UNVIERSITY OF CALIFORNIA, SAN DIEGO

Managing Resistance in the ICU:

An Evolutionary Approach to Rational Antibiotic Deployment

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

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2011

The Thesis of Ellsworth Campbell is approved and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California, San Diego

2011

DEDICATION

To all who follow their passions in the face of adversity and misfortune.

EPIGRAPH

“Go placidly amid the noise and haste

and remember what peace there may be in silence.”

-Max Erhman
Excerpt from Desiderata

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ABSTRACT OF THESIS

Managing Resistance in the ICU:

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Nosocomial infections account for 5 to 10% of all infections in the United States and act as a continuous reservoir for the maintenance of antibiotic resistance. Development, testing, and implementation of broad antimicrobial deployment strategies are crucial in the proper management of resistance over the long term. We present a population-based model which describes the spread of variably-resistant nosocomial pathogens amongst patients in an intensive care unit of a hospital. Our purpose is to identify treatment strategies which maximize the number of uninfected individuals while

maintaining low rates of multi-resistant infections. This was accomplished via the expansion of a previously published model by introducing pharmacodynamics, pharmacokinetics, and cross-resistance tradeoffs. Most importantly, we depart from this model's predecessors by treating the minimization of resistant-infected individuals as secondary to maximizing uninfected. We confirm that the benefit of a random mixing regimen over periodic cycling is minimal, while a hybrid of the two is slightly more effective. Finally, we show that time- and probability-based strategies are inferior to Multi-Drug cocktails in their ability to exploit resistance-associated fitness tradeoffs; thereby selectively favoring susceptible genotypes. These results provide an impetus to identify Multi-Drug cocktails which serve to minimize the incidence of multi-resistance while still maintaining curing efficacy.

I.

Introduction

Antibiotic resistance has been a widely publicized and studied phenomenon since it was first discovered in a clinical setting shortly after World War II [1]. Decades of antimicrobial misuse, precipitated by a fundamental naiveté towards their selective power, has led to a public health crisis that has only recently gained the necessary scientific and financial support to address it [2]. A recent flurry of theoretical research has been directed at identifying and comparing different antimicrobial deployment strategies [3-8]. Ecological theory dictates that the key to controlling resistance comes through forcing pathogens to cope with maximal environmental heterogeneity, thereby favoring the less-specialized sensitive genotypes [3]. This principle finds a variety of strategic applications to agriculture, oncology, and infectious diseases [9-11]. Unfortunately, these systems may be sufficiently divergent such that the identification of a single optimal treatment strategy is unlikely. Hospital-acquired, or nosocomial, infections propagate in an ecological niche brimming with selective pressure from antibiotics and are attributed with 2 million infections and 99,000 deaths per year [12]. Beyond its significance to human health, the inherent clinical nature of nosocomial infection allows for the development and application of well-controlled experiments. For these reasons, we believe this system to be ideal for the application of a mathematical model directed toward the ultimate goal of informing clinical resistance management experimentation.

In March 2011, the *Interagency Task Force on Antimicrobial Resistance* released an action plan draft for combating the threat; wherein it is suggested that increased surveillance and research-guided regulations will play a crucial role in the management

of antibiotic resistance [12]. Currently, ward- or hospital-wide antibiotic usage and surveillance protocols are uncommon but ambitious plans are currently in development [13]. Physicians currently lack the necessary infrastructure to inform their primary treatment based upon the prevalence of resistance in their own patient, let alone their immediate community [14]. We believe that theoretical modeling can provide a rubric with which to compare forthcoming clinical surveillance information. For example, a recent stochastic epidemiological model suggests that the cyclical usage of broad spectrum antibiotics can be extremely effective when combined with coarse data about the system's dynamics [5]. Here, we expand a population-based model of antimicrobial resistance in a hospital to compare treatment strategies such as mixing, cycling, and multi-drug cocktails [3]. This is accomplished via the implementation of a pharmacodynamic function, directed at resolving complex antimicrobial interactions, which is further modified to enforce a range of cross-resistance fitness tradeoffs [15]. Finally, we suggest that comparison of treatment strategies should be based upon the asymptotic proportion of uninfected individuals while taking the proportion of multi-resistant individuals into special consideration.

II.

Materials and Methods

We consider a population-based model of ODEs which describes a hospital's Intensive Care Unit (ICU). Two antimicrobial agents, drugs A and B, are employed and assumed to be of different classes and symmetrical efficacy. Patients can be colonized by four nosocomial pathogens: sensitive to both A and B (*s*), resistant to A and sensitive to B (r_1), resistant to B and sensitive to A (r_3), and resistant to both A and B (r_2). The patient population is subsequently compartmentalized into five states of colonization: *s*-colonized (*S*), r_1-colonized (R_1) r_2-colonized (R_2), r_3-colonized (R_3) and uninfected (*X*). In order to minimize stochastic effects, we assume infinite population size and represent the population as a system of five ordinary differential equations (*Eq 1a-e*, *Fig 1*).

$$\dot{S} = \mu(R_1 + R_2 + R_3 - 3S) + (G_s - \gamma)S + \beta SX + \sigma\beta cS(R_1 + R_2 + R_3) \quad \textbf{(1a)}$$

$$\dot{R_1} = \mu(S + R_2 + R_3 - 3R_1) + \left(G_{r_1} - \gamma\right)R_1 + \beta R_1 X(1-c) - \sigma\beta cSR_1 \quad \textbf{(1b)}$$

$$\dot{R_2} = \mu(S + R_1 + R_3 - 3R_2) + \left(G_{r_2} - \gamma\right)R_2 + \beta R_2 X(1-c) - \sigma\beta cSR_2 \quad \textbf{(1c)}$$

$$\dot{R_3} = \mu(S + R_1 + R_2 - 3R_3) + \left(G_{r_3} - \gamma\right)R_3 + \beta R_3 X(1-c) - \sigma\beta cSR_3 \quad \textbf{(1d)}$$

$$\dot{X} = -(G_s - \gamma)S - \left(G_{r_1} - \gamma\right)R_1 - \left(G_{r_2} - \gamma\right)R_2 - \left(G_{r_3} - \gamma\right)R_3 - \beta SX \quad \textbf{(1e)}$$

$$-\beta R_1 X(1-c) - \beta R_2 X(1-c) - \beta R_2 X(1-c) - \beta R_3 X(1-c)$$

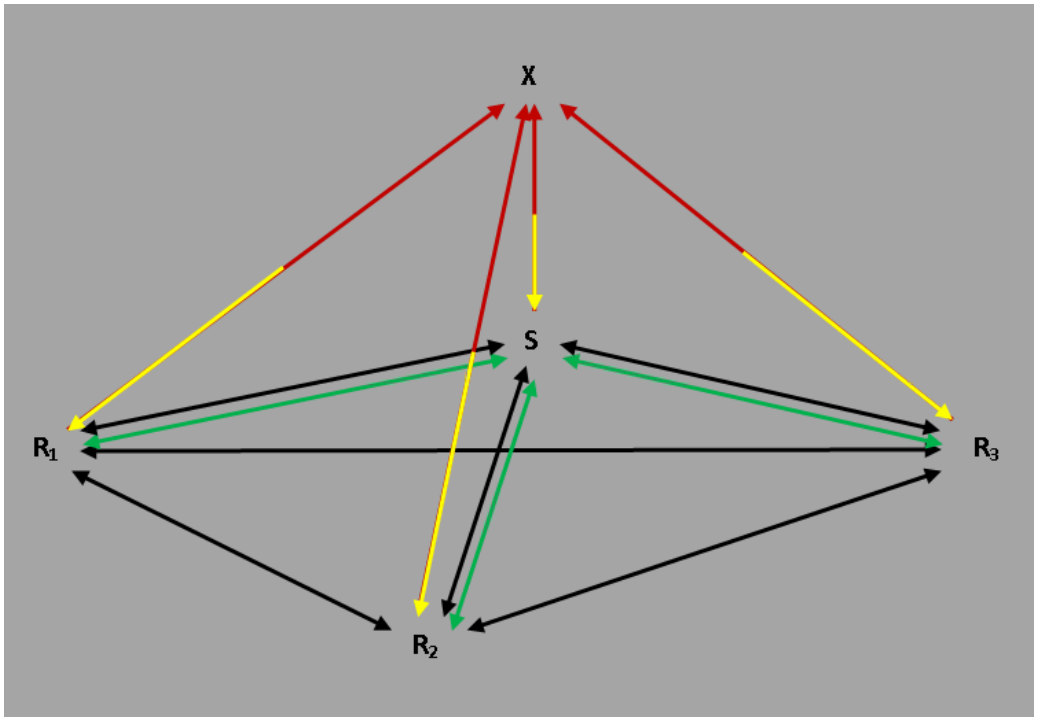

Figure 1: Stylized representation of the population-based ICU model.

