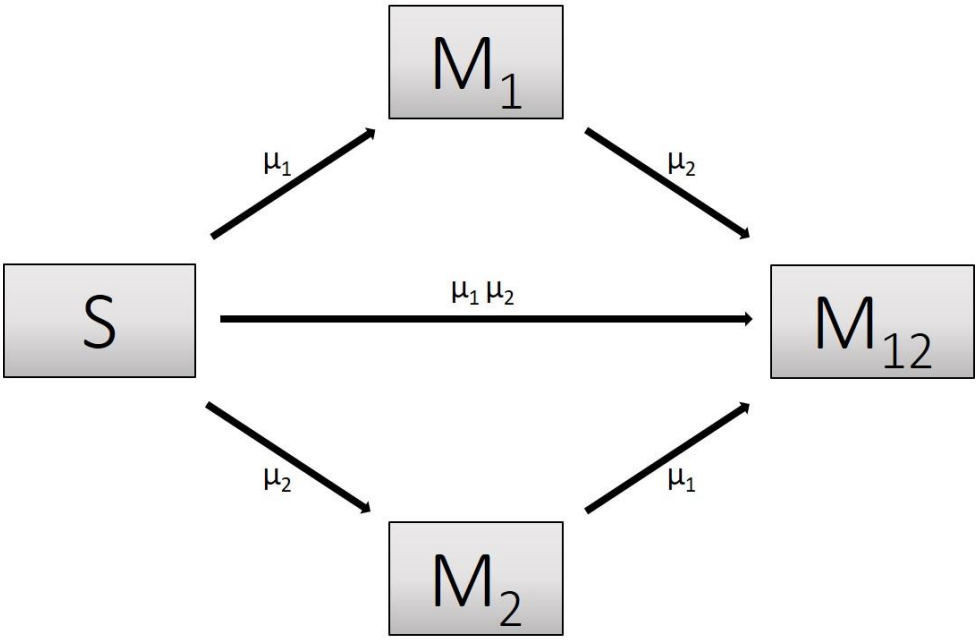
In this manuscript, we analyze the population dynamics of antibiotic cycling with the goal of clinical utility. We utilize clinically relevant parameters for presenting infections; high bacteria densities and antibiotic dosages in line with current treatment protocols. [8] Such models combined with PK / PD experiments would guide the clinical applicability of this proposed new treatment paradigm. Our model explicitly incorporates PD / PK parameters for individual strains, their mutation rates and bacterial fitness effects. Implicit within our model is immune control of bacterial density. We examine the sequential application of two drugs, A → B, each exhibiting different PD characteristics, to a hypothetical infection and estimate the rates of resistance ascent or population decline for single and multiply resistant clones in the simulated patient. The simulation results provide a foundation for validating this treatment framework. Time to clearance (of a hypothetical bacterial infection) represents the positive end of the scale, and time to fixation, T_{fix}, the negative end which the study focuses on for convenience. The presented framework is well-grounded in the underlying ecological, evolutionary, and pharmacological theory driving collateral sensitivity, and allows us to study the efficacy of treatment achievable under such regimen.

Current examples and proposals of MCS are for two drug cycling and we formulated our original model to study this. It is not only applicable to antimicrobial chemotherapy but also to cancer chemotherapy where investigators have been studying treatment strategies of various cancers based on MCS considerations, although with mixed experimental results. [9]

MATERIAL AND METHODS

We construct a compartment model where a susceptible population of bacteria, S, is exposed successively to two antimicrobial drugs, A1 and A2, and simulate treatment over a period of 10 days in most cases, and longer where necessary. All strains compete for a limited nutrient and grow at their respective maximum rates in the absence of antibiotic pressure. Resistance confers a fitness disadvantage of 10% for singly resistant mutants with a multiplicative factor of 10% per additional resistance mutation(s). The pharmacokinetics are provided by equations relevant to an *in vitro* kinetic model or chemostat [10] at a rate mimicking human glomerular filtration (GFR). The at times neglected pharmacodynamics is incorporated by modulating minimum growth rates relative to each strain's respective MIC, as opposed to by the MIC alone. Collateral sensitivity is accounted for by switching the

120 MIC of the relevant (pre-exposed) strain at periods corresponding to when the antibiotic is cycled and CS
121 is assumed to be instantaneous. A flow diagram representing the model is presented in Figure 1.



