Ecological theory suggests that antimicrobial cycling will not reduce antimicrobial resistance in hospitals

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Hospital-acquired infections caused by antibiotic-resistant bacteria pose a grave and growing threat to public health. Antimicrobial cycling, in which two or more antibiotic classes are alternated on a time scale of months to years, seems to be a leading candidate in the search for treatment strategies that can slow the evolution and spread of antibiotic resistance in hospitals. We develop a mathematical model of antimicrobial cycling in a hospital setting and use this model to explore the efficacy of cycling programs. We find that cycling is unlikely to reduce either the evolution or the spread of antibiotic resistance. Alternative drug-use strategies such as mixing, in which each treated patient receives one of several drug classes used simultaneously in the hospital, are predicted to be more effective. A simple ecological explanation underlies these results. Heterogeneous antibiotic use slows the spread of resistance. However, at the scale relevant to bacterial populations, mixing imposes greater heterogeneity than does cycling. As a consequence, cycling is unlikely to be effective and may even hinder resistance control. These results may explain the limited success reported thus far from clinical trials of antimicrobial cycling.

Nosocomial infection is a major contributor to mortality, morbidity, length of hospital stay, and the economic cost of health care (1, 2). Antibiotic resistance exacerbates the problem. Resistance increases the chance that a patient receives insufficient therapy and thus further increases the risks and costs associated with nosocomial infection (2–6). As such, the evolution and spread of antibiotic-resistant bacteria poses a grave threat, particularly in intensive-care units. In these units, antibiotic use is high, opportunities for transmission are abundant, and many patients are immunocompromised or otherwise susceptible to infection by opportunistic pathogens (7, 8).

Although several new antimicrobial drugs have recently been introduced and additional ones are forthcoming, experience suggests that, as new drugs become widely deployed, resistance to these agents will emerge and spread as well. Successful control of antibiotic resistance will require both the continued development of new drugs and the judicious use of our current arsenal of antibiotics.

Many authors have suggested that antimicrobial cycling, in which the empiric use of two or more classes of antibiotics is alternated over a time scale of months to years, may slow the evolution and spread of resistant and multiply resistant bacterial strains (7, 9–13). The motivation is straightforward. Should resistance to one class of drugs reach high frequency in a hospital ward, a scheduled switch of antibiotic classes would soon follow, leaving most of the bacterial strains in the hospital susceptible to the new therapy (14, 15). Moreover, fluctuating patterns of antimicrobial use may reduce the rate at which drug-sensitive strains can acquire resistance to single or multiple antibiotics. In addition to the intuitive appeal of these arguments, more than two decades of experience have shown that a one-time formulary shift can effectively control a hospital epidemic of antibiotic-resistant strains (16–22), as can a single rotation through a series of alternative drugs (23).

Nonetheless, we have little substantive evidence, empirical or theoretical, that repeated cycling will be effective as a long-term strategy to slow the emergence and spread of antibiotic resistance. Several intervention trials of cycling are currently underway, but the results reported to date are mixed. These studies either fail to show any advantage to cycling (24) or merely hint at possible advantages (15, 25). Because each of these studies uses a quasi-experimental design (with historical controls), it is difficult to distinguish the effects of cycling from the general effect of having a well-publicized, specific antimicrobial policy (26). Concomitant interventions in some of the studies (23, 27, 28) further complicate inference about the specific effects of cycling programs (29, 30). No theoretical models of cycling in a hospital setting have been published, and theoretical work on cycling in the greater community suggests that cycling will actually facilitate the spread of resistant strains (31).

In this article, we develop a mathematical model of antimicrobial cycling in a hospital setting. We show that cycling is unlikely to reduce rates of resistance carriage relative to alternative drug-use programs and present an ecological model to explain these findings. The primary purpose of the mathematical models used in this article is to isolate and illustrate the fundamental ecological processes that will be responsible for the success or failure of antimicrobial cycling programs, rather than to develop precise quantitative predictions. Thus, we concentrate on the general mathematical properties of the dynamical system, rather than on parameter estimation and forecasting.