Each node is a population of patients. Red arrows indicate transition from infected to uninfected states by drug or immune clearance. Yellow arrows indicate primary infections. Black arrows indicate resistance character-state changes via mutation or horizontal gene transfer. Green arrows indicate competition via secondary infections. Note that competition during secondary infection is a stalemate between resistant genotypes because cost is uniform.

Table 1: Table of Constant and Variable Definitions

μ	Rate of resistance acquisition	ψ_{max}	Maximum growth rate w/o drugs
k	Bacterial response time to changes in drug concentration	ψ_{min}	Minimum growth rate, in the presence of extremely high drug concentrations
γ	Immune clearance rate	*G()*	Strain-specific growth function
β	Transmission rate	α	Cross-Resistance fitness tradeoff parameter
σ	Rate of secondary infection	c	Cost of maintaining resistance
π	Rate of drug switching		

Maintenance of drug resistance characters is metabolically costly and affects a genotype's ability to compete with its wild-type relative. Cost (*c*) is manifest in our model when two genotypes compete for the same host; which occurs at a frequency given by the rate of primary and secondary infections ($\beta \cdot \sigma$). Note that cost is uniform for all resistant genotypes in order to emphasize the impact of drug multi-resistance tradeoffs discussed below. We assume no pre-existing resistance but allow it to evolve at a rate given by μ. This is uniform between resistance characters to simulate that resistance can evolve by mutation or via horizontal gene transfer. Finally, we assume that a nosocomial pathogen is highly virulent towards an immunocompromised patient hospitalized in an ICU and thus primary infection is assumed on contact ($\beta=1$). The system has been closed to patient influx/efflux in order to focus on drug- and strategy-mediated competition.

We further depart from our model's predecessors by replacing the drug clearance terms with net bacterial growth rates (G_{r_i}). Growth rates are determined by a pharmacodynamic function that is based on published E_{max} models and time-kill data.

$$G = \varphi_{max} - \frac{\overbrace{(\varphi_{max}-\varphi_{min})\cdot\left(\frac{[drug]}{MIC_{drug}}\right)^{k}}^{\tau_{drug}}}{\left(\frac{[drug]}{MIC_{drug}}\right)^{k} - \frac{\varphi_{min}}{\varphi_{max}}} \quad \textbf{(2)}$$

If a genotype's Minimum Inhibitory Concentration (MIC) is equal to the drug concentration the pharmacodynamic function will yield zero net growth.

$$G_{\left(\frac{[drug]}{MIC_{drug}}\right)=1} = \varphi_{max} - \tau_{drug} = 0 \quad \textbf{(3)}$$

This function is then modified by applying a cross-resistance scaling parameter (α) to the MIC of the multi-resistant strain (r_2).

$$\tau_{drug}^{r_2} = \frac{(\varphi_{max}-\varphi_{min})\cdot\left(\frac{[drug]}{\alpha\cdot MIC_{drug}}\right)^k}{\left(\frac{[drug]}{\alpha\cdot MIC_{drug}}\right)^k - \frac{\varphi_{min}}{\varphi_{max}}} \quad \textbf{(4)}$$

This parameter denotes the fitness tradeoff associated with the simultaneous maintenance of multiple resistance characters. Note that the cross-resistance tradeoff has a range defined by $f_{0<\alpha\leq 2}(G_{r_2})$, thereby allowing the fitness tradeoff to capture all genotypes contained in the linear variable space between full susceptibility and full resistance [Fig 2].

An antimicrobial cycling strategy is modeled by allowing each strain two different net bacterial growth rates that are alternated over a given periodicity (π).

$$G_{r_i}^{t_0\rightarrow t_0+\pi} = \varphi_{max} - \tau_A \quad \textbf{(5a)}$$

$$G_{r_i}^{t_\pi\rightarrow t_\pi+\pi} = \varphi_{max} - \tau_B \quad \textbf{(5b)}$$

Mixing strategy assumes random drug selection (A or B) and therefore the drug usage ratio within the ICU $\langle p(A): p(B)\rangle$ remains at 1. In contrast to cycling, we assume that each patient will maintain their current treatment until cleared to the uninfected state. We then develop a hybrid of the aforementioned strategies to exploit the increased temporal and spatial heterogeneity that they afford. This strategy consists of a patient population that is first divided and started on a broad spectrum antibiotic (A or B). Patients' treatments are then periodically cycled (π) on a synchronized schedule (A → B vs. B → A). This requires the expansion of our model to allow for two populations that are

defined by the infecting strain as well as the drug treatment group (e.g. R_1^A & R_1^B; ODEs not shown).

We consider two theoretical Cocktail regimens wherein both drugs are administered simultaneously. Note that under these regimens, drugs are administered at one half dosages with respect to all other treatment strategies. Put another way, the cumulative amount of administered drugs remains constant across all treatment regimens. Separate Cocktail Ck_{sep} is defined as the net bacterial growth rate φ_{max} less the effects of each drug as determined by the pharmacodynamic function. We believe this function captures drug pairings which have a non-interactive effect of Loewe's Additivity.

$$G_{r_2}^{Ck_{sep}} = \varphi_{max} - \frac{\overbrace{(\varphi_{max}-\varphi_{min})\cdot\left(\frac{½\cdot[A]}{MIC_A^{r_2}}\right)^k}^{\tau_A}}{\left(\frac{½\cdot[A]}{MIC_A^{r_2}}\right)^k - \frac{\varphi_{min}}{\varphi_{max}}} - \frac{\overbrace{(\varphi_{max}-\varphi_{min})\cdot\left(\frac{½\cdot[B]}{MIC_B^{r_2}}\right)^k}^{\tau_B}}{\left(\frac{½\cdot[A]}{MIC_B^{r_2}}\right)^k - \frac{\varphi_{min}}{\varphi_{max}}} \quad \textbf{(6)}$$

Combined Cocktail (Ck_{cmb}), differs in that it assumes that the paired drugs limit each other's efficacy and are thus combined into a single pharmacodynamic function. We believe this function captures antimicrobial pairings which de-couple different cellular metabolic function; thereby resulting in a concerted but attenuated antimicrobial effect.

$$G_{r_2}^{Ck_{cmb}} = \varphi_{max} - \frac{(\varphi_{max}-\varphi_{min})\cdot\left(\frac{½\cdot[A]}{MIC_A^{r_2}}+\frac{½\cdot[B]}{MIC_B^{r_2}}\right)^k}{\left(\frac{½\cdot[A]}{MIC_A^{r_2}}+\frac{½\cdot[B]}{MIC_B^{r_2}}\right)^k - \frac{\varphi_{min}}{\varphi_{max}}} \quad \textbf{(7)}$$

In order to guide our analysis of simulation data, we developed a more simplistic fitness model based on net bacterial growth rates determined by the pharmacodynamic function. Genotypic fitness under the periodic cycling regimen is defined as the

geometric mean of net bacterial growth rates when exposed to each drug individually (8). Fitness under random mixing is given by the arithmetic mean of a strain's growth rates when exposed to each drug individually (9). Fitness under cocktails is defined by the growth rate determined by each type's pharmacodynamic function (10-11).

$$w_{cyc}^{r_i} = \sqrt{e^{\pi\left(G_{r_i}^{A}+G_{r_i}^{B}\right)}} \quad \textbf{(8)}$$

$$w_{mix}^{r_i} = \left(e^{G_{r_i}^{A}} + e^{G_{r_i}^{B}}\right)/2 \quad \textbf{(9)}$$

$$w_{Ck_{sep}}^{r_i} = e^{(G_{r_i}^{A+B})} \quad \textbf{(10)}$$

$$w_{Ck_{cmb}}^{r_i} = e^{\left(G_{r_i}^{AB}\right)} \quad \textbf{(11)}$$

Given that net bacterial growth rates can be negative, each component is then turned into an exponential function, thereby avoiding irrational numbers. These fitness calculations allow a rough insight as to how different strains will fare, in relation to one another, in the presence of drugs on a specific treatment schedule.