122
123 **Figure 1:** Flow diagram describing the population dynamics of sensitive (S) bacteria exposed to
124 antibiotics and cells resulting from mutation / selection of resistance against antibiotic 1 (M_1), antibiotic
125 2 (M_2), or both (M_{12}). Rates of mutation are independent of each other, here described by μ_1 and μ_2, and
126 derived from Lee *et al* (2012). [11] The rate of double resistance arisal is a product of the individual
127 mutation rates μ_1 and μ_2.
128
129 **Treatment Model Description**
130 Our treatment protocol consists of exposing a sensitive population of $N_0 = 2 \times 10^8$ CFU ml^{-1} of bacteria to
131 each antibiotic as described below. We set the flow rate to 0.2 l h^{-1} and assume a basal mutation rate, μ,
132 of 3.9×10^{-10} $nucleotide^{-1}$ $generation^{-1}$, a conservative estimate as compared to the estimated range of μ.
133 [11] A single mutation is assumed to be sufficient to induce clinically significant resistance and that there is
134 an insignificant likelihood of reversion to full sensitivity. [12]
135 For sensitive cells, the MIC is set to 1 μg ml^{-1}. When applying drug A or drug B, the MIC of the cells
136 resistant to drug B or drug A respectively, is set to 0.5 μg ml^{-1} and the cells are thus more sensitive than
137 the sensitive cell population. The MIC of resistant cells is always set at 16 μg ml^{-1} prior to switching
138 when antibiotic is cycled. We assume a 10% reduction in fitness by imposing a penalty on maximum

growth rate. For each resistance 'acquisition' an additive fitness penalty is incurred with the triply resistant isolate being least 'fit'.

Antibiotics are pulsed at concentration, C μg ml^{-1} ($3 \leq c \leq 10$) into the chemostat for one hour, followed by 5 hours of medium alone. This is repeated four times per day and the antibiotic is switched daily (Pharma7d, 6h). The pharmacodynamic responsiveness of the exposed population to antibiotic concentration is reflected in the Hill coefficient, β, which defines how 'rapidly' the maximum efficacy is attained; the sensitivity of the bacterial population at concentrations close to the MIC.
We simulate MCS-guided treatment with daily, and 3 day cycling between antibiotics.
We follow each population throughout the treatment period and estimate time to clearance or the time to fixation of multiple drug resistance for either 2 or 3 drug rotations. The inhibitory effect of each antibiotic is reflected in the term, Ψ_{min}, which also reflects the maximal kill rate for each antibiotic. Values for β and Ψ_{min} are derived from experimental studies on *Escherichia coli* and *Staphylococcus aureus*. [13,14] We model the intrinsic growth of the population using a Monod model with resource conversion efficiency, $e = 5 \times 10^{-7}$ μg, and a Monod constant, κ of 0.25 μg ml^{-1} (See Levin et. al, 1977 [15]). Initially, there are no resistant isolates and we assume that the bacteria do not engage in horizontal gene transfer, a critical assumption for a fair assessment of CS.
Shown below is the coupled system of non-linear differential equations which describe the treatment regimen involving two antimicrobials. We carefully simulate solutions using Matlab. The system is stiff for some parameter values, and we utilize a stiff solver in such cases.