Modeling Antibiotic Cycling in a Hospital Setting

The ecology of hospital-associated bacteria differs from the ecology of the microbes responsible for most community-acquired infections, in three ways. First, a hospital is an open system, with a daily influx and efflux of patients. By contrast, a community is relatively closed, in that individuals enter or leave at much lower rates. Second, unlike the obligate pathogens responsible for many community-acquired infections, most bacterial species responsible for hospital-acquired infections are commensals that induce pathology only if they opportunistically colonize sterile sites, such as the bloodstream or the lower respiratory tract. Thus many patients enter the hospital asymptomatically colonized with the species responsible for nosocomial infection, and antimicrobial use is typically uncorrelated with colonization status. In some cases, prior colonization with sensitive strains is partially protective against colonization by resistant strains. Third, antimicrobial drugs are used at a much higher rate in the hospital than in the community at large.

To account for these features, we previously developed a mathematical model of the transmission and spread of antimicrobial resistance in a hospital setting (32, 33). The model is tailored for the transmission dynamics of organisms that are frequently transmitted between hosts in the hospital, most importantly the Gram-positive cocci such as *Staphylococcus*

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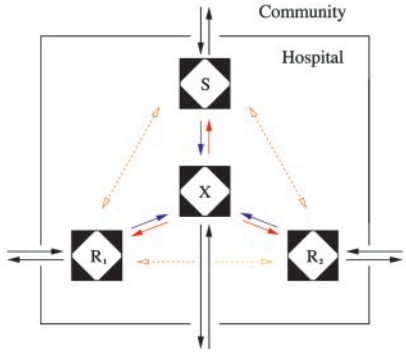

$$
\begin{aligned}
dS/dt &= (m - S)\,\mu - (\tau_1 + \tau_2 + \gamma)\,S + \beta\,S\,X + \sigma\,\beta\,(c_1 R_1 + c_2 R_2)\,S \\
dR_1/dt &= (m_1 - R_1)\,\mu - (\tau_2 + \gamma)\,R_1 + \beta\,(1 - c_1)\,R_1\,X - \sigma\beta\left(c_1 S + (c_1 - c_2)\,R_2\right) R_1 \\
dR_2/dt &= (m_2 - R_2)\,\mu - (\tau_1 + \gamma)\,R_2 + \beta\,(1 - c_2)\,R_2\,X - \sigma\beta\left(c_2 S + (c_2 - c_1)\,R_1\right) R_2 \\
dX/dt &= (1 - m - m_1 - m_2 - X)\,\mu + (\tau_1 + \tau_2 + \gamma)\,S + (\tau_2 + \gamma)\,R_1 + (\tau_1 + \gamma)\,R_2 \\
&\quad -\beta\,X\left(S + (1 - c_1)R_1 + (1 - c_2)R_2\right)
\end{aligned}
$$

Fig. 1. Schematic diagram of the model and the corresponding differential equations. The color coding associates mathematical terms with ecological processes. Red represents infection, yellow represents supercolonization, blue represents clearance, and black represents influx and efflux.

aureus and *Enterococcus* species (34). The model may also be used to consider the dynamics of Gram-negative bacteria, although, in its present form, it excludes the processes of endogenous selection of resistance by mutation or by outgrowth of a subpopulation, which may be especially important in these organisms (35). The model successfully accounts for a number of surprising features of hospital-acquired infections, including the rapid rate of change in response to interventions, the efficacy of nonspecific control measures such as hand-washing, and the observation that use of one drug is an individual risk factor for acquisition of resistance to other drugs, even in the absence of cross-resistance or associated linkage selection.

