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Dynamics and Control of Antibiotic Resistance in Structured Metapopulations

David L. Smith, Maciej F. Boni, and Ramanan Laxminarayan

Abstract. The evolution of resistance to antimicrobial drugs is a major public health concern. Mathematical models for the spread of resistance have played an important role as a conceptual tool for understanding how and why resistance emerges and spreads. Here, we present a new, general mathematical model for the spread of resistance within a population that accounts for several biologically plausible effects of antimicrobial drug use. Except for the evolution of *de novo* resistance, the model is mathematically identical to Lotka-Volterra competition. The simple model is extended to include the spread of resistance among several patches, and the evolution of multi-drug resistance. The models are used to illustrate some simple ideas about the spatial spread and spatial control of resistance and the evolution of multi-drug resistance.

1. Introduction

General concern about the evolution of resistance to antimicrobial drugs is growing because the frequency of resistant infections has increased [**27, 35**]. During the 1980s and 90s chloroquine resistance was responsible for a global rise in malaria mortality [**28, 35**]. At the same time, vancomycin-resistant enterococci (VRE) spread epidemically among hospitalized patient populations [**9, 27, 29**]. The spread of VRE, called "superbugs" because they were naturally resistant or had acquired resistance to all approved antimicrobials, was accompanied by widespread fear that methicillin-resistant *Staphylococcus aureus* (MRSA) would acquire vancomycin resistance genes from VRE to create another, more virulent superbug, vancomycin-and-methicillin-resistant *S. aureus* (VRSA), an event that occurred in 2003 [**11**]. VRE and the fear of VRSA led to the alarmist speculation that we might be entering a "post-antimicrobial era".

Meanwhile, the rate that new antimicrobials drugs are being approved has declined [**34**]. Although two new drugs were approved for gram-positive bacterial infections (including enterococci and *S. aureus*) in 1999 and 2000, and a handful of new antimicrobial agents are in the pipeline, these new agents do not represent novel mechanisms of action. For now, most malaria, enterococcal, and *S. aureus*

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infections are treatable, but the high frequency of resistance will inevitably lead to some treatment failure when patients take the wrong antimicrobial agent. However, excess morbidity and mortality caused by resistant infections and the slow response of the drug industry to a superbug suggest our efforts to keep up with evolution of resistance through pharmaceutical innovation may be futile and that part of the solution is better management of existing antimicrobials. The underlying cause of the evolution of resistance is the use of antimicrobial drugs – drug pressure creates a niche for resistant bacteria in a population that is being treated [**8**].

The evolution of resistance, defined broadly as the change in the frequency of resistance, involves the emergence of novel resistance and subsequent spread – two different problems with their own conceptual problems. The evolutionary origins of novel resistance are related to mutation and within-host selection [**15**]. Spread is related to human-to-human transmission, the movement of humans, inter- and intra-specific microbial competition, and other aspects of microbial ecology and host immunity. Emergence and spread are complex phenomena that occur within bacterial populations, within and among humans. Such phenomena are difficult to understand and analyze without the use of mathematical models [**10, 22, 26, 36**]. Indeed, VRE and anti-malarial resistance have initiated a new era of research on the population dynamics of drug resistance [**2, 3, 4, 5, 7, 15, 17, 18, 24, 25, 30, 31**]

Once resistance has emerged, public health responses should begin to shift focus from preventing emergence to reducing transmission and limiting spread. The measures to prevent the origins of new resistant pathogens and the spread of existing resistance types may not always be the same. Novel or *de novo* resistance, defined as the emergence of resistance by mutation within a population that was sensitive, encompasses one basic process for eukaryotes, but it can happen in two or more ways in prokaryotes [**20**]. The dominant view of the origins of antimicrobial resistance is that antimicrobials select for pre-existing mutations. High-level resistance may require several mutations, and since each one is rare, the process probably occurs step by step in partially resistant pathogens. Thus, the persistence of these partially resistant pathogens in a population plays a role in the origins of resistance [**16**]. This perspective is sufficient for *Plasmodium* and other eukaryotic pathogens, but bacteria are different [**20**]. In some cases, high-level resistance has emerged in bacteria when genes were acquired from other bacteria [**11**]. Novel resistance from the horizontal transmission of high-level resistance genes requires exposure to the genes as well as selection. Thus, the emergence of high-level resistance in one bacteria species is related to the prevalence of resistance in other species or microbial communities [**30**].

The spread of resistance from host to host is responsible for the majority of resistant infections. Resistant strains can spread in a population, just as their sensitive relatives do, once they have evolved, as long as they are not inhibited from infecting a host by the presence of another genotype. Thus, the underlying ecological model for the evolution of resistance is intra-specific competition [**2, 7**]. The spread of resistance can also play a role that is similar to mutation. As a result of drug chemotherapy, an infection will clear or recrudesce as an infection that remains drug-sensitive if no resistant mutant is present. Exposure to resistant strains just before or during drug treatment can "seed" a resistant infection that will respond as if it were pre-existing mutant. Thus, horizontal transmission of

resistant pathogens is as important to understand as within-host selection. Here, we develop simple models that capture some of these effects.

The amount of antimicrobials used in a population is important, but it is equally important to understand where those antimicrobials are used and how humans move around. Our main focus is on the spread of resistance in structured populations, such as hospitals or networks of rural towns, building on the efforts of others to understand the spread and persistence of resistance to antimicrobial drugs in bacteria [**31**]. We have developed models that focus on the spread of resistance, or epidemiological models [**20**]. Possible candidate organisms for applying these models include *Plasmodium falciparum*, gram positive bacteria that are leading causes of hospital-acquired infections, enterococci, and *S. aureus*. Despite the enormous differences between these different pathogens, there are important similarities in their epidemiology. The immune responses are relatively weak, persistence times are relatively long, and asymptomatic infections are far more common than symptomatic infections. Thus, the pathogen dynamics can be usefully understood with SIS models. One important difference is that the use of antimicrobials to treat VRE and MRSA tends to cause more "collateral damage" by selecting for resistance in non-target bacteria. This is also true for some anti-malarials, but many anti-malarial drugs are used only for infections with plasmodia.

These models are intended to illustrate some general principles that affect the emergence and spread of resistance. We begin by formulating a new, general model for the spread of resistance in well-mixed populations. Next, we extend the model to a spatial context and focus on the spread of resistance among populations. Finally, we consider resistance to two drugs in space, and illustrate some interesting phenomena. The results should be interpreted with circumspection since antimicrobial policy will also be affected by many other concerns.

2. Well-mixed populations

We begin with a model for the spread of resistance in a well-mixed population, and derive equations for the spread of resistance. We assume that individuals are either uninfected, infected by drug-sensitive pathogens, or infected by drug-resistant pathogens. This model posits the strongest form intra-specific competition: no individuals are simultaneously infected with both drug-sensitive and drug-resistant "strains". Let U denote the proportion of patients who are uninfected, W the proportion who are infected by a drug-sensitive strain (wild-type), and X the proportion who are colonized by a drug-resistant strain. We assume the population is constant, so $U = 1 - X - W$.

We assume that populations are locally well-mixed. Let β denote the contact parameter for directly transmitted pathogens and vectorial capacity for vector transmitted pathogens. Let λ denote the rate at which infections clear. With no further assumptions, the dynamics are described by the following coupled differential equations:

$$\begin{aligned} \dot{W} &= \beta W U - \lambda W \\ \dot{X} &= \beta X U - \lambda X. \end{aligned} \tag{2.1}$$

The superdot denotes the derivative with respect to time.

Equations (2.1), are only a common starting point for understanding the evolution of resistance. Total prevalence of the pathogen, $P = W + X$ is described by

a simple equation:

$$\dot{P} = \beta PU - \lambda P \tag{2.2}$$

The basic reproductive number for the pathogen is $R_0 = \beta/\lambda$, and if $R_0 > 1$, prevalence approaches the equilibrium $1 - 1/R_0$. There is no selection for or against resistance in Eqs. 2.1 because drug-sensitive and drug-resistant pathogens are competitively equivalent.

Intra-specific competition is modified by antimicrobial drug use and by antimicrobial resistance. Let ρ denote the rate that people are prescribed antimicrobials, and let ξ denote the fraction of the population under chemo-prophylaxis, defined as having concentrations of the drug that favor drug-resistant strains over drug-sensitive ones (see Appendix 1). Here, treatment and prophylaxis are not directly related to infection status. The assumption reflects the fact that most people who carry VRE, MRSA, or malaria infections are asymptomatic. Moreover, VRE and MRSA carriers are often treated for other infections, and selection for VRE or MRSA is a collateral effect. In areas where malaria is hyperendemic, malaria is often presumed to be the underlying cause of fever, so anti-malarials are often taken without respect to infection status. For malaria, new treatment programs being contemplated deliver anti-malarials to pregnant women and children, without respect to their infection status [**32, 33**]. For these reasons, we have not made the prescription rate a direct function of infection status.

Antimicrobial use and antimicrobial resistance fundamentally change intra-specific competition in one or more of the following ways:

(1) **Treatment and Clearance of Drug Sensitive Pathogens:** Infections clear in those who are undergoing chemo-prophylaxis. Total clearance rates increase to $(\lambda + \nu\xi)W$, where $\nu\xi$ is always less than or equal to ρ, the antimicrobial prescription rate, since if all infections are cleared instantaneously upon starting treatment, then $\nu\xi = \rho$. (The constraint helps to avoid errors in Eqs. 3 when considered in isolation from Eqs. 14).
(2) **Chemo-prophylaxis of Susceptibles:** An obvious effect of taking antimicrobials is that uninfected individuals are protected from infection by drug-sensitive strains. The incidence of infection with drug-sensitive strains is lowered by chemo-prophylaxis to $\beta WU(1 - \xi)$
(3) **Chemo-prophylaxis of Drug-Sensitives:** A secondary effect may be that those who are colonized by drug-sensitive strains do not transmit as efficiently while they are chemo-prophylaxed: shedding from prophylaxed individuals occurs at the rate $\zeta\xi$. Shedding from susceptibles occurs at the rate $(1-\xi+\zeta\xi)W$ shed. Combined with chemo-prophylaxis of susceptibles, the incidence of infection with drug-sensitive strains is lowered further to $\beta(1 - \xi)(1 - \xi + \zeta\xi)WU$. If susceptibles don't shed at all, then incidence is $\beta(1 - \xi)^2WU$.
(4) **Biological Cost of Resistance – Clearance:** A biological cost of resistance is often incorporated into these models by assuming a higher rate of spontaneous clearance for resistant pathogens, $(\phi + \lambda)X$.
(5) **Biological Cost of Resistance – Super-infection:** A biological cost of resistance may allow drug-sensitive strains to displace drug-resistant ones. This allows individuals to convert directly from resistant to sensitive without ever clearing an infection. We assume that this only occurs when

neither host is chemo-prophylaxed. We further assume that the resident has an inherent advantage; the probability of conversion, per contact, is $q \leq 1$. Thus, resistant hosts become sensitive at the rate $q(1-\xi)(1-\xi+\zeta\xi)\beta XW$.