Analysis and comparison of treatment strategies were conducted via simulations performed in MatLab (v.7.10.0.499 - R.2010a, MathWorks Natick, MA) utilizing the built-in numerical ODE solver (ODE45). The asymptotic proportion of uninfected individuals ($\widehat{X}$) was used as the primary metric of comparison with careful consideration given to the asymptotic proportion of multi-resistant infected individuals ($\widehat{R}2$). Time-dependent simulations were run over an interval such that $\lim_{t=1\rightarrow 10^6} f(\widehat{patient})$. $\widehat{X}$ & $\widehat{R}2$ are considered to be the mean of the population proportions over the last 10^2 time intervals, which we define as a 24-hour day.

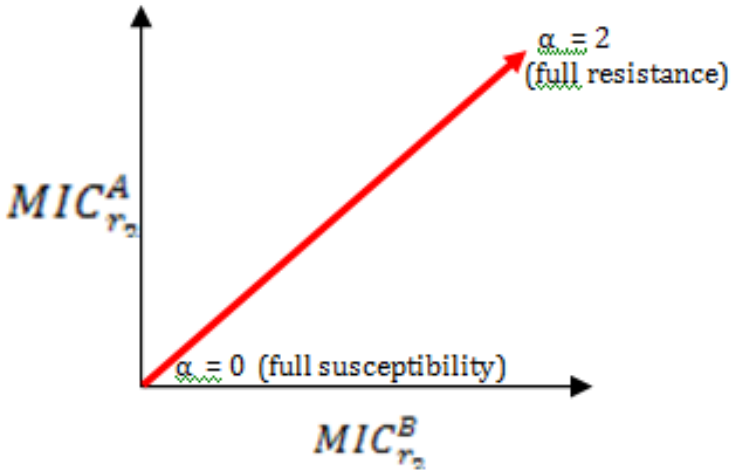

Figure 2: Range of possible cross-resistance fitness tradeoffs for r_2

Variable cross-resistance places the multi-resistant strain's (r_2) level of resistance anywhere along the continuum indicated in red. This range represents all genotypes between the susceptible and super-resistant types.

III.

Results

I. Model Generalizations

A. Cross-Resistance Tradeoffs

Central to our analysis of various treatment regimens is determining the effect of multi-resistant strains in competition with less specialized genotypes. More pointedly, we would like to elucidate the role of variable cross-resistance as it pertains to competition under different treatment strategies. Simulations of our model indicate a hierarchy of treatment regimens when multi-resistant strains are not allowed to evolve [Fig 3]. Upon introduction of a competitive multi-resistant genotype, we begin to see a divergence in the efficacy of different strategies because of the dominant role a multi-resistant strain (r_2) commands when cross-resistance tradeoffs allow [Fig 4]. We identified tradeoff thresholds which allow r_2 a sufficient fitness advantage such that it precipitates a restructuring of the patient distribution. Our simulations indicate that each strategy has two corresponding α-thresholds where the level of cross-resistance: (θ_1) Allows r_2 to compete for hosts and maintain a population at equilibrium. (θ_2) Allows fixation of the r_2 genotype thereby rendering a change in strategy futile [Fig 5]. For all tradeoffs where $\alpha \leq \theta_1^{strat}$ the proportion of uninfected individuals remains static [Fig 6a]. When $\alpha > \theta_1^{strat}$, the benefit to the proportion of uninfected individuals undergoes diminishing returns [Fig 6b]. Finally, as α approaches its maximal value, all but one of our cocktail strategies approaches the control of the single drug treatment [Fig 6c].

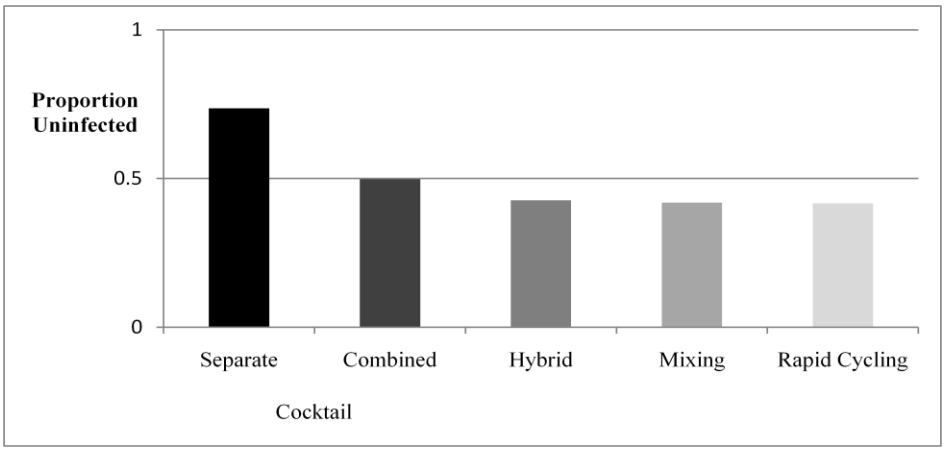

Figure 3: Proportion of uninfected individuals under various strategies without multi resistant genotypes.

Histogram indicating a hierarchy of treatment efficacy when multi-resistant strains are not allowed to evolve. Cocktail and Mixing are average asymptotic proportions while Hybrid and Cycling long-term averages when initial dynamics are ignored.

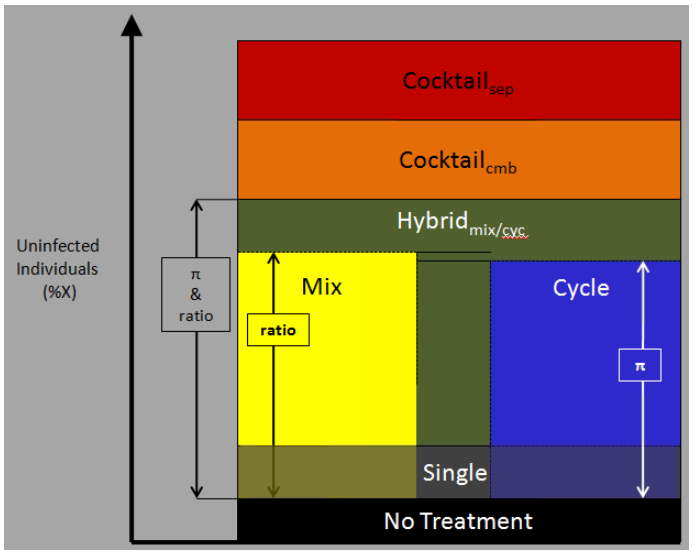

Figure 4: Stylized hierarchy of various treatment strategies.