Here, we extend the earlier model to see whether antimicrobial cycling can be effective at controlling resistance. We consider the following scenario. Two antimicrobials, drug 1 and drug 2, are available. Strains resistant to each drug individually are present. Because a strain resistant to both drugs would be equally unaffected by both drugs (and therefore not directly affected by the particular drug treatment policies in use), we assume that dual resistance has not yet emerged. Our model, illustrated in Fig. 1, tracks several groups of patients according to their colonization status. The X group or compartment represents patients who are uncolonized by the bacterial species of interest. The S compartment represents the patients colonized by susceptible bacteria of this species. For the purposes of this model, the uncolonized label refers to epidemiological properties rather than microbiological ones: the uncolonized group includes not only those patients who are entirely uncolonized by the species of interest, but also those who carry sufficiently small populations that (*i*) they are unlikely to transmit to other patients and (*ii*) they are more likely than fully colonized patients to be superinfected by new strains.

The two R compartments, R_1 and R_2, represent patients colonized by strains resistant to drug 1 and drug 2, respectively. Individual patients may enter the hospital in any of the states X, S, R_1, and R_2, and they do so at rates $\mu(1 - m - m_1 - m_2)$, μm, μm_1, and μm_2 per day. Here μ represents the rate of patient turnover in the hospital. Regardless of colonization state, patients leave the hospital after an average stay of $1/\mu$ days. If untreated, patients colonized with susceptible bacteria remain colonized on average $1/\gamma$ days. Drug 1 is used at a rate τ_1 per day and drug 2 at a rate τ_2 per day; drug use clears any strain not resistant to that drug.

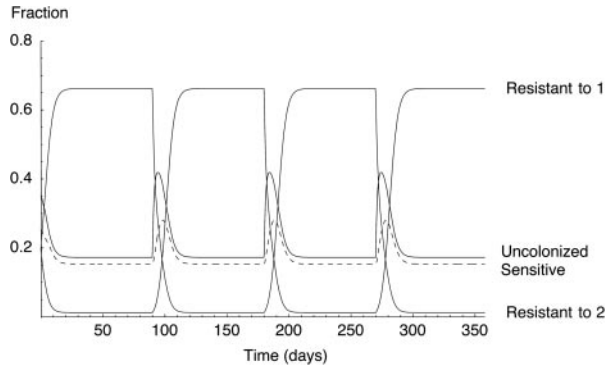

Fig. 2. Strain frequencies over time, for a cycling program with a drug switch every 90 days and 80% compliance ($\alpha = 0.8$). Parameter values: $\beta = 1$, $c = 0$, $\gamma = 0.03$, $m = 0.7$, $m_1 = .05$, $m_2 = .05$, $\tau_1 + \tau_2 = 0.5$, $\mu = 0.1$, $\sigma = 0.25$, and $\alpha = 0.8$.

Uncolonized individuals are colonized at rates proportional to the frequencies of each strain, with a rate constant of β. Parameters c_1 and c_2 represent the "fitness cost" to a bacterium of being resistant in the absence of drug use. Colonized individuals may have their bacterial population replaced by "supercolonization" with bacteria transmitted in the hospital; the parameter σ determines the rate of this secondary colonization relative to that of primary colonization.

For cycling protocols, the parameter α represents physician compliance with the cycling program and is equal to the fraction of patients that receive the currently indicated drug instead of a randomly chosen therapy. Thus when $\alpha = 1$, all patients receive the currently indicated therapy, and, when $\alpha = 0$, half of the patients receive drug 1 and half receive drug 2.

In this model, we assume that strains resistant to both antibiotics have not yet appeared. In reality, multiple resistance is common in intensive-care units and other hospital wards. In some cases, multiple resistance may include resistance to both classes being considered for cycling or mixing. Although it is important to devise antimicrobial policies for these situations, the cycling and mixing policies considered here are unlikely to differ significantly in impact because the dually resistant strain will be impervious to the use of these antibiotics regardless of the protocol.

Results

By numerically solving the differential equations given in Fig. 1, we can see how the expected frequency of each bacterial strain changes over time. Fig. 2 shows that cyclic use of antibiotics results in cyclic incidence of strain frequencies. Each time a new drug is instituted, the frequency of the strain resistant to that drug climbs, whereas the frequency of the strain resistant to the unused drug declines. Immediately after each switch of drugs, strains resistant to the newly instituted therapy are rare, and the new antibiotic is temporarily more effective than usual. As a result, the curve representing the fraction of uncolonized patients surges upward briefly after each switch.