(6) **Transfer of Resistance Factors** *or* **Super-infection under Chemo-prophylaxis:** Drug-sensitive bacteria may acquire resistance factors from drug-resistant ones by gene transfer, for example, on a plasmid. We assume that this transfer is most likely if the host is being treated with antimicrobial drugs. Acquisition of resistance factors would be indistinguishable from super-infection during or just before chemo-prophylaxis that allows drug-resistant pathogens to displace drug-sensitive ones. We assume that this occurs through ordinary contact, and that the probability of conversion by either mechanism is r. Thus, people change status from sensitive to resistant at the rate $r\xi\beta XW$.

(7) **Novel Resistance:** The use of drugs may favor the evolution of resistance within a host, either through mutation or through the inter-specific transfer of high-level resistance factors. People change status from infected with drug-sensitive to drug-resistant pathogens at the rate $c\xi W$.

This list may not include all the advantages or disadvantages of resistance, but it describes several plausible mechanisms. In sum, antimicrobial drug use provides an advantage to resistant strains by reducing transmission and increasing clearance rates of drug-sensitive pathogens. This is countered by a biological cost of resistance that leads to more rapid clearance of resistant bacteria. Finally, drug use can change the rate that people change status without becoming uncolonized through super-infection, from infected with resistant to sensitive, or vice versa.

TABLE 1. State variables and parameters for Eqs. 2.3 and Eqs. 3.1.

U	Proportion of the population that is uninfected
W	Proportion that is infected with sensitive pathogens
X	Proportion that is infected with resistant pathogens
β	Contact parameter
ξ_a	Proportion of the population that is prophylaxed by antibiotic a
ν	The proportion of prophylaxed populations that clear infections
λ	The rate that infections are naturally cleared
ϕ	Cost of resistance, higher clearance
q, r	Probability of displacement by superinfection
ζ	Reduced transmission by prophylaxed susceptibles
c	Probability that treatment leads to *de novo* resistance
$1/\sigma_i$	Average time spent in i^{th} population
$\psi_{i,j}$	Proportion of immigrants to i^{th} population that come from the j^{th} population

Incorporating all these effects gives the more complicated equations describing local competition under some specific level of antimicrobial drug use:

$$\begin{aligned}\dot{W} &= \beta(1-\xi)(1-\xi+\zeta\xi)WU + (q(1-\xi)(1-\xi+\zeta\xi) - r\xi)\,\beta XW \\ &\quad - (\lambda+\nu\xi)W - c\xi W \\ \dot{X} &= \beta XU - (q(1-\xi)(1-\xi+\zeta\xi) - r\xi)\,\beta XW - (\lambda+\phi)X + c\xi W.\end{aligned} \tag{2.3}$$

A slightly more formal derivation of these equations is provided in Appendix 1.

Importantly, in the new environment the basic reproductive numbers for each type change, depending on the amount of antimicrobial drug that is used.

$$\begin{aligned}\mathcal{R}_{0,w} &= \frac{\beta(1-\xi)(1-\xi+\zeta\xi)}{\lambda+\nu\xi} \\ \mathcal{R}_{0,x} &= \frac{\beta}{\lambda+\phi}\end{aligned} \tag{2.4}$$

We denote the basic reproductive number in the new environment with a different script and a subscript for each strain, to distinguish it from the basic reproductive number of the wild-type in an untreated population R_0

One could consider the susceptible population as a resource, and treat the dynamics as an example of "resource-based competition." The competitive dynamics would be entirely determined by the basic reproductive number for each strain in a particular treatment environment. $\mathcal{R}_{0,w}$ and $\mathcal{R}_{0,x}$. The pathogen with the highest $\mathcal{R}_{0,s}$ would also have the highest carrying capacity and would draw the susceptible population below the other strain's threshold level and prevent it from invading.

Super-infection makes this sort of analysis incorrect because coexistence is determined by the ability of each strain to invade the other at carrying capacity, and each strain is able to use the other pathogen as a resource. For example, the resistant strain can invade all susceptibles as well as sensitives, $U+r\xi W$, while persistence times, the average waiting time until a strain is either cleared or replaced, are shorter, approximately $1/(\lambda+\phi+q(1-\xi)(1-\xi+\zeta\xi)W)$. Similarly, the resource for sensitive strains invading a population is $U+q(1-\xi)^2X$, and persistence times are approximately $1/(\lambda+\nu\xi+r\xi X)$. Thus, it is no longer possible to say which pathogen will be dominant by simply finding the one with the highest $\mathcal{R}_{0,s}$.

Goldilocksian Coexistence. The dynamics of this system are transparent once Eqs 2.3 are rewritten in the following way:

$$\begin{aligned}\dot{W} &= r_w W\left[1-\left(W+\alpha_w X\right)/K_w\right] - c\xi W \\ \dot{X} &= r_x X\left[1-\left(X+\alpha_x W\right)/K_x\right] + c\xi W\end{aligned} \tag{2.5}$$

In Eqs 2.5, the parameters are recast as maximum growth rates, carrying capacities and competition coefficients (Table 2). In other words, ignoring the background evolution of novel resistance (i.e. assuming $c=0$), the underlying dynamics are mathematically equivalent to the well-understood Lotka-Volterra competition equations.

Ignoring the evolution of novel resistance (i.e. $c=0$) antimicrobial drug use reduces the prevalence of sensitive pathogens from $1-1/R_0$ to K_w, if resistance never appears. If $K_x-\alpha_x K_w>0$, resistance will eventually be able to invade once it appears. If resistance is already present at a low frequency, the frequency will not begin to increase until antimicrobials reduce the prevalence of the sensitive

TABLE 2. The coefficients when Eqs. 2.3 are rewritten as Lotka-Volterra competition equations.

$i=$	w	x
r_i	$\beta(1-\xi)(1-\xi+\zeta\xi)-\nu\xi-\lambda$	$\beta-\phi-\lambda$
K_i	$1-1/\mathcal{R}_{0,w}$	$1-1/\mathcal{R}_{0,x}$
α_i	$1-q+\frac{r\xi}{(1-\xi)(1-\xi+\zeta\xi)}$	$1+q(1-\xi)(1-\xi+\zeta\xi)-r\xi$

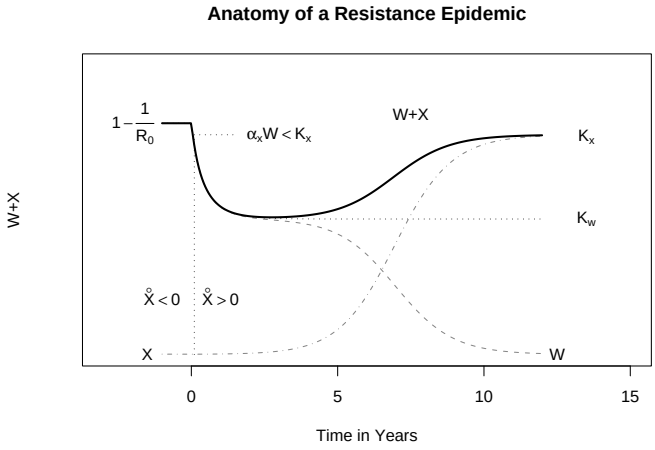

FIGURE 1. The anatomy of an epidemic of resistance. Before antimicrobials, the prevalence of a sensitive phenotype is assumed to be at equilibrium, $1-1/R_0$. Once drug use starts, at time $t=0$, the prevalence of sensitive bacteria declines and would eventually reach a new equilibrium, K_w, if resistance never invaded (the dotted line shows W without competition from X). Once the prevalence of sensitive bacteria decline below a threshold ($W<K_x/\alpha_x$), the prevalence of resistance (dot-dash, X) begins to increase (i.e. $\dot{X}>0$) and approaches a new equilibrium, K_x . The frequency of resistance is initially rare, but in this case, it eventually goes to fixation and drives the sensitive bacteria extinct (dashed lines show W with competition). Total prevalence ($W+X$, solid dark line) drops when the antimicrobial is initially introduced, but eventually rebounds. The parameters and initial conditions are the following: $q=r=\lambda=1/500$ days, $\phi=\lambda/5$, $\xi=3\%$, $\zeta=0$, $\nu=0.1$, $R_0=5$, $W(-365)=K_w$, and $X(-1)=0.001$ or 0.

phenotype to $W<K_x/\alpha_x$. If $K_w-\alpha_w K_x<0$, the resistant types will increase to a new equilibrium K_x and the sensitive phenotypes will be eliminated (Figure 1).