Various treatment strategies' proportion of uninfected individuals. Under the cycling strategy, switching periodicity (π) has a large impact on the efficacy of treatment. Under Mixing, the ratio at which drugs are employed plays a decisive role. Therefore, Hybrid maintains the same limitations.

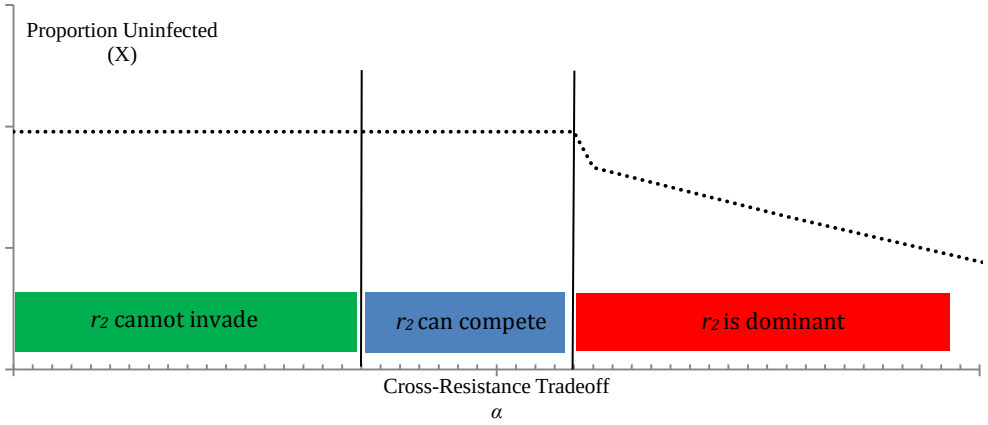

Figure 5: Proportion of uninfected and multi-resistance competition.

This shows the general features of all treatment strategies when the uninfected population is considered with respect to cross-resistance fitness tradeoffs. As tradeoff increases, the multi-resistant strain becomes increasingly competitive.

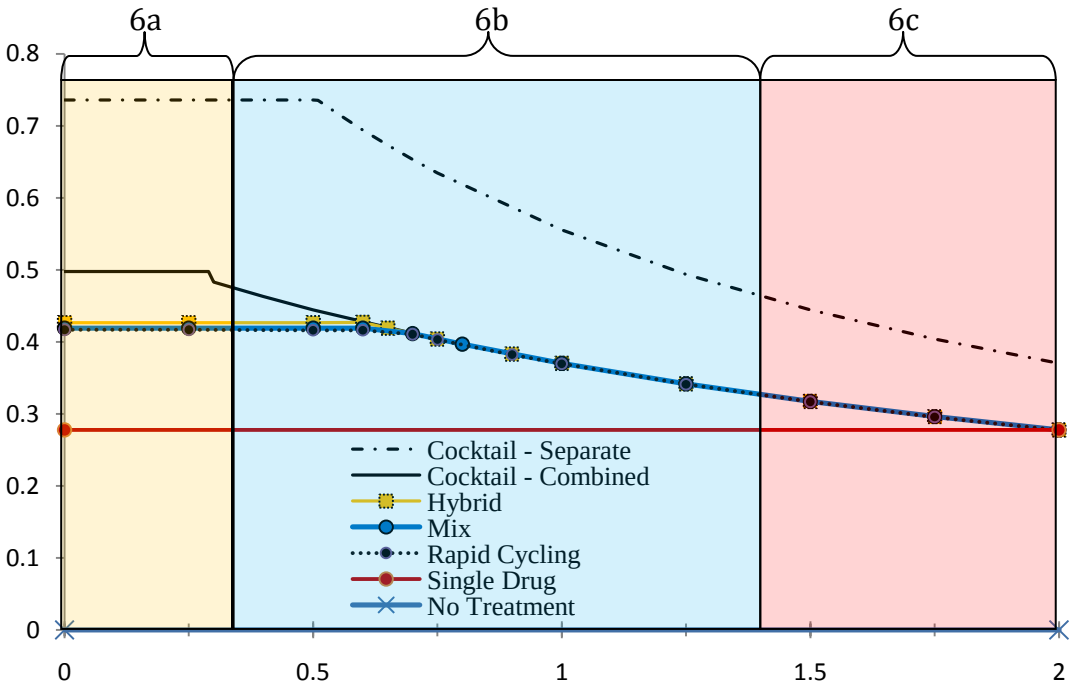

Figure 6: Proportion of uninfected under all treatment strategies.

Proportion of uninfected individuals under all strategies with respect to cross-resistance tradeoff. **(A)** Indicates the range of tradeoffs which do not impact the uninfected proportion **(B)** Highlights that the benefit to the uninfected population due to different treatment strategies undergo diminishing returns as the tradeoff increases. **(C)** Shows that a change in treatment strategy at high tradeoff values is futile, unless powerful cocktails are employed.

B. No Treatment

To assess various treatment strategies we begin by obtaining measurements of the asymptotic proportion of all patients in the specific case of no drug treatment. We intend this to act as a baseline of comparison as well as a control of the model's competition dynamics. When no drugs are employed, each genotype has an equivalent net growth rate but differ in overall fitness due to the cost of resistance. Because the cost of all resistance characters are uniform, when no drugs are employed we expect the resistant populations to be competitively excluded and only appear at the rate of resistance acquisition (μ). We find that the asymptotic proportion of uninfected individuals is given by $\hat{X}_{nt} \cong (\gamma - \varphi_{max})$, or the clearance rate of a patient's immune system less the net bacterial growth rate of the susceptible strain in the absence of drugs. The result of untreated nosocomial infections in an intensive care ward can be seen as a worst case scenario, therefore we assume φ_{max} to be equal to that of γ. Put another way, the net bacterial growth is equal to the immune clearance rate. As a result, a lack of treatment results in the proportion of uninfected individuals dropping to zero.

C. Single Drug Treatment

If a single broad spectrum antibiotic is regularly employed without surveillance we assume the resistant mutant to be under powerful selection until its minimum inhibitory concentration (MIC) meets the drug concentration employed. The singly-resistant r_1 has an MIC equal to the treatment dosage; we assume symmetrical levels of resistance between r_1 and r_3, therefore $\hat{R}_1 \propto \hat{R}_3$. We find that the mutant resistance allele quickly sweeps to fixation amongst infected patients under a single-drug treatment regimen. Such a strategy represents a worst possible treatment strategy wherein an

infected patient's fate would be entirely dependent upon their compromised immune system to clear the infection. Our simulations indicate that the drug A-only regimen maximizes the $\hat{R}_1$ patient state with only a small proportion of uninfected individuals. As with no treatment, we expect $\hat{X}_{sng} \sim (\gamma - G_{r_1})$). However the proportion of uninfected individuals under single treatment is greater than that of no treatment because the net bacterial growth rate does not negate the effect of immune clearance. This is a result of the drug's bacteriostatic effect when the genotype's MIC reaches the dosage concentration ($G_{r_1} = 0$; *See Method; Eq.3*).

II. Time- and Probability-based Treatment Strategies

A. Cycling

Using a single drug indefinitely results in an unacceptably high proportion of resistant-infected hosts. The intuitive and most commonly practiced response is to then switch the primary broad spectrum antibiotic to reduce the frequency of resistance. Our model indicates that after such a change there is an immediate, though fleeting benefit to the proportion of uninfected individuals as the dominant population feels the full force of the secondary drug [fig. 7c]. For example, if an ICU were to switch drugs every 30 days we expect the uninfected population to reach a minimum in less than three weeks. In addition, the average proportions of the resistant-infected patients are roughly equivalent when averaged over the long term ($\hat{R}_1^{cyc} \approx \hat{R}_3^{cyc}$) [Fig. 7b]. When cycling antimicrobial agents on a pre-determined schedule, $\hat{X}_{cyc}$ is maximized when the drug rotation interval (π) is shortest, regardless of the imposed tradeoff. In addition, as $\pi \to \infty$ we observe that $\hat{X}_{cyc} \sim \hat{X}_{sng}$ (i.e. as the drug rotation interval increases, the strain resistant to the currently deployed drug experiences the same environment it would under a single drug

regimen) [Fig. 7a, 8a-c]. As with other strategies, $\hat{R}_2^{cyc}$ is of little concern until the cross-resistance tradeoff of r_2 surpasses the first fitness threshold $(\alpha \geq \theta_1)$. Beyond the second threshold, which allows for fixation of the r_2-genotype, the short-lived benefit that $\hat{X}_{cyc}$ receives from switching disappears because it is equally fit under both environmental conditions $(\alpha \geq \theta_2)$.