Mathematical Modeling

For all antibiotics**,**

$$\psi_i(\mathrm{Ab}_i, \mathrm{MIC}_i) = \frac{\psi_{\max_i}(\mathrm{t}) - \left(\psi_{\max_i} - \psi_{\min_i}\right)\left(\frac{Ab_i}{MIC_i}\right)^{\beta_i}}{\left(\frac{Ab_i}{MIC_i}\right)^{\beta_i} - \left(\frac{\psi_{\min_i}}{\psi_{\max_i}}\right)} \quad , \qquad (1)$$

where *i* represents each respective antibiotic (in this iteration, 1 or 2)

$$H(R) = x/(x + k_m) \qquad (2)$$

Antibiotic 1

Parameters for dosing Ab_1

$MIC_S = 1; MIC_1 = 15;\ MIC_2 = 0.5;\ MIC_{12} = 15$

$$\frac{dR}{dt} = Cw - e\,H(R)(v_{max_S}\,S + v_{max_{M_1}}M_1 + v_{max_{M_2}}M_2 + v_{max_{M_{12}}}(M_{12})) - wR \quad (3)$$

$$\frac{dS}{dt} = v_{max}H(R)\,\psi_1(Ab_1, MIC_S)S - \mu_1 S - \mu_2 S - \mu_1\mu_2 S - wS \quad (4)$$

$$\frac{dAb_1}{dt} = c\ square(t) - wAb_1 \quad (5)$$

$$\frac{dM_1}{dt} = v_{max_{M_1}}H(R)\,\psi_1\big(Ab_1, MIC_{M_1}\big)M_1 - \mu_2 M_1 + \mu_1 S - wM_1 \quad (6)$$

$$\frac{dM_2}{dt} = v_{max_{M_2}}H(R)\,\psi_1\big(Ab_1, MIC_{M_2}\big)M_2 - \mu_1 M_2 + \mu_2 S - wM_2 \quad (7)$$

$$\frac{dM_{12}}{dt} = v_{max_{M_{12}}}H(R)\,\psi_1\big(Ab_1, MIC_{M_{12}}\big)M_{12} + \mu_2 M_1 + \mu_1 M_2 + \mu_1\mu_2 S - wM_{12} \quad (8)$$

$$\frac{dAb_2}{dt} = -wAb_2 \quad (9)$$

Antibiotic 2

Parameters for dosing Ab_2

$MIC_S = 1; MIC_1 = 0.5;\ MIC_2 = 15;\ MIC_{12} = 15$

$$\frac{dR}{dt} = Cw - e\,H(R)(v_{max_S}\,S + v_{max_{M_1}}M_1 + v_{max_{M_2}}M_2 + v_{max_{M_{12}}}(M_{12})) - wR \quad (10)$$

$$\frac{dS}{dt} = v_{max_S}\,H(R)\,\psi_2(Ab_2, MIC_S)S - \mu_1 S - \mu_2 S - \mu_1\mu_2 S - wS \quad (11)$$

$$\frac{dAb_1}{dt} = -wAb_1 \quad (12)$$

$$\frac{dM_1}{dt} = v_{max_{M_1}}H(R)\,\psi_2(Ab_2, MIC_1)M_1 - \mu_2 M_1 + \mu_1 S - wM_1 \quad (13)$$

$$\frac{dM_2}{dt} = v_{max_{M_2}}H(R)\,\psi_2(Ab_2, MIC_2)M_2 - \mu_1 M_2 + \mu_2 S - wM_2 \quad (14)$$

$$\frac{dM_{12}}{dt} = v_{max_{M_{12}}}H(R)\,\psi_2(Ab_2, MIC_{12})M_{12} + \mu_2 M_1 + \mu_1 M_2 + \mu_1\mu_2 S - wM_{12} \quad (15)$$

$$\frac{dAb_2}{dt} = c\ square(t) - wAb_2 \quad (16)$$

where,

R	= Internal Resource concentration	C	= Resource source concentration
Ab_1	= Antibiotic 1 concentration	Ab_2	= Antibiotic 2 concentration
S	= Sensitive cells	M_1	= Resistant mutants to Ab_1