Coexistence requires a Goldilocksian balance:

$$\alpha_x<K_x/K_w<1/\alpha_w \tag{2.6}$$

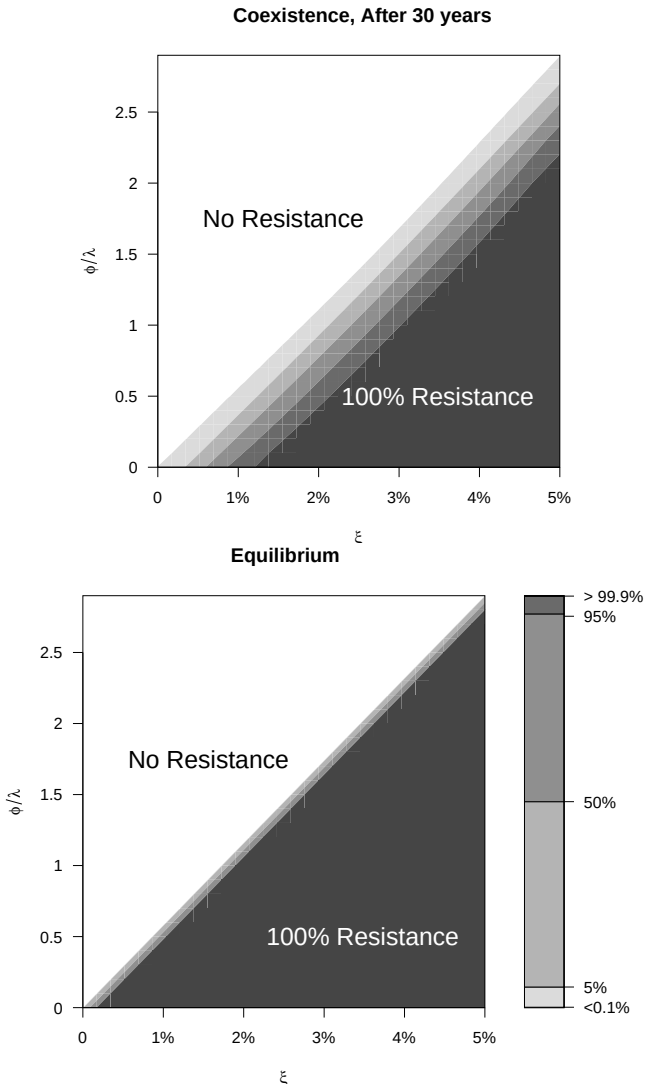

FIGURE 2. Coexistence (light gray) depends on a Goldilocksian balance between the rate of antimicrobial drug use and the cost of resistance, but when generation times ($1/\lambda$) are long, two strains can coexist for a very long time. The colors indicate the frequency of resistance–the darker, the higher frequency of resistance (see the key). Here, we've plotted the frequency of resistance after 30 years, and the equilibrium.

It is possible to translate this into a formula related to the proportion of the population under chemo-prophylaxis, ξ, but the resulting expressions are complicated. Crudely summarized, if selection pressure is too strong, sensitive phenotypes will be eliminated. If selection pressure is too weak, resistance will remain absent. Coexistence does not occur unless selection pressure is just right (Figure 2).

In natural populations, resistance has rarely become fixed. One explanation for coexistence is that the biological cost of resistance is extremely high. Some circumstantial evidence undermines this hypothesis, for a very high biological cost would be relatively easy to measure. A high biological cost of resistance has been reported relatively rarely [**1**]. One possible reason for a low biological cost of resistance is that compensatory mutations can arise that minimize the biological cost of resistance [**21**].

An alternative hypothesis is that coexistence is a transient phenomenon–resistance has had insufficient time to become fixed (Figure 2b). This sort of transient resistance also requires that the rate of antimicrobial use be delicately balanced, although the constraints are not quite as Goldilocksian (Silverlocksian?).

Another mechanism that could also explain coexistence is that prescription rates adjust to the frequency of resistance, for example, patients switch to another drug or avoid treatment when resistance becomes very frequent, coexistence would be more robust. An alternative explanation, explored below, is population heterogeneity.

3. Resistance in Structured Populations

The rate of antimicrobial drug use and local transmission can vary, with important implications for the dynamics and control of resistance. To understand epidemics in structured populations, we extend the previous model to link several locally well-mixed populations. Let subscript i denote the i^{th} population, and let $1/\sigma_i$ denote the average length of stay in the i^{th} population. We have assumed that the size of each local population remains constant, so every individual who leaves one population is replaced by an arrival from elsewhere. Let $\psi_{i,j}$ denote the proportion of all immigrants (sensitive or resistant) to population i that come from population j. The migration fractions are implicitly related to the relative population sizes and the migration rates between each pair of populations (see Appendix 2).

We assume that transmission rates (β_i) and the proportion chemo-prophylaxed (ξ_i) can vary from place but other parameters are fixed, no matter where a person resides at the time. The local dynamics are described by the following:

$$\begin{aligned}
\dot{W}_i =& \beta_i(1-\xi_i)^2 W_i U_i + \left(q(1-\xi_i)^2 - r\xi_i\right)\beta_i X_i W_i \\
& - \nu\xi_i W_i - \lambda W_i - c\xi_i W_i - \sigma_i(W_i - \sum_j \psi_{i,j} W_j)) \\
\dot{X}_i =& \beta_i X_i U_i - \left(q(1-\xi_i)^2 - r\xi_i\right)\beta_i X_i W_i \\
& - (\phi+\lambda)X_i + c\xi_i W_i - \sigma_i(X_i - \sum_j \psi_{i,j} X_j).
\end{aligned} \tag{3.1}$$

Spatial Coexistence. Coexistence between sensitive and resistant phenotypes is relatively easy when migration rates are very low and antimicrobial use is heterogeneous. For example, consider a two-patch model where no antimicrobials are used in patch one, but antimicrobial use in patch two is high enough to fix resistance. Coexistence is trivial if the patches remain separated. With high migration rates, the population behaves as if well-mixed, with respect to coexistence. The amount of migration required to undermine this spatial coexistence is surprisingly small in the two-patch model (Figure 3).

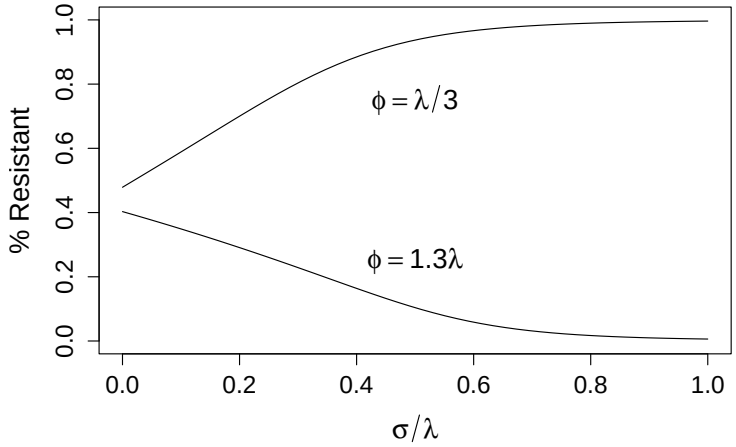

FIGURE 3. Spatial heterogeneity in antimicrobial use promotes coexistence when migration rates are low. Here, the equilibria are plotted as a function of the migration rate. For these parameters, the population is approximately well-mixed when migration occurs on approximately the same time scale as clearance.

An alternative explanation is that the human population is composed of many subgroups that vary in the amount of time spent in the prophylaxed location. For example, the elderly population spend more time, on average, than the non-elderly in hospitals and long-term care facilities [**31**]. Those who are frequently hospitalized play a role in the spread of antimicrobial resistant hospital-acquired infections that is analogous to those who are most sexually active in spreading sexually-transmitted diseases.

The lessons learned from hospital-acquired infections may be played out in structured populations where the sub-populations have a spatial relationship. A simple illustration of the principle is the frequency of resistance on an array (Figure 4). To keep the point as simple as possible, we allow two patches to be treated, but we vary the distance separating the treated patches. When the two treated patches are close together, individuals who have acquired resistance in one patch are more likely to enter the other, where they continue to transmit. Thus, the closer two patches are to one another, the more they amplify each other. A similar phenomenon happens at the edge, where we assume that no individuals leave at the edges so individuals are more likely to return to the treated patch. To put it simply, the prevalence of antimicrobial resistance in one subpopulation is affected by the rate of antimicrobial use in surrounding populations [**31**].

The spread of resistance in one relatively simple spatial network was described as a part of a study in Tanzania – the prevalence of resistance in two treated areas, and in surrounding areas provide some evidence that these principles are at work in real populations [**12**]. Enzi, an untreated town between two treated towns had a higher frequency of resistance than the treated towns, or than any of the untreated

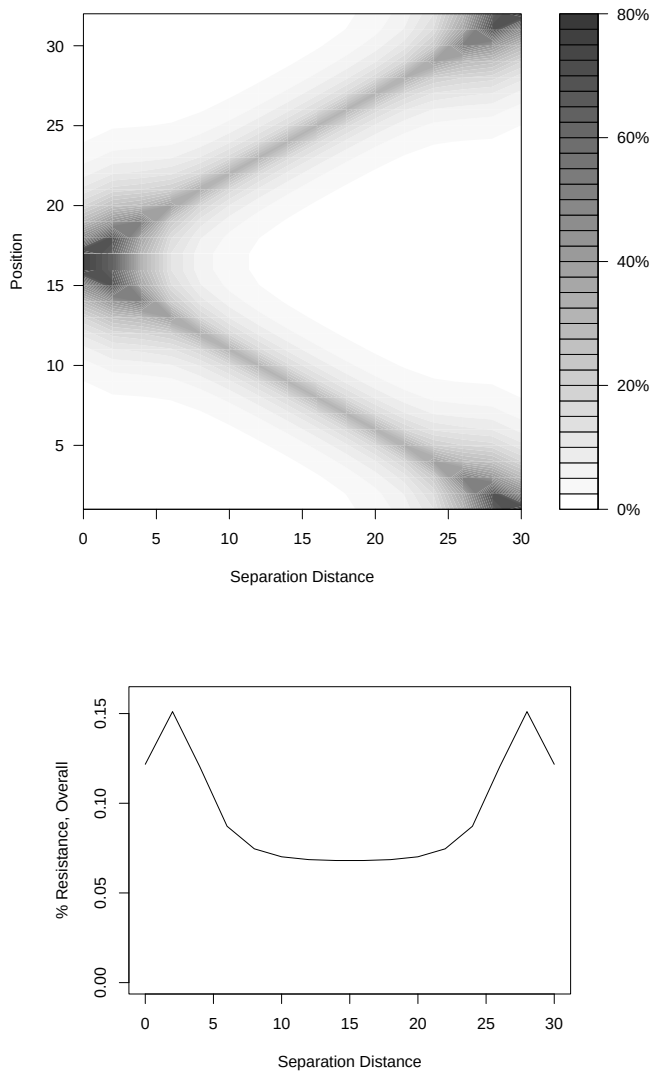

FIGURE 4. The frequency of resistance is higher when some hosts spend more time in populations where antimicrobials are heavily used. In the top graph, we show the frequency of resistance simulated on an array where two treated patches surrounded by untreated patches were separated by the indicated distance (vertical transects). When the patches are close to one another, hosts infected with resistant phenotypes are more likely to re-enter a treated patch. When the patches are near the edge, a similar phenomenon occurs because of a reflecting boundary condition. Curiously, resistance overall is lower when the two patches are exactly adjacent or at the very edge because the effects of spillover on the adjacent, untreated populations are limited. The model is available upon request.