B. Random Mixing and Cycle-Mix Hybrid

Previous theoretical models directed at identifying optimal treatment strategies have found little benefit to cycling protocols over that of mixing. Periodic cycling can be understood as a discrete subset of outcomes within the random mixing strategy itself, and therefore optimal cycling essentially is optimal mixing. We observe that optimal periodic cycling results are nearly indistinguishable from that of optimal mixing. A hybrid of the two strategies (*see Methods)* yields only a slight improvement over both random mixing and infinitely rapid cycling in terms of the uninfected population [Fig 9]. We also find that this hierarchy is reflected in each strategy's θ_2-threshold [Table 1]. Thus the cycling strategy allows the multi-resistant allele to rise to fixation with a slightly weaker tradeoff than under the mixing strategies. Note that the only functional difference between the hybrid strategy and its competitors is increased environmental heterogeneity due to forced periodic cycling of an already randomized treatment.

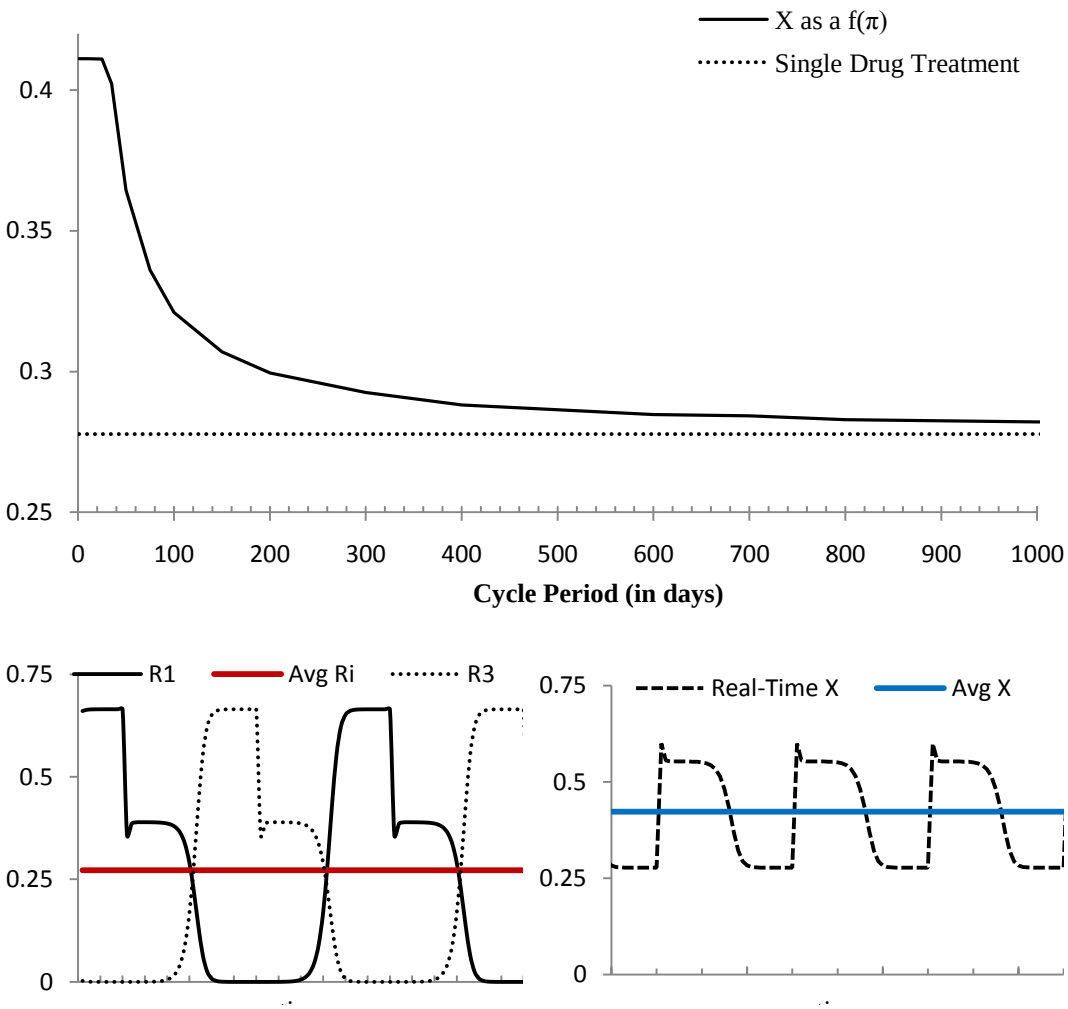

Figure 7: Proportion of populations in response to cycle periodicity.

Proportion of various patient populations with respect to the drug-switching rate. **(A)** Indicates that as period increases, the proportion of uninfected individuals asymptotes with that of single drug treatment. **(B)** Shows that the R_1 and R_3 population, under cycling, are mirrors of each other and maintain the same average proportion over time. **(C)** Highlights the momentary benefit that the uninfected proportion gains immediately after a switch.