195 M_2 = Resistant mutants to Ab_2 M_{12} = Double resistant mutants

196 μ_1 = Mutation rate (to Ab_1) μ_2 = Mutation rate (to Ab_2)

197 With initial resource, R_0 = 100 µg ml^{-1}, and Sensitive bacteria, S_0 = 2 x 10^8 CFU ml^{-1}

198

199 *Chemostat parameters*

200 $w = 0.2$ ml h^{-1} {flow rate},

201 $C = 500$ µg ml^{-1} {resource reservoir}

202 $Ab_i = 3$ µg ml^{-1} h^{-1} **OR** 10 µg ml^{-1} h^{-1} {per hour dosed antibiotic}

203 (**Note: MIC$_S$=1**)

204

205 $\psi_{\min_1} = -1$ **OR** -3 ; $\psi_{\max_1} = 1; \beta_1 = 1$ **OR** 3

206 $\psi_{\min_2} = -1$ **OR** -3; $\psi_{\max_2} = 1$; $\beta_2 = 1$ **OR** 3

207 $\mu_1 = 3.9 \text{ x } 10^{-10}$ bp^{-1} h^{-1} {spontaneous mutation rate => to Ab_1}

208 $\mu_2 = 3.9 \text{ x } 10^{-10}$ bp^{-1} h^{-1} {spontaneous mutation rate => to Ab_2}

209

210 *Parameter values*

211 $e = 5 \text{ x } 10^{-7}$ µg {per cell division glucose requirement [15]}

212 $v_{max_S} = 0.7$ h^{-1} {maximum growth rate of sensitive cells S}

213 $v_{max_{M_1}} = v_{max_{M_2}} = 0.9\ (v_{max_S})$ h^{-1} {maximum growth rate singly resistant cells}

214 $v_{max_{M_{12}}} = 0.9^2\ (v_{maxS})$ h^{-1} {maximum growth rate doubly resistant cells}

215 $k_m = 0.25$ µg ml^{-1} {Monod constant, taken from [15]}

216

217

218 **RESULTS**

219

220 **In silico assessment of two drug cycling.**

221 We simulate treatment with two antimicrobials exhibiting varying PD parameters and encompassing

222 both bacteriostatic (S) and bactericidal (C) drug types (See Table 1 and *Materials & Methods*). We

223 consider both daily cycling and three-day cycling of each antibiotic. We define the time to fixation, T_{fix},

224 as the time between first application of drug 1 and the time at which the double resistant strain exceeds

225 0.9 N_0. The simulation results are summarized in Table 1 and displayed in Figure 2.