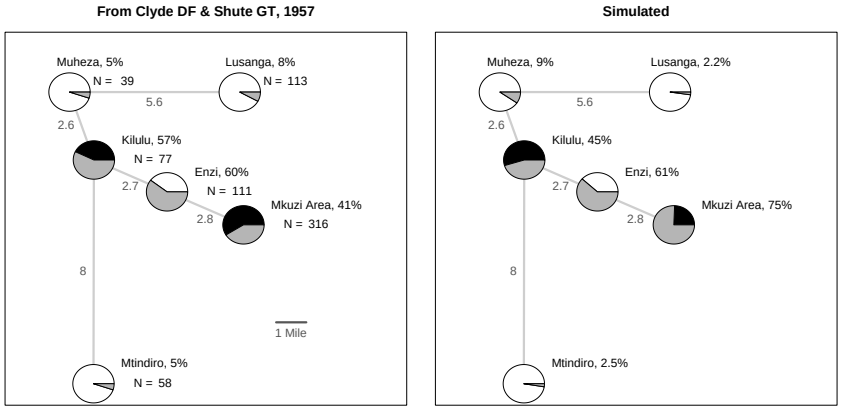

FIGURE 5. The frequency of resistance in Enzi (left) and in a simulation (right). Dosing with pyrimethamine in two cities (black background) led to high frequency of resistance (grey). Surrounding, untreated cities (black background) generally had much lower resistance, suggesting limited spread. The exception was Enzi, situated between the two treated populations, where the high frequency of resistance suggested spread of resistant phenotypes from the flanking treated populations. No resistance was found in Pongwe 12 miles east ($N = 48$), or in a northwest belt 5–9 miles away ($N = 65$). The gray lines show the roads connecting the towns at the time of the study. right) Some of these patterns can be reproduced by trial and error. The model is available upon request.

towns nearby (Figure 5, left). By trial and error, we found migration parameters and treatment frequencies that generated prevalence patterns that were close to those in the study (Figure 5, right). Try as we might, we could not find parameters that made the frequency of resistance for untreated Enzi higher than the treated cities flanking it. Mkuzi is a large and heterogeneous area. It is possible that treatment rates were very high in those parts of Mkuzi that were near Enzi but lower further away, but resistance was reported for the aggregated Mkuzi population. This is entirely speculative, but a relatively simple structured model does approximately reproduce the observed patterns.

The same principles apply to more complicated networks of interacting populations, including the flows of people on landscapes, the flow of patients among health-care institutions, and the flow of patients within a hospital.

4. Multi-drug Resistance in Structured Populations

The lessons from structured populations have an important applications antimicrobial drug policies. The previous results suggest the decision of what drug to recommend will depend, in part, on the frequency of resistance and antimicrobial resistance in neighboring populations. A primary concern here is the evolution of multi-drug resistance (MDR). Recent models for the evolution of MDR have focused on developing strategies to prevent the rapid evolution of MDR-strains [**6, 7**].

While some of these models have managed to simplify the dimensionality of such systems substantially, it is not always clear that such reductions in model size are desirable and that lower-dimensionality models are good approximations of the true models. To illustrate the complexity of MDR-models, we present a summary of the variables necessary to model a human-bacterial interaction with two antimicrobials. In the appendix, we describe some common approximations. Finally, we present some results on antimicrobial prescribing strategies in a structured population.

To model the different chemo-prophylaxis scenarios with two antimicrobials, we need to break up the host population into four population classes. We call our two antimicrobials x and y, and we denote by subscripts which type of chemo–prophylaxis a host is undergoing, if any. For example, a host colonized by the wild-type and currently taking antimicrobial y will be in population class W_y. We use the letter z to denote both antimicrobials; hosts in the class W_z are prophylaxed by antimicrobials x and y simultaneously. Subscript n indicates no prophylaxis.

To determine infection status, we need a further sub-division into five population classes: one class for uncolonized individuals, and four classes for the four possible types of resistance in the bacteria. Hosts in the class U are currently uncolonized; hosts in W are colonized by the wild-type strain; hosts in X and Y are colonized by a strain resistant to antimicrobial x or y, respectively; and, hosts in the population class Z are colonized by a bacterial strain resistant to both antimicrobials x and y. Five classes for infection status and four classes for prophylaxis status result in an unwieldy 20 population classes (see Table 3). The full model with 20 differential equations can be seen in Appendix 3. Below we present a lower-dimensional, collapsed version of this model.

TABLE 3. Asterisks (*) denote individuals who are effectively prophylaxed. These hosts cannot shed/transmit their pathogen; their microbial populations can evolve resistance if these hosts come into contact with another host infected with a strain resistant to their antimicrobial.

col. strain / proph. state	not proph.	proph. by ab x	proph. by ab y	proph. by abs x and y
none	U_n	U_x	U_y	U_z
wildtype, ab-sensitive	W_n	W_x (*)	W_y (*)	W_z (*)
resistant to ab x	X_n	X_x	X_y (*)	X_z (*)
resistant to ab y	Y_n	Y_x (*)	Y_y	Y_z (*)
resistant to abs x and y	Z_n	Z_x	Z_y	Z_z

We set $q = 0$, so that we do not have reversion to sensitives via a superinfection mechanism, $c = 0$ so that there is no evolution of novel resistance, and $r = 1$ so that drug-resistant strains can always invade prophylaxed hosts colonized by a sensitive population. We once again compartmentalize our host population by their treatment status. We say that a fraction ξ_n are not prophylaxed, a fraction ξ_x are prophylaxed by antimicrobial x, a fraction ξ_y are prophylaxed by antimicrobial y, and a fraction ξ_z are prophylaxed by both antimicrobials; $\xi_n + \xi_x + \xi_y + \xi_z = 1$.

This method allows us to make the same ξ-like approximation that is presented in Appendix 1.

Dimensionality reduction in our dynamical system relies on collapsing the infected classes into forces of infection and the susceptible classes into effective susceptible populations as has been done elsewhere [**13, 14, 23**]. The forces of infection are defined as

$$\begin{aligned}\Lambda_w &= \beta W_n \\ \Lambda_x &= \beta(X_n + X_x) \\ \Lambda_y &= \beta(Y_n + Y_y) \\ \Lambda_z &= \beta(Z_n + Z_x + Z_y + Z_z).\end{aligned} \tag{4.1}$$

Let Q_s be the class susceptible to the strain s. Then,

$$\begin{aligned}Q_w &= U_n \\ Q_x &= U_n + U_x + W_x + Y_x \\ Q_y &= U_n + U_y + W_y + X_y \\ Q_z &= U_n + U_x + U_y + U_z + W_x + W_y + W_z + X_y + X_z + Y_x + Y_z\end{aligned} \tag{4.2}$$

are the four effective susceptible classes as perceived by each of the four pathogenic strains. After some rearranging and approximating (Appendix 3), the 8 classes yield the closed dynamical system

$$\begin{aligned}\dot{Q}_w =& -Q_w(\Lambda_w + \Lambda_x + \Lambda_y + \Lambda_z) \\ &+ \frac{\lambda}{\beta}\left(\Lambda_w + \frac{\lambda+\phi_1}{\lambda}\frac{\xi_n}{\xi_n+\xi_x}\Lambda_x + \frac{\lambda+\phi_1}{\lambda}\frac{\xi_n}{\xi_n+\xi_y}\Lambda_y + \frac{\lambda+\phi_2}{\lambda}\xi_n\Lambda_z\right) \\ \dot{Q}_x =& -Q_x(\Lambda_x + \Lambda_z) - Q_w(\Lambda_w + \Lambda_y) \\ &+ \frac{\lambda}{\beta}\left(\Lambda_w + \frac{\lambda+\phi_1}{\lambda}\Lambda_x + \frac{\lambda+\phi_1}{\lambda}\frac{\xi_n}{\xi_n+\xi_y}\Lambda_y + \frac{\lambda+\phi_2}{\lambda}(\xi_n+\xi_x)\Lambda_z\right) \\ \dot{Q}_y =& -Q_y(\Lambda_y + \Lambda_z) - Q_w(\Lambda_w + \Lambda_x) \\ &+ \frac{\lambda}{\beta}\left(\Lambda_w + \frac{\lambda+\phi_1}{\lambda}\frac{\xi_n}{\xi_n+\xi_x}\Lambda_x + \frac{\lambda+\phi_1}{\lambda}\Lambda_y + \frac{\lambda+\phi_2}{\lambda}(\xi_n+\xi_y)\Lambda_z\right) \\ \dot{Q}_z =& -Q_z\Lambda_z - Q_y\Lambda_y - Q_x\Lambda_x - Q_w\Lambda_w \\ &+ \frac{\lambda}{\beta}\left(\Lambda_w + \frac{\lambda+\phi_1}{\lambda}\Lambda_x + \frac{\lambda+\phi_1}{\lambda}\Lambda_y + \frac{\lambda+\phi_2}{\lambda}\Lambda_z\right) \\ \dot{\Lambda}_w =& \beta Q_w\Lambda_w - \lambda\Lambda_w \\ \dot{\Lambda}_x =& \beta Q_x\Lambda_x - (\lambda+\phi_1)\Lambda_x \\ \dot{\Lambda}_y =& \beta Q_y\Lambda_y - (\lambda+\phi_1)\Lambda_y \\ \dot{\Lambda}_z =& \beta Q_z\Lambda_z - (\lambda+\phi_2)\Lambda_z,\end{aligned} \tag{4.3}$$

where ϕ_1 is the cost of resistance (i.e. higher clearance) of the singly-resistant strains X and Y, and ϕ_2 is the cost of resistant of the doubly-resistant strain Z.