To determine whether cycling is effective at reducing resistance carriage, we compare the average fraction of patients carrying resistant bacteria, given by the time integral $\int R_1(t) + R_2(t)\,dt$, that occur under cycling to the average fraction carrying resistant bacteria under an alternative treatment protocol. This alternative protocol, which we label "mixing," randomly assigns drug 1 to half of the treated patients and assigns drug 2 to the other treated patients. Mixing is a reasonable approximation of current usage patterns in most units and, as such, serves as a

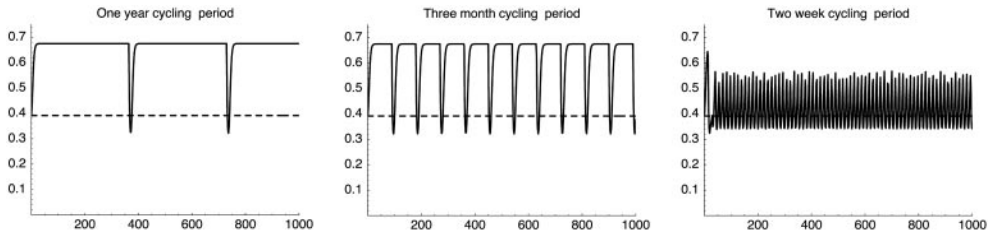

Fig. 3. Fraction of patients carrying resistant bacteria, for cycle lengths of 1 yr, 3 months, and 2 weeks, respectively. Solid lines, total fraction of patients colonized with resistant bacteria under cycling; dashed lines, total fraction of patients colonized with resistant bacteria under a 50–50 mixing regime. By this measure, mixing outperforms cycling. Parameters are as in Fig. 2.

natural baseline for comparison when judging the efficacy of cycling.

Fig. 3 shows the total number of resistant cases when cycling occurs with periods of 365, 90, and 14 days, respectively. The total fraction of resistant strains in the hospital (solid lines) is higher for longer cycle periods. During the majority of each cycle, the total fraction resistant is greater than the total fraction resistant under mixing (dashed lines). This trend seems to hold quite generally over a wide range of parameters (data not shown).

The overall effect of cycle period is illustrated in Fig. 4, a parametric plot showing average total resistance over time as a function of cycle period. In Fig. 4, the average fraction of patients colonized by a resistant strain is greater for cycling of any period than for 50–50 mixing and, indeed, increases monotonically with cycle period. As cycle period approaches zero, total resistance approaches that observed for 50–50 mixing; this convergence is unsurprising, in that mixing is equivalent to cycling with a very short period.

To assess the robustness of these results, we randomly selected 100 parameter sets to provide broad coverage of parameter space, as follows. For each parameter set, incoming strain frequencies were selected from a uniform distribution on the three-dimensional simplex. The β values were selected from a log uniform distribution on [0.001,1], the costs of resistance to each of the two drugs were selected independently from a uniform distribution on [0, 0.5], the total rate of drug use was selected from a uniform distribution on [0,1], and the rate of supercolonization (relative to colonization) was selected from a uniform distribution on [0,1]. In all 100 parameter sets, we observed the qualitative pattern illustrated in Fig. 4: total resistance is lower for mixing than for any cycling period and increases monotonically with cycle length. Across parameter sets, the magnitude of this increase ranged from negligible to an additional 30% incidence of resistant colonization as a result of cycling.

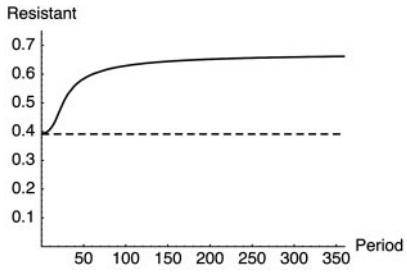

Fig. 4. Average total resistance as a function of cycle period, calculated numerically. Solid lines, average total fraction of patients colonized with resistant bacteria under cycling; dashed lines, total fraction of patients colonized with resistant bacteria under a 50–50 mixing regime. Parameters are as in Fig. 2.