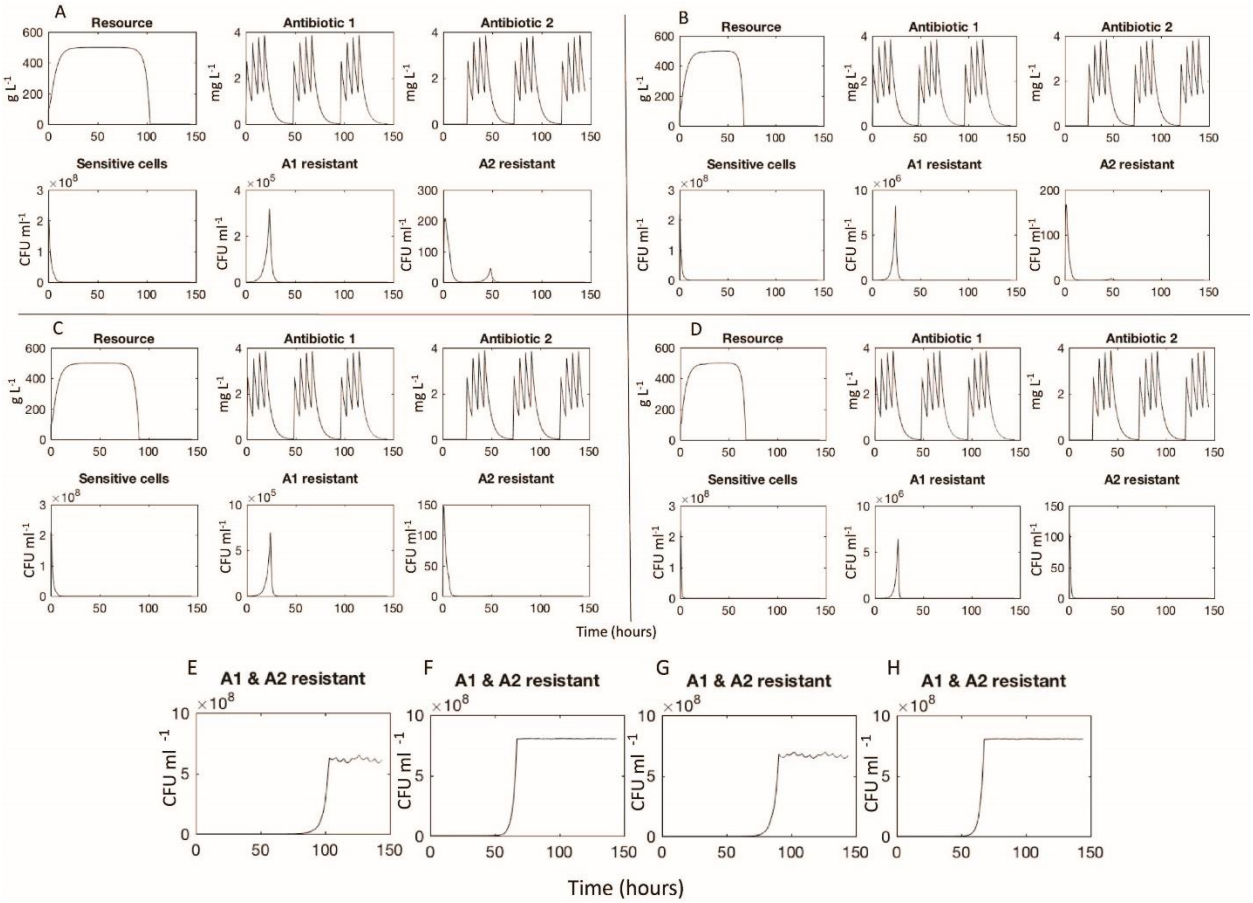


Figure 2. Selected simulations of our mathematical model showing changes in resource and antibiotics concentrations as well as population densities or sensitive and mono-resistant cells. Each panel A-D represents different runs for input antibiotic concentrations of 3 µg ml^{-1} (**A**: β = 1, S / S; **B**: β = 3, S / S; **C**: β = 1, C / C; **D**: β = 3, C / C). **E-H** represent the population densities of doubly resistant cells => T_{fix} for runs **A-D** respectively.

For low antibiotic dosing (~ 3x MIC of sensitive cells), fixation of double mutants, i.e. resistance to both applied antibiotics occurs rapidly, T_{fix} ranging between 67 hours and 115 hours. This occurred despite the increased susceptibility imposed by CS theory. The fixation of doubly resistant strains is seen to be invariable, although it takes 50% longer when drugs are cycled every three days than when cycling daily. The use of drugs with smaller Hill coefficients (β) leads to a 25% delay in fixation. Less significant are the differences between the various combinations of static and cidal drugs. For drugs with small Hill coefficients however, there is a 5% delay in fixation when one static drug is used and a further 5% delay when both cycled drugs are bacteriostatic.