These equations have six free parameters (we can scale out λ), and they can serve as a useful guide as to how the microbial population structure would respond to various antimicrobial-prescribing strategies. The equations also allow us to approximate basic reproduction ratios for the four strains, relative to that for the

wild-type in an untreated population, $R_0 = \beta/\lambda$. In a different environment defined by some treatment rates, the basic reproductive numbers of the pathogens are:

$$\begin{aligned}
\mathcal{R}_{0,w} &= R_0\xi_n, \\
\mathcal{R}_{0,x} &= R_0(\xi_n + \xi_x)\frac{\lambda}{\lambda+\phi_1}, \\
\mathcal{R}_{0,y} &= R_0(\xi_n + \xi_y)\frac{\lambda}{\lambda+\phi_1}, \\
\mathcal{R}_{0,z} &= R_0(\xi_n + \xi_x + \xi_y + \xi_z)\frac{\lambda}{\lambda+\phi_2} = R_0\frac{\lambda}{\lambda+\phi_2}.
\end{aligned} \tag{4.4}$$

Notice that the basic reproductive number for each type is lower than R_0, but the highest $\mathcal{R}_{0,s}$ varies, depending on the amount and type of antimicrobial being used. The biological cost of resistance is assumed to increase with the number of drugs to which the pathogen is resistant. Countering this cost, the population that is susceptible to infection increases with the number of antimicrobials to which a strain is resistant. Since MDR strains are resistant to every antimicrobial, they can infect any individual. These values underline the important effect prophylaxis and treatment can have on the rates of spread of the antimicrobial-sensitive pathogens.

MDR Dynamics in Space. We now consider the spread of resistance in two equally sized patches, or spatial locations (named 1 and 2) to illustrate how antimicrobial prescribing strategies and resistance patterns vary in the simplest spatial model. Instead of eight state variables we now need sixteen; we call them $Q_{w,1}, \Lambda_{y,2}$, etc. Our Q-equations will now look like

$$\dot{Q}_{w,1} = -Q_{w,1}(\Lambda_{w,1} + \Lambda_{x,1} + \Lambda_{y,1} + \Lambda_{z,1}) + \frac{\lambda}{\beta}(\cdots) + \sigma(Q_{w,2} - Q_{w,1}). \tag{4.5}$$

Similarly, our Λ-equations will be

$$\dot{\Lambda}_{y,1} = \beta Q_{y,1}\Lambda_{y,1} - (\lambda+\phi_1)\Lambda_{y,1} + \sigma(\Lambda_{y,2} - \Lambda_{y,1}). \tag{4.6}$$

Note that now we will have eight parameters (six free parameters) describing prescribing frequencies: $\xi_{n1}, \xi_{x1}, \xi_{y1}, \xi_{z1}$ in patch 1 and $\xi_{n2}, \xi_{x2}, \xi_{y2}, \xi_{z2}$ in patch 2.

Treatment strategies for MDR-models specify what fraction of the population is treated, as well as how the different antimicrobials will be distributed among treated hosts. Some common multi-drug treatment strategies are (1) load balancing (also called 50-50 treatment [**7**] and mixing [**6**]), where half of the treated hosts are given antimicrobial x and the other half are given antimicrobial y; (2) combination therapy, where all treated hosts are given both antimicrobials simultaneously; and (3) sequential treatment or antimicrobial cycling, where hospitals treat all hosts with one antimicrobial for a given period of time, then switch to the second for some time, switching back and forth between two or cycling through three or more. Single-drug treatment is of course also an option. In the two-patch model, we will consider the case where antimicrobial x is used at one location and antimicrobial y in the other; this can be thought of as "load balancing in space".

Sequential treatment is believed to be the poorest strategy [**6, 7**], in that it drives the evolution of double-resistants the most quickly. Load-balancing and combination therapy are slightly better, though combination therapy puts more "direct" favorable selection pressure on the double-resistants, while a load-balancing

strategy puts indirect selection pressure on the double-resistants. Load-balancing in space has not yet been studied. We present two simple examples of spatial treatment regimes and their resulting bacterial strain structures.

Load Balancing in Space. We consider a scenario of two hospitals where patients sometimes get transferred from one to the other, or two cities between which individuals frequently migrate. Patch 1 chooses to use antimicrobial x to treat all its patients, while patch 2 uses antimicrobial y.

When there is no migration between the two patches (hospitals), single-drug use will drive the evolution of single-resistants. If treatment levels are high enough, strain X will fix in patch 1 and strain Y will fix in patch 2. In this scenario, when we allow individuals to migrate between patches, these fixation dynamics can change. From the perspective of hosts in the W - and Z-classes, the patch these hosts occupy is irrelevant since treatment in both patches is effective against hosts infected with the wild type, and ineffective against hosts infected with the double-resistant. However, hosts infected with a strain resistant to only one antimicrobial see the two patches quite differently. In patch 2, for example, strain Y will dominate and eventually fix; if a host from patch 2 carrying a strain resistant to antimicrobial y migrates to patch 1, his potential susceptible pool changes from all hosts in patch 2 to a fraction ξ_{n1} of the hosts in patch 1. The fraction ξ_{x1} of hosts in patch 1 who are prophylaxed by antimicrobial x will not be able to contract an infection from the new immigrant since his strain is susceptible to the antimicrobial x. This means that migration is detrimental to the single-resistant strains.

This effect can be seen in Figure 6. Here we chose $\beta = 2$, $\lambda = 1$, $\zeta = 0$, $\phi_1 = 1/4$, and $\phi_2 = 1/2$, so that in the absence of antimicrobial treatment, the strains' basic reproduction ratios are $\mathcal{R}_{0,w} = 2$, $\mathcal{R}_{0,x} = \mathcal{R}_{0,y} = 8/5$, and $\mathcal{R}_{0,z} = 4/3$. In patch 1, we designate the single-drug treatment regime via $\xi_{n1} = 2/3$ and $\xi_{x1} = 1/3$; in patch 2, we have $\xi_{n2} = 2/3$, and $\xi_{y2} = 1/3$, so that $2/3$ of all hosts remain non-prophylaxed while $1/3$ receive single-drug treatment with the drug depending on their location. Under these treatment frequencies, the replacement numbers is patch 1 are

$$\mathcal{R}_{0,x} = 8/5 \quad > \quad \mathcal{R}_{0,w} = \mathcal{R}_{0,z} = 4/3 \quad > \quad \mathcal{R}_{0,y} = 16/15,$$

and in patch 2,

$$\mathcal{R}_{0,y} = 8/5 \quad > \quad \mathcal{R}_{0,w} = \mathcal{R}_{0,z} = 4/3 \quad > \quad \mathcal{R}_{0,x} = 16/15.$$

When the $\mathcal{R}_0$-values are ordered in this way, it becomes clear why migration is unfavorable to the single-resistants. They have the highest basic reproduction ratio in one patch and thus increase in relative frequency in this patch, but upon migration they have the lowest reproduction ratio in the other patch and are out-competed by the other three strains.

In Figure 6, we see the slow increase and then decrease in frequency of the single-resistant strains. The remarkable result in this simple model setup is that the addition of spatial structure causes a population-wide reversion to the wild-type strain, a demonstration that load balancing in space can reduce resistance. Without spatial structure, these parameter values would allow coexistence of all strains.

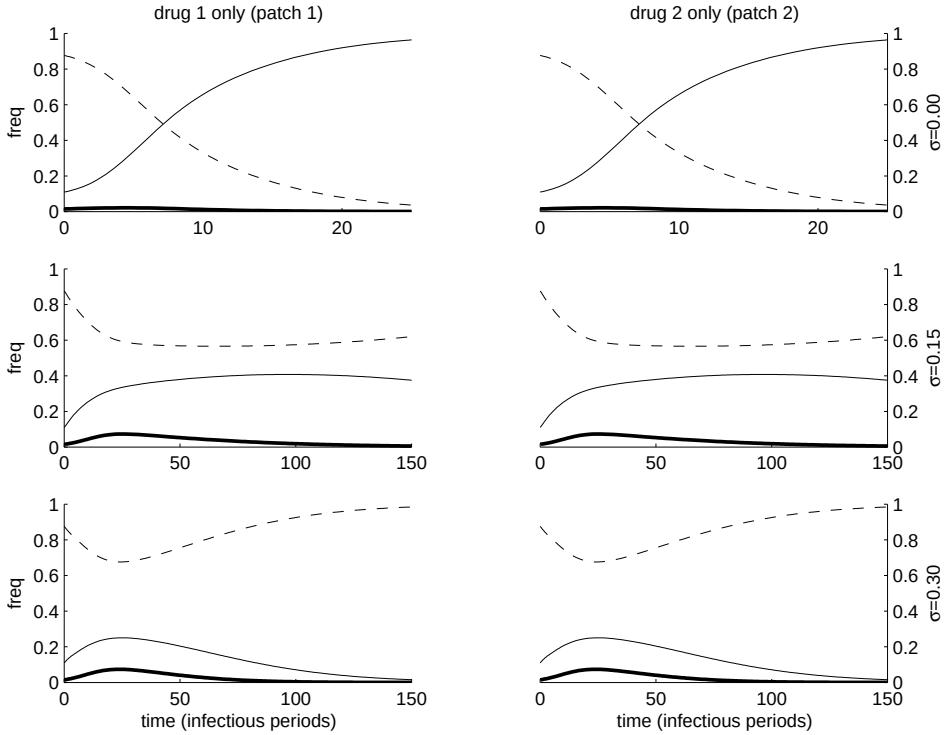

FIGURE 6. Load Balancing in Space. Dashed line represent the frequency of the wild type, solid lines the frequency of single-resistants, and the thick line represents the frequency of double-resistant strains (strength of line indicates strength of resistance). In all figures, $\beta = 2, \lambda = 1, \phi_1 = 1/4, \phi_2 = 1/2, \xi_{n1} = \xi_{n2} = 2/3, \xi_{x1} = \xi_{y2} = 1/3$. In the first row, there is no migration between patches and each spatial location undergoes selection for a particular single resistant: the single-resistant to antimicrobial x flourishes in patch 1, while the single-resistant to antimicrobial y flourishes in patch 2. In the second and third rows, the patches are coupled via a migration parameter, and the single-resistants can no longer flourish since upon migration they observe a higher prophylaxed population. Wild type strains fix for both $\sigma = 0.15$ and $\sigma = 0.30$.