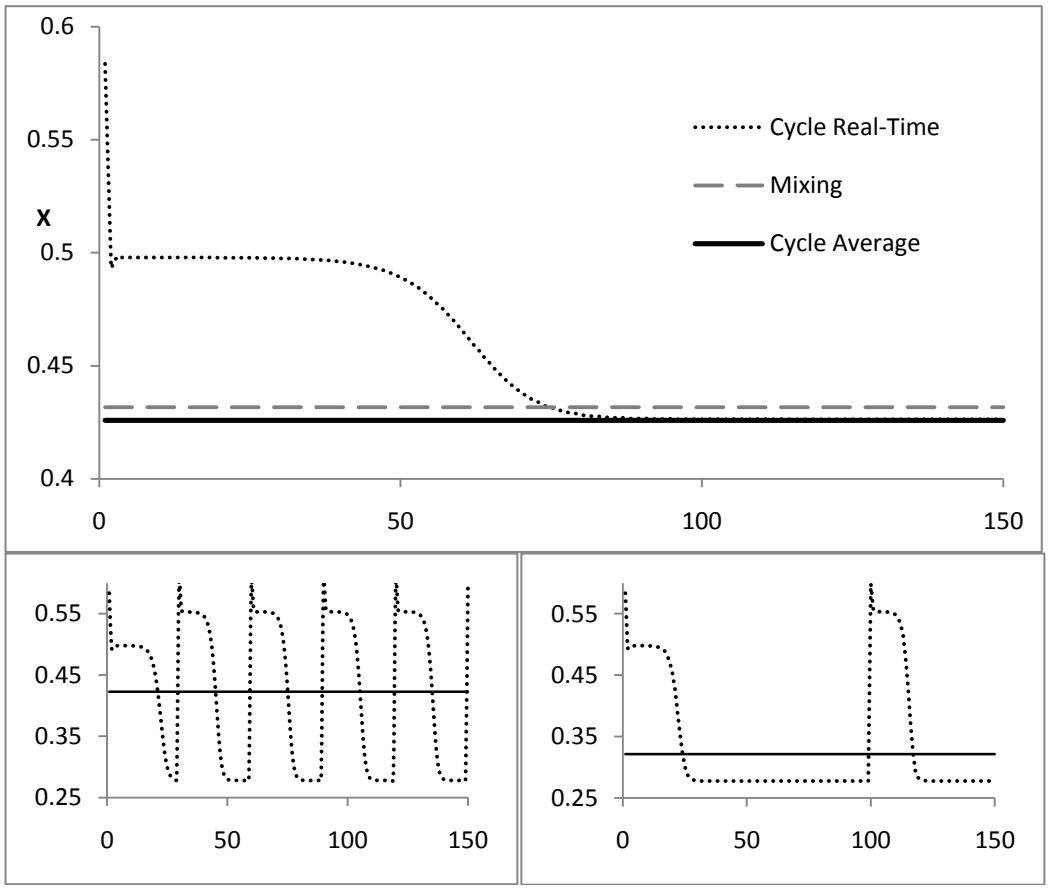

Figure 8: Proportion of uninfected in response to cycling periodicity

Proportion of uninfected patient population in real-time and average over the long term. **(A)** Shows the proportion of uninfected individuals when drugs are switched multiple times each day ($\pi = 0.1$). Note that it asymptotes below the average attained by Mixing. **(B)** Shows the proportion of uninfected individuals when drugs are switched every three weeks ($\pi = 21$). **(C)** Indicates the proportion of uninfected individuals when drugs are switched every 100 days ($\pi = 100$).

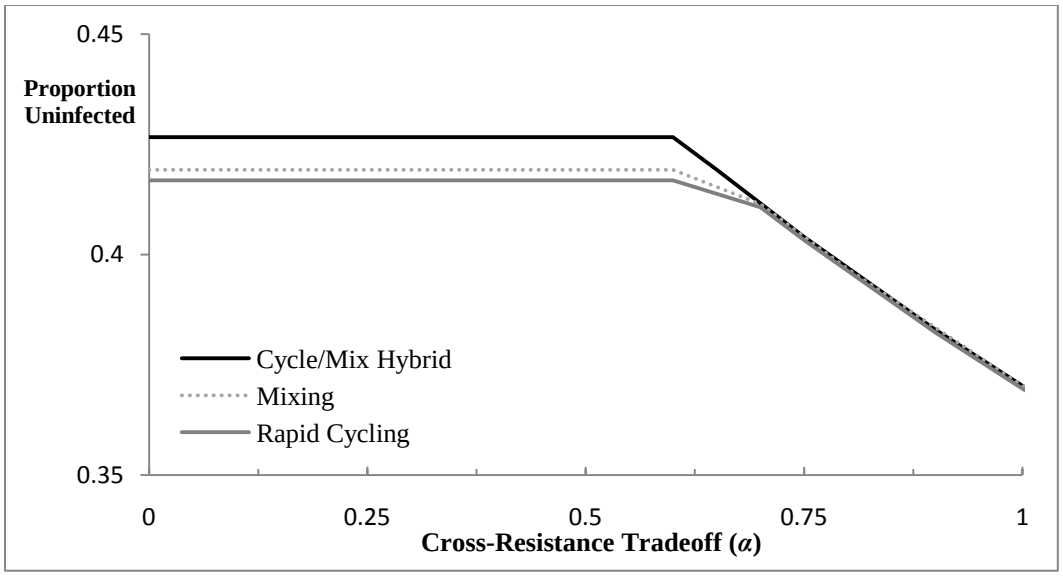

Figure 9: Proportion of uninfected in response to cycling, mixing, and hybrid.

The proportions of each antimicrobial deployment strategy under optimal conditions indicates a clear hierarchy with respect to the uninfected population. Note that the differences shown may be negligible in small hospital wards.

Table 2: Tradeoff thresholds for optimal Mixing & Cycling.

When secondary infections are not taken into account, as is the case with the simplistic fitness model, periodic cycling allows r_2 to achieve dominance with a more readily attained cross-resistance tradeoff (lower α). When the full simulation is taken into account, this difference disappears. The discrepancy highlights the inflated role of secondary infections under the periodic cycling regimen.

$\alpha = \theta_2$	**Cycle**	**Mixing**
Fitness (net growth)	0.500	0.565
Simulation	0.660	0.660
Discrepancy	0.160	0.095

III. Multi-Drug Cocktails and Tradeoff Thresholds

A. Comparison of Multi-Drug Cocktail Strategies

Maximizing antibiotic heterogeneity that nosocomial pathogens experience over time is of crucial significance in regards to curing patients and minimizing resistance. We suggest that forcing a pathogen to cope with the antibiotics of two environments simultaneously may be the optimal path because it fosters intraspecific competition. Therefore, we investigate two competing strategies in order to capture drug pairings which are dependent and independent of each other (Ck_{cmb} and Ck_{sep}, respectively) [Fig 10]. We find that an ICU which employs Ck_{sep} will vastly improve the proportion of uninfected individuals; however, for a given range of tradeoff Ck_{cmb} also represents a significant improvement over previously described strategies $\left(\hat{X}_{ck_{sep}} > \hat{X}_{ck_{cmb}} \gg \hat{X}_{hyb}\right)$ [Fig. 4]. We find that under both cocktail strategies the tradeoff thresholds which allow r_2 to survive (θ_1) and to thrive (θ_2) are lower than previously described strategies. This difference is most pronounced with Ck_{cmb} given that when $\alpha < \theta_2$, r_2's primary competitor is that of the susceptible strain, thereby requiring the least costly cross-resistance tradeoff threshold to dominate.

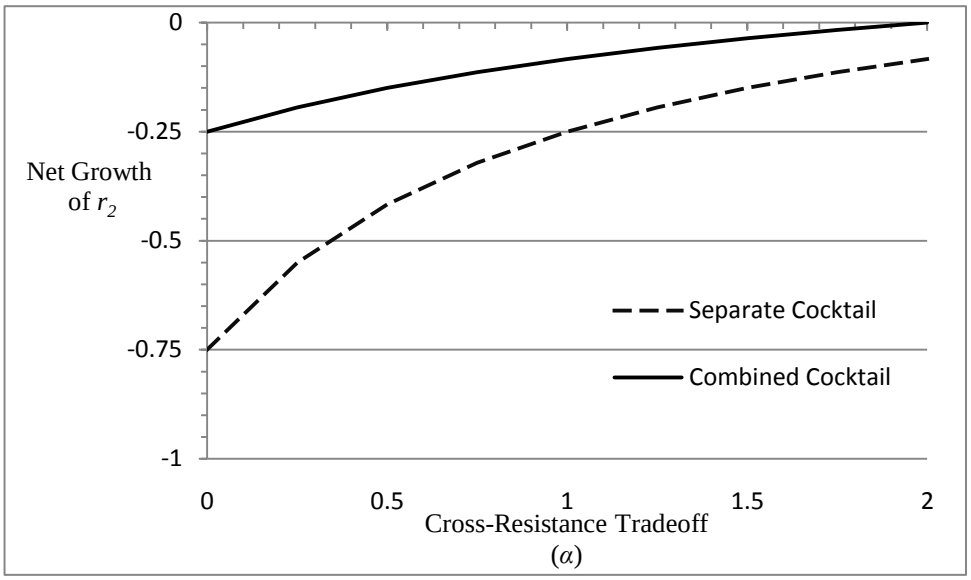

Figure 10: Net growth of multi-resistant genotype in response to tradeoff. Under the separate cocktail, and for all cross-resistance tradeoffs, the net growth rate of the multi-resistant strain is significantly lower than under the combined cocktail. In addition, note the lack of linearity as the tradeoff becomes more costly.