242
243 **Table 1.** Two antibiotic exposure over ten days with daily cycling between Antibiotic 1 and Antibiotic 2
244 for one day (**Id**) and three day (**3d**) cycling protocols. **C** denotes a bactericidal antibiotic while **S** denotes
245 a bacteriostatic antibiotic. We report resistance fixation T_{fix} in each simulation. For further clarification,
246 see text.
247

Min growth rate ψ_{min_1}	Min growth rate ψ_{min_2}	Hill Coefficient β	Drug classification	T_{fix}@ c = 3μg ml^{-1} (1d, 3d)	T_{fix}@ c = 10μg ml^{-1} (1d, 3d)
-1	-1	3	S/S	67h, 102h	95h, 113h
-1	-3	3	S/C	67h, 101h	90h, 107h
-3	-1	3	C/S	68h, 101h	90h, 112h
-3	-3	3	C/C	68h, 101h	87h, 107h
-1	-1	1	S/S	96h, 115h	438h, >1000h
-1	-3	1	S/C	90h, 110h	294h, 1198h
-3	-1	1	C/S	90h, 115h	233h, 1124h
-3	-3	1	C/C	85h, 109h	237h, 523h

248
249 For moderate to high dosing schemes (~ 10x MIC of sensitive cells), for drugs having large β, doubly
250 resistant mutants fixate rapidly, within four days of treatment initiation. This is not the case for drugs
251 with small β values with which resistance only emerges (and fixates) long after a traditional clinical
252 treatment regimen is completed, i.e. > 7-10 days. T_{fix} also decreases when using two bactericidal (C / C)
253 drugs as compared to other combinations of cidal and static drugs (C / S or S / C). The best strategy of all
254 from our simulations is the use of two bacteriostatic drugs (S / S) with a low β, while cycling them every
255 three days. Under these conditions, no resistance is observed for any conceivable infection treatment
256 duration, which we interpret as meaning that such regimen with MCS guiding protocol may actually
257 inhibit the development of resistance.
258
259 Lastly, we note that significant short duration population spikes (> 10^5 CFU ml^{-1}) occurred for some singly
260 resistant strains.