Combination Therapy and Load Balancing. The previous example seems like a rational choice of prescribing strategies if each hospital has the luxury of choosing either drug for its patients. However, if each patch harbors both types of resistant hosts, as well as doubly-resistant hosts, treatment decisions will have to be made on a per-patient basis and both drugs will have to be used in each patch. In this scenario, combination therapy and within-patch load balancing are better options [**6, 7**]. We examine the consequences of implementing a combination-therapy strategy in one patch and a load-balancing strategy in the other.

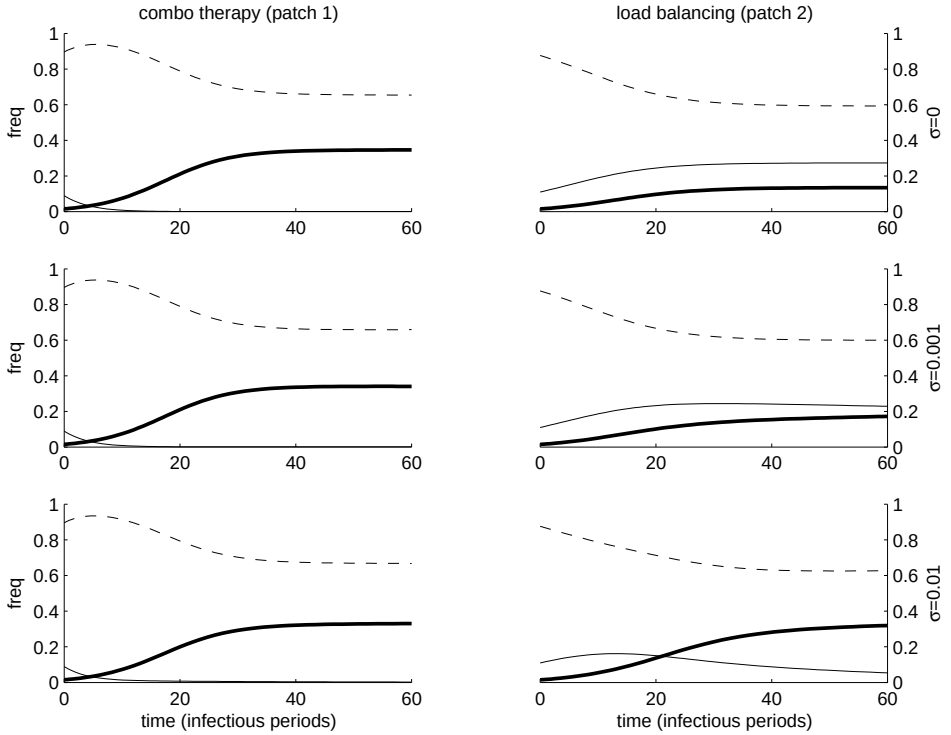

FIGURE 7. Combination Therapy vs. Load Balancing in space. Dashed line represent the frequency of the wild-type, solid lines the frequency of single-resistants, and the thick line represents the frequency of double-resistant strains. In all figures, $\beta = 2, \lambda = 1, \phi_1 = 1/4, \phi_2 = 1/2, \xi_{n1} = \xi_{n2} = 2/3$. In patch 1 (left column) we have $\xi_{x1} = \xi_{y1} = 0, \xi_{z1} = 1/3$. In patch 2 (right column) we have $\xi_{x1} = \xi_{y1} = 1/6, \xi_{z1} = 0$. We see that as coupling between the patches is increased, the combination-therapy scheme is unaffected, but the microbial population structure under the load-balancing regime does in fact change, and it begins to resemble the population structure under combination therapy. In all plots, for both patches, 25% of hosts are infected at equilibrium.

We use the same parameter values for transmission, recovery, and costs of resistance as in the first example. In patch 1, we represent combination therapy by $\xi_{n1} = 2/3$ and $\xi_{z1} = 1/3$. In patch 2, we have load balancing: $\xi_{n2} = 2/3$ and $\xi_{x2} = \xi_{y2} = 1/6$. Under combination therapy in patch 1, we have $\mathcal{R}_{0,w} = \mathcal{R}_{0,z} = 4/3$ and $\mathcal{R}_{0,x} = \mathcal{R}_{0,y} = 16/15$, so the wild types and double-resistants coexist. Under load balancing in patch 2, all strains have an $\mathcal{R}_0 = 4/3$ and all coexist. The first row of Figure 7 shows the strain frequencies in each patch when there is no migration.

Migration homogenizes the population structures in each patch. Because there are more double-resistants in patch 1, there is a net migration of hosts infected with the double-resistant from patch 1 to patch 2. Likewise, there is a net migration of hosts infected with a single-resistant from patch 2 to patch 1.

Double-resistants moving into a load-balancing scheme thrive and provide the resident single-resistants with more opportunity to acquire a second resistance mechanism. As migration increases, most of the single-resistants in patch 2 evolve into double-resistants. Single-resistants moving from load balancing to combination therapy undergo a reduction in $\mathcal{R}_0$; they migrate to a new environment where they are the least fit and cannot increase in frequency.

As coupling between the patches becomes stronger, the direct selection pressure for double-resistants under combination therapy overwhelms the balance achieved by using only single-drug treatments in patch 2. The bacterial strain structure under combination therapy is unaffected by immigration. The strain structure under load balancing, however, becomes destabilized by the arrival of double-resistants, and it begins to resemble the strain structure under combination therapy.

5. Discussion

Here, we have presented and explored some new mathematical models for the spread of resistance in a well-mixed population that explicitly consider the window-of-opportunity for resistance created by chemo-prophylaxis. The use of antimicrobials selects for resistance within a host, but if the rate of use is high enough, a resistant strain can sustain a chain of transmission within a population. Antimicrobial drug use does this by either reducing the proportion colonized by sensitive bacteria or by making it possible for resistance to invade sensitive strains directly through superinfection or the transfer resistance elements. The among-host component of selection and associated transmission are generally considered to be more important than the novel resistance due to within-host component of selection in setting the frequency of resistance within a population.

In most places, the average rate of antimicrobial drug use is too low to favor resistance, but since the rate of drug use is heterogeneous, resistance is favored in some places that are effectively sources for resistance. Thus, from the perspective of resistance, population is structured into a set of sources and sinks. Source-sink dynamics favor coexistence because some people spend more time in sources than others [**30**].

Sources create a local spillover effect, where the prevalence of resistance in nearby populations is always higher. Two sources that are near one another amplify each other. The corollary is that it is better to use different antimicrobials than your neighbors. At the very least, management decisions for antimicrobial resistant pathogens should consider what nearby populations do. Even better, the decisions should be planned and coordinated at regional scales with the purpose of making antimicrobial use as heterogeneous as possible.

For MDR, the implications are more serious. Other papers have emphasized the importance of making drug use as heterogeneous as possible [**6, 7, 19**]. Here, we have shown that load balancing can be effectively done by using different antimicrobials in different hospitals or cities that interact through migration. Moreover, because of chemo-prophylaxis, load balancing has certain advantages over combination therapy, at least when it comes to selection for resistance at the population level. In combination therapy, the total levels of both antimicrobials increase and select for both single-resistant mutants as well as the MDR mutant. In load balancing, total selection pressure is reduced, and the single-mutants are more common, guaranteeing that at least one treatment option is available.

Our models are meant to illustrate some general principles and point out some intriguing possibilities, and should not be interpreted directly as policy recommendations. Antimicrobial policy must also be determined by other concerns, including the interests of the patient, the different efficacies of the antimicrobials, whether antimicrobials have a broad or narrow spectrum, economic considerations, and other factors.