B. Beneficial Cross-Resistance Tradeoffs

In order to algebraically solve for fitness thresholds under the Ck_{cmb} strategy we must first make a few assumptions about the effects of fitness on competition. We assume that, given rough measurements of pharmacodynamic fitness, certain genotypes will be competitively excluded under specific treatment strategies. We also assume that the role of background resistance acquisition at equilibrium is negligible. Finally, we assume that a tradeoff may exist which favors the susceptible over the multi-resistant genotype. If such a tradeoff exists, we ask what value of α is necessary for r_2 to break free of its competitive exclusion by s $\left(\dot{R}_2 > S\,, where\ \dot{S} = 0\right)$. If we use our system of ODEs and invoke the aforementioned assumptions we find that this will occur if the tradeoff is such that

$G_{r_2} > G_s - c[G_s - \gamma + \sigma(\beta - \gamma - G_s)]$. Therefore we can say that only the susceptible genotype is capable of growing when the cross-resistance tradeoff falls below the first threshold $[0 < \alpha < \theta_1]$. Under these conditions, our model is therefore reduced to two populations and is defined by the following system:

$$\hat{X}_{Ck_{cmb}} = \frac{\gamma - G_S}{\beta} \quad \textbf{(1a)}$$

$$\hat{S}_{Ck_{cmb}} = 1 - \hat{X}_{Ck_{cmb}} \quad \textbf{(1b)}$$

$$1 = \hat{X}_{Ck_{cmb}} + \hat{S}_{Ck_{cmb}} \quad \textbf{(1c)}$$

We then simplify and algebraically solve for the uninfected individuals and find that the solution, at equilibrium, exactly replicates the asymptotic proportion of uninfected individuals given by the simulation model.

C. Intermediate Cross-Resistance Tradeoffs

Next, we consider the ranges of cross-resistance tradeoffs which allow for competition between genotypes under the *Ck*$_{cmb}$ treatment regimen [$\theta_1 < \alpha < \theta_2$]. Given the prevalence of competition between genotypes in ICUs, this range of cross-resistance tradeoff is most likely to capture reality. Under these conditions, our system contains three populations of patients and is thus defined by the following algebraic system:

$$1 = \hat{X} + \hat{S} + \hat{R}_2 \quad \textbf{(4a)}$$

$$\hat{S} = \frac{\gamma - \beta\hat{X}(1-c) - G_{R_2}}{\sigma\beta c} \quad \textbf{(4b)}$$

$$\hat{R}_2 = \frac{\gamma - \beta\hat{X} - G_S}{\sigma\beta c} \quad \textbf{(4c)}$$

Again, by ignoring background mutation and assuming equilibrium we can then come to a quadratic solution given by (5).

$$0 = \hat{X}^2[\beta^2(1-c)] + \hat{X}\left[\beta\left(G_S - cG_s + \gamma c - 2\gamma + G_{R_2}\right)\right] - G_{R_2}(G_S + \gamma) - \gamma G_S - \gamma^2\sigma\beta c \quad \textbf{(5)}$$
$$\theta_1 < \alpha < \theta_2$$

The result of this algebraic solution is identical to the simulation outcome for the equivalent range of cross-resistance tradeoffs.

D. Detrimental Cross-Resistance Tradeoffs

Now that we've determined when it is most effective to employ the *Ck*$_{cmb}$ treatment strategy we must also identify the cross-resistance threshold that might render it ineffective or dangerous (θ_2). This would occur when r_2 has a cross-resistance tradeoff such that it can competitively exclude *s*. Put another way: When will α be sufficiently low as to allow *s* to invade an r_2-dominated ICU $\left(\dot{S} > \dot{R}_2\,, where\ \dot{R}_2 = 0\right)$? Using the

same assumptions as for θ_1, we can also solve for θ_2 and find that it occurs when the inequality given by (2) is satisfied.

$$G_{R_2} < \frac{\langle G_S(1-c) + c\gamma - \sigma c\gamma + \sigma c\beta(1-c)\rangle}{1-\sigma c} \quad \textbf{(2)}$$

Thus we can conclude that r_2 will competitively exclude all other genotypes when its tradeoff exceeds the θ_2-threshold. The proportion of uninfected individuals is then given by (3).

$$\lim_{\theta_2<\alpha<2,} \hat{X}_{Ck_{cmb}} = \frac{\gamma - G_{R_2}}{\beta(1-c)} \quad \textbf{(3)}$$

While this strategy achieves a higher proportion of uninfected individuals than other single-drug strategies, note that multi-resistance is fixed beyond this threshold.

IV.

Discussion

Theoretical studies which provided the framework for our research suggest that periodic cycling of antibiotics provides no practical benefit over random mixing. Such a determination is made based on the goal of minimizing the spread of resistant-infected patients. The expansion of this framework in the direction of optimal control theory has shed light on the value of informing the cyclical use of antibiotics via resistance surveillance [5-7]. We feel that our model complements these studies by highlighting the value of multi-drug cocktails through the implementation of concentration-dependent bacterial growth and cross-resistance tradeoffs. We would like to guide the use of optimal cocktail strategies based on the aforementioned fitness thresholds. If system surveillance indicates that multi-resistance is evolving at a high cross-resistance tradeoff, such that $\alpha < \theta_1$, then we would advocate the Ck_{sep} strategy. This would maximize the proportion of uninfected patients with minimal risk of a dominant multi-resistant strain evolving. If surveillance indicates that the tradeoff lies somewhere between the two thresholds, therefore indicating an increased risk of a competitive multi-resistant genotype, it may be best to employ the Ck_{cmb} strategy. If the tradeoff surpasses the threshold which allows competitive exclusion by r_2, more information is needed to discern optimality due to the risk posed by a dominant multi-resistant genotype. However, there is a significant range of fitness tradeoffs where the Ck_{cmb} strategy improves upon time-dependent and probability based strategies.

Clinical manifestations of nosocomial pathogens with variable resistance characteristics are unlikely to manifest identical morbidity and mortality among their hosts. Reliance upon the level of resistance might therefore allow for a situation where the incidence of resistance is low but favors a strain where mortality is all but

guaranteed. When considered, this metric requires increasingly complicated methods of analysis in order for optimality to be discerned in a clinical setting, let alone achieved. We therefore use the proportion of uninfected patients as the standard by which treatment strategies are compared. There is a similar danger with this metric, in that we may optimize the amount of uninfected individuals by employing a strategy which favors multiply-resistant strains; thereby limiting patient recovery due to a lack cost-effective treatment options [16]. Can we accept an antimicrobial deployment strategy which effectively dooms the unlucky few to a grim prognosis that is largely dependent a compromised immune system? Public opinion will likely play a greater role in answering this question than medical professionals and theoreticians. We suggest that the primary means of comparison be based on the proportion of uninfected individuals at equilibrium, but warn that the level of multiple resistance should be carefully considered with respect to the larger community.

Lack of treatment and the indefinite use of a single antibiotic serve as theoretical measurements of worst-case scenarios where disease and resistance are rampant. Time-dependent and probabilistic treatment regimens, such as periodic cycling and random mixing, currently serve as upper bounds to our current clinical capabilities. Our results indicate that in a small hospital ward (<50 patients) random mixing, cycling, or their hybrid are functionally equivalent. We may therefore choose to mimic random mixing in clinical practice; however, random events and human error will prevent us from making it a reality. Likewise, but under cycling, we can appreciate that using a single antibiotic for too long will result in high resistance; but how quickly must we cycle to reap the benefits? While it is theoretically informative

that optimality is reached as the cycle period (π) approaches zero, it is of limited clinical significance because of its logistical and financial implausibility. We must then ask the question: If there is little to no difference under optimal conditions, under which strategy would we most readily approach optimality? The current *modus operandi* in ICUs is to allow the physician to choose the treatment which best fits their patient's symptoms. This lack of top-down coordination closely resembles the hypothetical random mixing strategy. Therefore, it seems that mixing is the most logistically feasible means by which optimality can be approached.