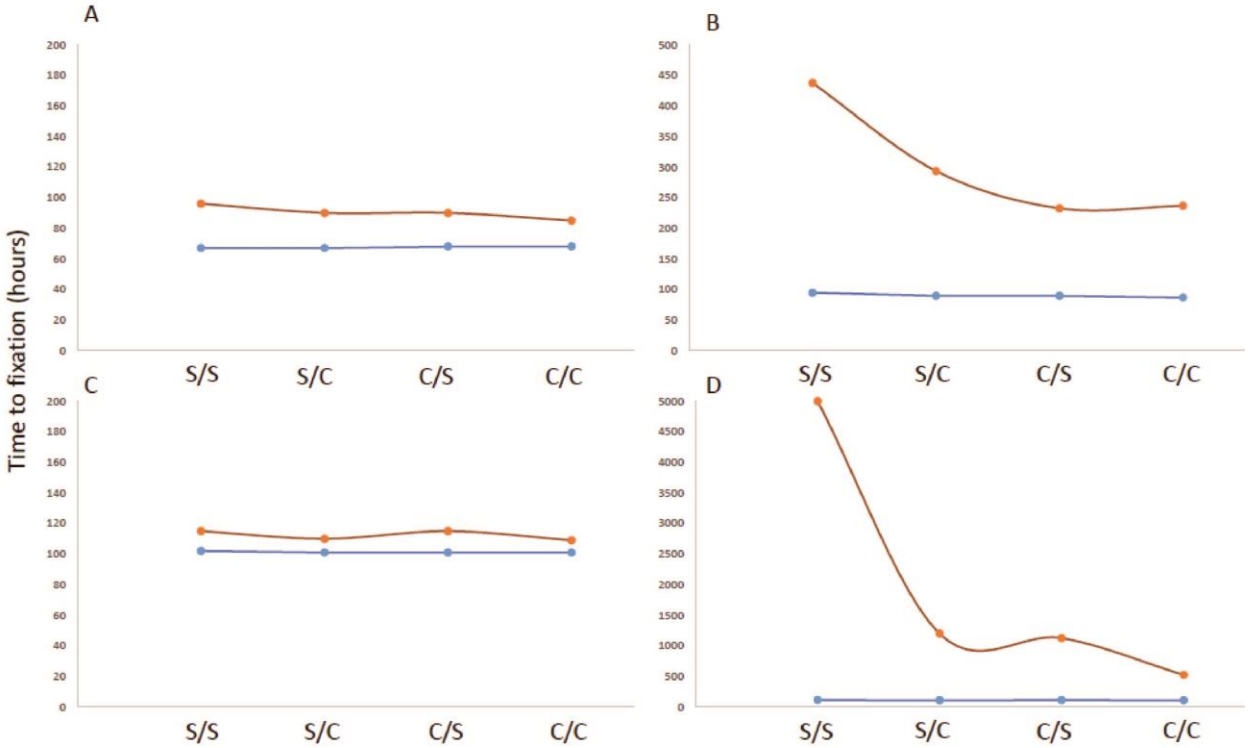
261 **DISCUSSION**

262 The emerging crisis that is pan antibiotic resistance, compounded by a dearth of new antibiotics in the
263 development pipeline [16] is necessitating new approaches to chemotherapy to minimize resistance

264 emergence and dissemination. Many clinical treatment strategies have been proposed, ranging from
265 combinatorial therapy to antibiotic cycling incorporation in the treatment of everyday infections in
266 order to mitigate resistance emergence. Antibiotic cycling can be implemented either on the level of
267 individual patients or the institutional unit. Earlier modeling studies of unit cycling cast doubts on the
268 utility of this strategy [17] and results of hospital trials have been mixed.
269 The MCS approach is an exciting treatment option but multiple cycle testing with clinically relevant
270 PK/PD consideration has not been explored. Our mathematical model allows us to do precisely this,
271 focusing on the PD properties relevant to treatment of a hypothetical infection. Our modeling strategy
272 not only considers the classical Minimum Inhibitory Concentration but also the dose responsiveness of a
273 population of sensitive and resistant bacteria in a resource-limited setting. This provides a broader
274 perspective than the usual one parameter, MIC, summary on the PD.
275 Several drug pair combinations have been identified as exhibiting MCS as earlier stated. Results from
276 WGS based studies have identified two possible mechanisms steering MCS: i) intragenic suppression of
277 resistance, [7] and, ii) epistasis derived reversal of resistance (towards the other drug). [2] These are based
278 on both the mutation target size and the breadth of the path each adaptive walk has traversed.
279 Intragenic suppression is limited by the smaller number of mutations that can lead to an MCS phenotype
280 for a related antibiotic while epistatic suppression is more dependent on an underlying genetic diversity.
281 These are also discussed in [7] and the observed saddlepoint in their published evolutionary landscape
282 points to potential treatment failure; evolutionary unpredictability as such is not considered herein.
283 These only serve to bolster the importance of our comprehensive pharmacodynamic study.
284
285 Pharmacodynamic properties of antibiotics are broadly delineated as bacteriostatic or bactericidal for
286 ease of pharmacological understanding. Most bacteriostatic drugs are weakly -cidal and while
287 bactericidal drugs are often preferred to bacteriostatic drugs, in immunocompetent patients, there is no
288 advantage in using the former. From our simulations, it appears that when exploiting MCS, the
289 bacteriostatic drugs are superior to their -cidal counterparts, as they inhibit resistance development. To
290 our knowledge, this is the first report or observation of such, emerging albeit from an *in silico* study.
291 As presented in the Results section, we observe the rapid fixation of resistance in all cases of low drug
292 concentration exposure, underlining the importance of adequate dosage. The large population sizes
293 commonly associated with infections, also considered in our study, would require an immunocompetent
294 host (patient) to clear. At first glance, our model lacks both innate and adaptive immunity (approached
295 in [18]) but the innate immunity is implicit in form of resource concentration limitation on population size.