6. Acknowledgements

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7. Appendices

Appendix 1. Let ρ denote the rate that antimicrobial prescription begins, and let $1/\delta$ denote the duration of the protective effects. We subdivide a population into the states of chemo-prophylaxed or not, denoted with subscripts x or n. We further subdivide the population into those who are uncolonized, infected by sensitive phenotypes, or infected by resistant phenotypes. Also, let $U = U_n + U_x$, $W = W_n + W_x$ and $X = X_n + X_x$. The following six equations describe the dynamics for well-mixed populations:

(7.1)
$$\begin{array}{lllll}
\dot{U}_n = & -\beta W_n U_n - \beta X U_n & +\lambda W_n + \lambda X_n + \phi X_n & -\rho U_n + \delta U_x \\
\dot{U}_x = & -\beta X U_x & +\lambda W_x + \lambda X_x + \phi X_x + \nu W_x & +\rho U_n - \delta U_x \\
\dot{W}_n = & \beta W_n U_n + q\beta W_n X_n & -\lambda W_n & -\rho W_n + \delta W_x \\
\dot{W}_x = & -r\beta X W_x & -\lambda W_x - \nu W_x & +\rho W_n - \delta W_x \\
\dot{X}_n = & \beta X U_n - q\beta W_n X_n & -\lambda X_n - \phi X_n & -\rho X_n + \delta X_x \\
\dot{X}_x = & \beta X U_x + r\beta X W_x & -\lambda X_n - \phi X_x & +\rho X_n - \delta X_x
\end{array}$$

In the variables U, X, and W, the dynamics are

$$\begin{array}{ll}
\dot{U} = & -\beta W_n U_n - \beta X U + \lambda(W + X) + \phi X + \nu W_x \\
\dot{W} = & \beta W_n U_n + q\beta W_n X_n - r\beta X W_x - \lambda W - \nu W_x \\
\dot{X} = & \beta X U - q\beta W_n X_n + r\beta X W_x - \lambda X - \phi X
\end{array} , \tag{7.2}$$

Assuming that the rate of drug use is constant and that the pharmacodynamics are fast, the proportion of some class that is protected is given by the equation

$$\dot{y}_x = \rho(y - y_x) - \delta y_x. \tag{7.3}$$

And we use the equilibrium, assuming $\dot{y}_x$ is fast relative to $\dot{y}$:

$$\bar{y}_x = \frac{\rho}{\rho + \delta} y. \tag{7.4}$$

We let $\xi = \rho/(\rho + \delta)$, and $W_x = \xi W$, $W_n = (1 - \xi)W$, $U_n = (1 - \xi)U$, and $X_n = (1 - \xi)X$. We substitute these into (7.2), and write down the much simpler system of equations (2.3). We leave it to others to show when this approximation fails.

Appendix 2. The equations describe a closed population where local population sizes are also constant, but not necessarily equal. Let N_i denote the size of the i^{th} population; the net emigration is $\sigma_i N_i$.

Let $\psi_{i,j}$ denote the fraction of immigrants to the i^{th} population that come from population j, and $\sum_j \psi_{i,j} = 1$. Let $\omega_{i,j}$ denote the fraction of emigrants from population i that go to population j. To balance migration:

$$\psi_{i,j}\sigma_i N_i = \omega_{i,j}\sigma_j N_j. \tag{7.5}$$

Appendix 3. The full model with 2 antimicrobials, 4 prophylaxed states, and 5 colonization states is described below via 20 differential equations describing the dynamics of the 20 population classes described in Table 2. In the equations below, $\lambda_{s,e}$ is the recovery rate of strain s in environment e. These will be simplified later.

$$\begin{aligned}
\dot{U}_n &= -\beta W_n U_n - \beta(X_n + X_x)U_n - \beta(Y_n + Y_y)U_n - \beta(Z_n + Z_x + Z_y + Z_z)U_n \\
&\quad +\lambda_{w,n}W_n + \lambda_{x,n}X_n + \lambda_{y,n}Y_n + \lambda_{z,n}Z_n \\
\dot{U}_x &= -\beta(X_n + X_x)U_x - \beta(Z_n + Z_x + Z_y + Z_z)U_x \\
&\quad +\lambda_{w,x}W_x + \lambda_{x,x}X_x + \lambda_{y,x}Y_x + \lambda_{z,x}Z_x \\
\dot{U}_y &= -\beta(Y_n + Y_y)U_y - \beta(Z_n + Z_x + Z_y + Z_z)U_y \\
&\quad +\lambda_{w,y}W_y + \lambda_{x,y}X_y + \lambda_{y,y}Y_y + \lambda_{z,y}Z_y \\
\dot{U}_z &= -\beta(Z_n + Z_x + Z_y + Z_z)U_z \\
&\quad +\lambda_{w,z}W_z + \lambda_{x,z}X_z + \lambda_{y,z}Y_z + \lambda_{z,z}Z_z \\
\dot{W}_n &= +\beta W_n U_n - \lambda_{w,n}W_n \\
\dot{W}_x &= -\beta(X_n + X_x)W_x - \beta(Z_n + Z_x + Z_y + Z_z)W_x - \lambda_{w,x}W_x \\
\dot{W}_y &= -\beta(Y_n + Y_y)W_y - \beta(Z_n + Z_x + Z_y + Z_z)W_y - \lambda_{w,y}W_y \\
\dot{W}_z &= -\beta(Z_n + Z_x + Z_y + Z_z)W_z - \lambda_{w,z}W_y \\
\dot{X}_n &= +\beta(X_n + X_x)U_n - \lambda_{x,n}X_n \\
\dot{X}_x &= +\beta(X_n + X_x)(U_x + W_x) - \lambda_{x,x}X_x \\
\dot{X}_y &= -\beta(Y_n + Y_y)X_y - \beta(Z_n + Z_x + Z_y + Z_z)X_y - \lambda_{x,y}X_y \\
\dot{X}_z &= -\beta(Z_n + Z_x + Z_y + Z_z)X_z - \lambda_{x,z}X_z \\
\dot{Y}_n &= +\beta(Y_n + Y_y)U_n - \lambda_{y,n}Y_n \\
\dot{Y}_x &= -\beta(X_n + X_x)Y_x - \beta(Z_n + Z_x + Z_y + Z_z)Y_x - \lambda_{y,x}Y_x \\
\dot{Y}_y &= +\beta(Y_n + Y_y)(U_y + W_y) - \lambda_{y,y}Y_y \\
\dot{Y}_z &= -\beta(Z_n + Z_x + Z_y + Z_z)Y_z - \lambda_{y,z}Y_z \\
\dot{Z}_n &= +\beta(Z_n + Z_x + Z_y + Z_z)U_n - \lambda_{z,n}Z_n \\
\dot{Z}_x &= +\beta(Z_n + Z_x + Z_y + Z_z)(U_x + W_x + Y_x) + \beta(X_n + X_x)Y_x - \lambda_{z,x}Z_x \\
\dot{Z}_y &= +\beta(Z_n + Z_x + Z_y + Z_z)(U_y + W_y + X_y) + \beta(Y_n + Y_y)X_y - \lambda_{z,y}Z_y \\
\dot{Z}_z &= +\beta(Z_n + Z_x + Z_y + Z_z)(U_z + W_z + X_z + Y_z) - \lambda_{z,z}Z_z
\end{aligned}$$

In these classes we have eliminated the flow of hosts to prophylaxis (ρ), and from prophylaxis (δ). We will simply assume that the host population is divided into

four sub-groups undergoing varying degrees of prophylaxis: a fraction ξ_n is not prophylaxed, a fraction ξ_x is prophylaxed by antimicrobial x, a fraction ξ_y is prophylaxed by antimicrobial y, a fraction ξ_z is prophylaxed by antimicrobials x and y.

Ignoring the recovery dynamics momentarily, these 8 classes defined by (4.1) and (4.2) allow us to write our system down in 10 equations:

$$
\begin{aligned}
\dot{Q}_w &= -Q_w(\Lambda_w + \Lambda_x + \Lambda_y + \Lambda_z) \\
\dot{Q}_x &= -Q_x(\Lambda_x + \Lambda_z) - Q_w(\Lambda_w + \Lambda_y) \\
\dot{Q}_y &= -Q_y(\Lambda_y + \Lambda_z) - Q_w(\Lambda_w + \Lambda_x) \\
\dot{Q}_z &= -Q_z\Lambda_z - Q_y\Lambda_y - Q_x\Lambda_x - Q_x\Lambda_x \\
\dot{\Lambda}_w &= \beta Q_w \Lambda_w \\
\dot{\Lambda}_x &= \beta(Q_x - Y_x)\Lambda_x \\
\dot{\Lambda}_y &= \beta(Q_y - X_y)\Lambda_y \\
\dot{\Lambda}_z &= \beta Q_z\Lambda_z + \beta X_y\Lambda_y + \beta Y_x\Lambda_x \\
\dot{X}_y &= -X_y(\Lambda_y + \Lambda_z) \\
\dot{Y}_x &= -Y_x(\Lambda_x + \Lambda_z).
\end{aligned}
\tag{7.6}
$$

If we add in the recovery terms, we can no longer express the system in 10-dimensions, unless we make a similar approximation as in the one drug case, namely that the fractions ξ_i express the relative frequencies in the 5 types of disease classes (uncolonized, infected with wild type, infected with resistant to x, infected with resistant to y, infected with resistant to x and y). Making this approximation, we see that Z_x, for example, can be expressed as $\xi_x\,\beta^{-1}\Lambda_z$. And,

$$
X_n \approx \frac{\xi_n}{\xi_n + \xi_x}\,\frac{1}{\beta}\,\Lambda_x.
$$

Then, our approximation yields the differential equations:

$$
\begin{aligned}
\dot{Q}_w &= -Q_w(\Lambda_w+\Lambda_x+\Lambda_y+\Lambda_z) \\
&\quad +\frac{\lambda}{\beta}\left(\Lambda_w+\frac{\lambda_S}{\lambda}\frac{\xi_n}{\xi_n+\xi_x}\Lambda_x+\frac{\lambda_S}{\lambda}\frac{\xi_n}{\xi_n+\xi_y}\Lambda_y+\frac{\lambda_D}{\lambda}\xi_n\Lambda_z\right) \\
\dot{Q}_x &= -Q_x(\Lambda_x+\Lambda_z)-Q_w(\Lambda_w+\Lambda_y) \\
&\quad +\frac{\lambda}{\beta}\left(\Lambda_w+\frac{\lambda_S}{\lambda}\Lambda_x+\frac{\lambda_S}{\lambda}\frac{\xi_n}{\xi_n+\xi_y}\Lambda_y+\frac{\lambda_D}{\lambda}(\xi_n+\xi_x)\Lambda_z\right) \\
\dot{Q}_y &= -Q_y(\Lambda_y+\Lambda_z)-Q_w(\Lambda_w+\Lambda_x) \\
&\quad +\frac{\lambda}{\beta}\left(\Lambda_w+\frac{\lambda_S}{\lambda}\frac{\xi_n}{\xi_n+\xi_x}\Lambda_x+\frac{\lambda_S}{\lambda}\Lambda_y+\frac{\lambda_D}{\lambda}(\xi_n+\xi_y)\Lambda_z\right) \\
\dot{Q}_z &= -Q_z\Lambda_z-Q_y\Lambda_y-Q_x\Lambda_x-Q_x\Lambda_x \\
&\quad +\frac{\lambda}{\beta}\left(\Lambda_w+\frac{\lambda_S}{\lambda}\Lambda_x+\frac{\lambda_S}{\lambda}\Lambda_y+\frac{\lambda_D}{\lambda}\Lambda_z\right) \\
\dot{\Lambda}_w &= \beta Q_w\Lambda_w-\lambda\Lambda_w \\
\dot{\Lambda}_x &= \beta(Q_x-Y_x)\Lambda_x-\lambda_S\Lambda_x \\
\dot{\Lambda}_y &= \beta(Q_y-X_y)\Lambda_y-\lambda_S\Lambda_y \\
\dot{\Lambda}_z &= \beta Q_z\Lambda_z+\beta X_y\Lambda_y+\beta Y_x\Lambda_x-\lambda_D\Lambda_z \\
\dot{X}_y &= -X_y(\Lambda_y+\Lambda_z)-\lambda_A X_y \\
\dot{Y}_x &= -Y_x(\Lambda_x+\Lambda_z)-\lambda_A Y_x,
\end{aligned}
\tag{7.7}
$$