Recent theoretical findings are encouraging in that it seems that the theoretical limit of mixing can be far surpassed when drugs administration is based on system surveillance rather than pre-determined ratios and schedules (Informed Switching Strategies: ISS) [5]. Bonhoeffer *et al.*'s corroborate the prediction of ecological theory which states that maximal environmental heterogeneity is optimal; but also provides a corollary that antibiotic heterogeneity should be maximized with respect to a specific population and its stochastic history. It is important to note that optimal treatment strategies within the nosocomial realm may not be applicable to other human disease. For example, drug resistance from an oncology perspective is of less concern because resistant mutants persist in the environment beyond the lifespan of that patient. In the same respect, the short reproductive timescales of HIV may minimize the benefits of drug cycling strategies. Therefore the path necessary to achieve optimality of a given treatment regimen is dynamic with respect to the stochastic variation of the system as well as the life strategy of the pathogen in question.

Previous population-based models directed to study antibiotic resistance are traditionally based on drug administration rate, and assume clearance on contact. Our model differs by expressing drug-mediated clearance as net bacterial growth; thus allowing for fine tuning of the pharmacodynamic function to simulate a specific drug-pathogen relationship. Fine tuning is essential because the relationship between a pathogen's MIC and the administered drug concentration is extremely complex [16]. Our model is robust to such complexity because its pharmacodynamic function is based on the widely used and deeply explored Hill function (E_{max} models). We represent two forms of cocktails where the pharmacodynamic functions are held separate and combined; thereby representing cocktails whose drug pairs act independently and those which interact. For example, doxycycline (DOX) and ciprofloxacin (CPR) are considered a suppressive drug pair and are thus are considered to interact [22]. Doxycycline (DOX) targets the ribosome to inhibit protein synthesis while ciprofloxacin (CPR) targets cell machinery involved in DNA replication. If a DOX-resistant genotype is faced with CPR, it results in a costly overabundance of ribosomes due to a lack of DNA available for transcription. The effective "de-coupling" of these systems in DOX-r genotypes puts the single-resistant mutants at a competitive disadvantage when compared with the wild-type [22]. Consequently, with this multi-drug pairing a multi-resistant genotype is more likely to be in competition with the susceptible, rather than a singly-resistant one. Our combined cocktail strategy captures the fitness relationship generated by *in vitro* pairings of DOX and CPR.

It was previously thought that the occurrence of multi-resistance in the presence of two selecting agents was of little clinical significance due to the statistical rarity of

two independent mutations which confer resistance factors to different drugs. The extreme selective conditions of an ICU tend to ameliorate the cost of maintaining resistance characters by forcing resistant and wild-type resistant pathogens to compete in antimicrobial-free environments. This occurs most frequently when pathogens are vectored through medical equipment, visitors, and healthcare professionals. Resistance characters are consequently maintained at high allelic frequencies even when spatially and temporally removed from their selecting agent. These conditions, combined with the frequency with which resistance is transmitted by horizontal gene transfer (HGT), have led to high prevalence of multi-resistance with hospital acquired diseases such as tuberculosis. While HGT allows for increased incidence of cross-resistance it occurs by a mechanism which leaves it open to exploitation by carefully selected multi-drug cocktails. For example, the maintenance of resistance factors housed on distinct accessory plasmids may, in some cases, be more costly than one due to the metabolic costs they impose. If so, we can then identify the drug pairings which maximize the fitness tradeoff of cross-resistance, thereby favoring the less specialized genotype.

Multi-drug cocktails are most frequently exploited in HIV, TB, and cancer patients where they place such a stress on the target organism to raise the effective concentration of the pair above the Mutation Prevention Concentration (MPC) [18-20]. This method of treatment uses lower concentrations of individual drugs, thereby decreasing negative side effects, while still maintaining curing efficacy. We found that a multi-drug pair whose components work independently (Ck_{sep}) perform better in terms of maximizing the proportion of uninfected individuals. Conversely, drug pairs which interact (Ck_{cmb}) still provide a significant advantage over mixing and cycling

with the same drugs. Cocktails of the Ck_{sep}-type may not be commonly found for nosocomial infections and thus we expect clinically employed cocktail pairings to lie somewhere between the two strategies in terms of the proportion of uninfected patients $\hat{X}_{ck_{sep}} > \hat{X}_{ck_{clinic}} > \hat{X}_{ck_{cmb}}$.

It is important not to consider Ck_{sep} as a panacea of antibiotic deployment strategies because of its potential to select for a particularly nasty multi-resistant strain. Multi-resistance does not often sweep to fixation, and thus we presume that most tradeoffs *in vivo* would place an r_2 genotype under the θ_2-threshold. On the opposite end of the cocktail spectrum, Ck_{cmb} may pose an increased risk of cross-resistance because it achieves a θ_2-threshold at a much lower cross-resistance tradeoff, thereby capturing the increased likelihood of resistance with similar drugs. The r_2-genotype's increased ability to competitively exclude occurs because sufficiently small values of α allow all genotypes an equivalent net growth rate; however, the susceptible genotype does not incur the cost of resistance and is therefore more fit than resistant genotypes. In order to create an environment which selects for the susceptible strain and maximizes the number of uninfected individuals under the Ck_{cmb} strategy, r_2's cross-resistance tradeoff must fall below the strategy's θ_2-threshold. Note that tradeoffs above this threshold still warrant the use of Ck_{cmb} because it outperforms other strategies. If cross-resistance tradeoffs exist which favor susceptibility over resistance, in the face of effective treatment, then Ck_{cmb}-type drug pairings may prove invaluable in the management of multi-resistance. Thus we are in agreement with a growing chorus of resistance management-minded researchers in that the benefits of maximizing killing power may not balance out the increased risk of resistance [Reede, Kishony,

maybe more?]. Such powerful pairings may pose an unnecessary risk to patients and, due to the prevalence of HGT, the community at large.

The success or failure of a strategy seems largely dependent upon the threshold by which multi-resistant genotypes can competitively exclude other genotypes. Unfortunately, healthcare professionals cannot control the cross-resistance tradeoffs that manifest when multi-resistance evolves. It is not unreasonable to assume that tradeoffs exist over the majority of our tradeoff range; however, certain tradeoffs are more likely to evolve and rise to dominance than others. Our aim is to identify optimal treatment strategies when surveillance informs us of the specific range of cross-resistance tradeoffs in the system. Consequently, we illustrate how these thresholds can be identified without the need for cumbersome simulations. Unfortunately, this method is dependent upon having steady-state equilibria and thus is not readily applied to time-dependent strategies like periodic or informed switching. With regard to the multi-drug cocktail strategies we expect that realistic drug pairings will have thresholds which range between the bounds of Ck_{sep} and Ck_{cmb} ($\theta_2^{Ck_{sep}} > \theta_2^{Ck_{clinic}} > \theta_2^{Ck_{cmb}}$). It would be beneficial to find how various multi-drug treatment pairings, with respect to drug epistasis, impact the position of these thresholds along the tradeoff continuum.

Ideally, we would like to roll back the clock to a time before antimicrobial resistance evolved and approach antimicrobial resistance with "20-20 hindsight." However, evolutionary theory seems to dictate that we may be able to reduce the selective pressure that we apply during treatment while still maintaining treatment efficacy. In order to achieve this, we must first understand and exploit the fitness landscape that we generate through our treatment protocols. Recent theoretical studies

in combination with our own indicate that this may best be achieved through the use of increased surveillance and clinical adaptations of proposed strategies such as Informed Switching Strategies and cocktails. Biotic epistatic drug interactions will play a crucial role in the formation of the fitness landscapes imposed by various drug pairings. Furthermore, these landscapes are in constant flux because of the inordinate strength of stochastic events in hospital wards with small populations. Future research might benefit by focusing on comparisons between multi-drug cocktails and dynamic switching routines in a stochastic context. Finally, it may not be in the best interest of the patient or the community at large to maximize the bactericidal effects of our treatments; perhaps we could help the most patients through the informed direction of intraspecific competition such that it works for us rather than against us.

V.

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