296 From the estimates of T_{fix}, the single factor most significant in the early fixation of multiple resistance
297 using this MCS protocol is the cycling period (*cv* 67 h vs. 110 h for one day cycling and three day cycling
298 respectively). Following this is the effect of the Hill coefficient, β, on T_{fix} which, in line with, [19] challenges
299 the idea that increased clearance rates minimize the selection and fixation of resistant isolates. The
300 increased selective pressure imposed by higher order bactericidal drugs leads to a more rapid fixation of
301 resistance in the population. Our model explains this from a nutrient competition perspective; as
302 antibiotic 2 is being applied, the killing of the sensitive cells and those resistant to antibiotic 1, results
303 effectively in reducing the total population density and thus competition is relaxed.
304 This is also true for the other antibiotics and evolution of doubly resistant mutants is facilitated by the
305 large population sizes being acted upon by the imposed (conservative) mutation rate in our model (See
306 Figure 2). Once doubly resistant, this collateral sensitivity is lost and overall fitness of the population is
307 maximized under (either Ab_1 or Ab_2) antibiotic pressure.
308
309 It is known that the efficacy of beta lactam antibiotics is highest at concentrations around five times the
310 MIC and the results obtained from our low dose simulations are thus interesting for this highly clinically
311 utilized class of antibiotics. The PD parameter β describes the sensitivity of the antibiotic to changes
312 proximal to the point of inflection (zero growth) in the graph of the Hill function (See model description).
313 This plays a vital role in the success of MCS-guided treatment regimen although it is not evident *á priori*
314 (See Figure 3). As is seen upon replotting the data to reflect the effect of β on our estimate of efficacy,
315 the fixation time for bactericidal drugs in particular is heavily dependent on the drug dosage especially
316 for the 3d cycling protocol. These are strong indicators of the importance of drug choice under such
317 treatment regimen consideration.
318
319 In vivo, the immune system works hand in hand with antibiotics to clear the infection. In some of our
320 simulations, the single and double mutants remain at low enough densities to be easily cleared by
321 circulating immune cells. However, when fixation of double mutants occurs within a few days, the
322 likelihood of treatment failure would by definition, increase. Even in cases where fixation does occur,
323 the immune system, unaffected by AMR state may still clear. As such our model only serves as a
324 cautionary tale; heterogeneity in immunity and an immunocompromised state could exacerbate the
325 likelihood of treatment failure, as would antimicrobial heteroresistance, inoculum effects and high
326 frequency of AMR bacteria in the original infection.

327 In addition, horizontal gene transfer confounds the model's predictability as the rate of plasmid-
328 mediated resistance transmission, especially conjugable plasmids, is considerably higher than the
329 replication dependent vertical propagation of point mutations. We do not consider plasmid-based
330 transmission of antimicrobial resistance genes and neither do we include any resistant mutants initially,
331 only considering de novo mutations conferring resistance. These mutations we assume occur
332 independently of one another. Under continuous antibiotic exposure, selection drives the system
333 towards a fitness maximum through resistance compensation which is favored over reversion [12].
334



335
336 **Figure 3:** Categorical plot showing the effect of parameter values β and ψ_{min} on fixation time, T_{fix}, for
337 two drug concentrations; 3µg ml^{-1} (Panels ***A*** & ***C***) or 10µg ml^{-1} (Panels ***B*** & ***D***) of combinations of cidal (*C*)
338 and static (*S*) drugs (*x-axes*) were simulated with $\beta = 1$ (*Red*) or $\beta = 3$ (*Blue*) and cycled either every day
339 (Panels ***A*** & ***B***) or every 3 days (Panels ***C*** & ***D***).

340

341 The modeling framework we present in this manuscript treats two drug cycling but has already been
342 extended to three drug cycling in anticipation of triplet combinations that may be identified in due
343 course. Before clinical deployment however, the utility of MCS must be explored experimentally,
344 rationalized physiologically, and further tested for generality. Mathematical models of this sort will play

345 a crucial role in establishing the applicability and suggesting modifications to this and other proposed
346 treatment regimen.
347

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357 **DISCLOSURE**
358 The authors have no relevant or material financial interests to disclose.
359

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