where $\lambda_S=\lambda+\phi_1$ and $\lambda_D=\lambda+\phi_2$ are the recovery rates (in all environments where antimicrobials have no effect) for the single-resistant and for the double-resistant, respectively. λ_A is the recovery rate for a host undergoing effective antimicrobial treatment. Since $\dot{X}_y<0$ and $\dot{Y}_x<0$, we simply say that these two classes are zero, and we approximate the full system with the remaining eight equations; this is system (4.3) in the text.

References

[1] D. I. Andersson and B. R. Levin, The biological cost of antibiotic resistance, *Current Opinions in Microbiology* **2** (1999), 489–493.

[2] D. J. Austin, M. Kakehashi, and R. M. Anderson, The transmission dynamics of antibiotic-resistant bacteria: the relationship between resistance in commensal organisms and antibiotic consumption, *Proc. R. Soc. Lond. B* **264** (1997), 1629–1638.

[3] D. J. Austin and R. M. Anderson, Transmission dynamics of epidemic methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci in England and Wales, *J. Infect. Dis.* **179** (1999), 883–891.

[4] D. J. Austin, M. J. M. Bonten, R. A. Weinstein, S. Slaughter, and R. M. Anderson, Vancomycin-resistant enterococci in intensive-care hospital settings: transmission dynamics, persistence, and the impact of infection control programs, *Proc. Natl. Acad. Sci. USA* **96** (1999), 6908–6913.

[5] D. J. Austin, K. G. Kristinsson, and R. M. Anderson, The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance, *Proc. Natl. Acad. Sci. USA* **96** (1999), 1152–1156.

[6] C. T. Bergstrom, M. Lo, and M. Lipsitch, Ecological theory suggests that antimicrobial cycling will not reduce antimicrobial resistance in hospitals, *Proc. Natl. Acad. Sci. USA* **101** (2004), 13285–13290.

[7] S. Bonhoeffer, M. Lipsitch, and B. R. Levin, Evaluating treatment protocols to prevent antibiotic resistance, *Proc. Natl. Acad. Sci. USA* **94** (1997), 12106–12111.
[8] M. F. Boni and M. W. Feldman, Evolution of antibiotic resistance by human and bacterial niche construction, *Evolution* **59**(3) (2005), 477–491.
[9] M. J. Bonten, R. Willems, and R. A. Weinstein, Vancomycin-resistant enterococci: Why are they here, and where do they come from? *Lancet Infect. Dis.* **1** (2001), 314–325.
[10] M. J. Bonten, D. J. Austin, and M. Lipsitch, Understanding the spread of antibiotic resistant pathogens in hospitals: Mathematical models as tools for control, *Clin. Infect. Dis.* **33** (2001), 1739–1746.
[11] CDC, *Staphylococcus aureus* resistant to vancomycin — United States, 2002, *MMWR* **51** (2002), 565–567.
[12] D. F. Clyde and G. T. Shute, Resistance of *Plasmodium falciparum* in Tanganyika to pyrimethamine administered at weekly intervals, *Trans. R. Soc. Trop. Med. Hyg.* **51** (1957), 505–513.
[13] J. R. Gog and J. Swinton, A status-based approach to multiple strain dynamics, *J. Math. Biol.* **44** (2002), 169–184.
[14] M. G. M. Gomes and G. F. Medley, Dynamics of multiple strains of infectious agents coupled by cross-immunity: A comparison of models, in (S. Blower, C. Castillo-Chavez, K. L. Cooke, D. Kirschner, and P. van der Driessche, eds.), *Mathematical Approaches for Emerging and Reemerging Infections: Models, Methods and Theory*, IMA Volumes in Mathematics and its Applications, Springer-Verlag, New York, 2001, 171–191.
[15] I. M. Hastings and U. D'Allesandro, Modelling a predictable disaster: the rise and spread of drug-resistant malaria, *Parasitol. Today* **16** (2000), 340–347.
[16] I. M. Hastings, W. M. Watkins, and N. J. White, The evolution of drug-resistant malaria: the role of drug elimination half-life, *Philos. Trans. R. Soc. Lond. B* **29** (2002), 505–519.
[17] M. B. Hoshen, W. D. Stein, and H. Ginsburg, Mathematical modelling of malaria chemotherapy: combining artesunate and mefloquine, *Parasitology* **124** (2002), 9–15.
[18] J. C. Koella and R. Antia, Epidemiological models for the spread of anti-malarial resistance, *Malaria J.* **2** (2003), 3.
[19] R. Laxminarayan and M. L. Weitzman, On the implications of endogenous resistance to medications, *J. Health Econ.* **21** (2002), 709–718.
[20] B. R. Levin and C. T. Bergstrom, Bacteria are different: observations, interpretations, speculations, and opinions about the mechanisms of adaptive evolution in prokaryotes, *Proc. Natl. Acad. Sci. USA* **97** (2000), 6981–6985.
[21] B. R. Levin, V. Perrot, and N. Walker, Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria, *Genetics* **154** (2000), 985–997.
[22] B. R. Levin, Minimizing potential resistance: a population dynamics view, *Clin. Infect. Dis.* **33**(Supple 3) (2001), S161–S169.
[23] S. A. Levin, V. Andreasen, and J. Lin, The dynamics of cocirculating influenza strains conferring partial cross-immunity, *J. Math. Biol.* **35** (1997), 825–842.
[24] M. Lipsitch, C. T. Bergstrom, and B. R. Levin, The epidemiology of antibiotic resistance in hospitals: paradoxes and prescriptions, *Proc. Natl. Acad. Sci. USA* **97** (2000), 1938–1943.
[25] M. Lipsitch, Measuring and interpreting associations between antibiotic use and penicillin resistance in *Streptococcus pneumoniae*, *Clin. Infect. Dis.* **32** (2001), 1044–1054.
[26] M. Lipsitch and M. H. Samore, Antimicrobial use and antimicrobial resistance: a population perspective, *Emerg. Infect. Dis.* **8** (2002), 347–354.
[27] National Nosocomial Infections Surveillance System, National nosocomial infections surveillance (nnis) system report, data summary from january 1992 through june 2004, issued october 2004, *Am. J. Infect. Control.* **32** (2004), 470–485.
[28] R. Ndyomugyenyi and P. Magnussen, Trends in malaria-attributable morbidity and mortality among young children admitted to Ugandan hospitals, for the period 1990–2001, *Ann. Trop. Med. Parasitol.* **98** (2004), 315–327.
[29] L. B. Rice, Emergence of vancomycin-resistant enterococci, *Emerg. Infect. Dis.* **7** (2001), 183–187.
[30] D. L. Smith, A. D. Harris, J. A. Johnson, E. K. Silbergeld, and J. G. Morris, Jr., Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria, *Proc. Natl. Acad. Sci. USA* **99** (2002), 6434–6439.

[31] D. L. Smith, J. Dushoff, E. N. Perencevich, A. D. Harris, and S. A. Levin, Persistent colonization and the spread of antibiotic resistance in nosocomial pathogens: resistance is a regional problem, *Proc. Natl. Acad. Sci. USA* **101** (2004), 3709–3714.

[32] D. Schellenberg, C. Menendez, E. Kahigwa, J. Aponte, J. Vidal, M. Tanner, H. Mshinda, and P. Alonso, Intermittent treatment for malaria and anaemia control at time of routine vaccinations in Tanzanian infants: a randomised, placebo-controlled trial, *Lancet* **357** (2000), 1471–1477.

[33] C. E. Shulman, E. K. Dorman, F. Cutts, K. Kawuondo, J. N. Bulmer, N. Peshu, and K. Marsh, Intermittent sulphadoxine-pyrimethamine to prevent severe anaemia secondary to malaria in pregnancy: a randomised placebo-controlled trial, *Lancet* **353** (1999), 632–636.

[34] B. Spellberg, J. H. Powers, E. P. Brass, L. G. Miller, and J. E. Edwards, Jr., Trends in antimicrobial drug development: implications for the future, *Clin. Infect. Dis.* **38** (2004), 1279–1286.

[35] J. F. Trape, The public health impact of chloroquine resistance in Africa, *Am. J. Trop. Med. Hyg.* **64**(1–2 Suppl) (2001), 12–17.

[36] S. Yeung, W. Pongtavornpinyo, I. M. Hastings, A. J. Mills, and N. J. White, Antimalarial drug resistance, artemisinin-based combination therapy, and the contribution of modeling to elucidating policy choices, *Am. J.Trop. Med. Hyg.* **71** (2004), 179–186.

Fogarty International Center, National Institutes of Health, Bethesda, MD 20892
E-mail address: smitdave@mail.nih.gov

Department of Biological Sciences, Stanford University
E-mail address: maciek@charles.stanford.edu

Resources for the Future, Washington DC
E-mail address: ramanan@rff.org