Why Cycling Fails to Reduce the Incidence of Resistant Bacteria

The basic rationale for cycling is that fluctuating selection pressures will reduce the rate of adaptation or the ability of an evolving population to track its environment. Niederman (14) summarized this intuition in an early review on antimicrobial cycling:

> The "crop rotation" theory of antibiotic use has suggested that if we routinely vary our "to go" antibiotic in the ICU, we can minimize the emergence of resistance because selection pressure for bacteria to develop resistance to a specific antibiotic would be reduced as organisms become exposed to continually varying antimicrobials.

Niederman's explanation encompasses two separate potential advantages to cycling. First, cycling may limit the spread of resistance alleles currently present in the population. Control of already-present alleles is the aim of most resistance-management interventions and the subject of this section. Second, cycling may inhibit the formation of novel resistance alleles or resistance allele combinations. We address this possibility in *Emergence of New Resistance*.

Consider a set of alleles present in a population and subject to fluctuating selection. We might expect that the relative frequencies of these alleles will more closely match the demands of the environment when selective conditions fluctuate less rapidly. In a slowly changing environment, we might expect currently favored alleles to be at higher frequency, more of the time, than in a rapidly changing environment. Although such claims seem intuitively straightforward, we are unaware of a clear analytical treatment of this issue anywhere in the population biology literature. To demonstrate that the argument is indeed feasible, we have developed a simple illustrative model, presented in *Appendix*. We consider a symmetric model of fluctuating selective conditions, with two environments and two alleles, and we assume that individuals carrying each allele enter the population at some nonzero rate, as would be the case for a hospital when resistance is present in the community. We find that the average degree to which the population is adapted to the current environment increases monotonically with the period of environmental fluctuation. That is, slower environmental change results in a closer match between individuals and environment.

Thus, both Niederman's basic intuition about environmental fluctuation and the results from our appendix suggest that slower cycles lead to better-adapted populations. By contrast, our findings in *Results* suggest that resistent bacteria fare better under cycling than under a static mixing ratio. Why the discrepancy?

The answer lies in the scale at which heterogeneity is experienced by bacterial clones in a hospital. At the scale relevant to bacterial populations, mixing rather than cycling imposes greater fluctuation in selective conditions. We can see this principle by

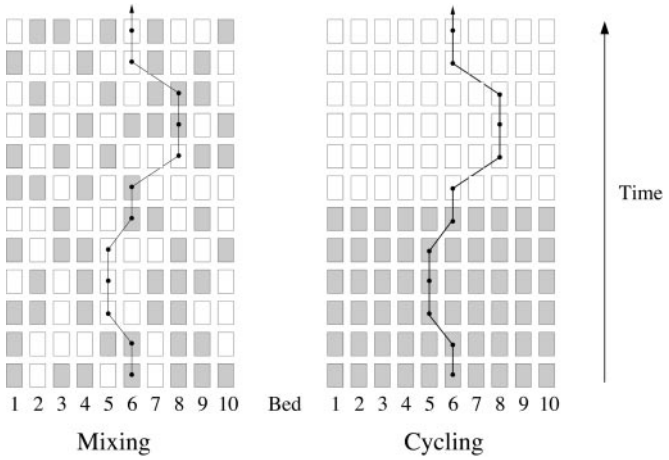

Fig. 5. Effects of cycling and mixing on the selective conditions faced by a bacterial clone. Cycling offers greater heterogeneity at the level of the ward, but mixing offers greater heterogeneity at the level of the individual patient.

envisioning the selective regime faced by a bacterial clone as it spreads among patients in a hospital. Under a cycling program, the clonal population experiences consistent selective conditions until the next cycle, usually a period of many months. Under a mixing program, by contrast, the clonal population will experience fluctuating selective conditions on a much shorter timescale as it spreads from patient to patient, some of whom are under treatment with one drug and others under treatment with another.

Fig. 5 illustrates the time course of a single clone in a hospital setting. Each square represents a patient in a hospital bed; gray squares receive treatment with drug 1, whereas white squares receive treatment with drug 2. In a ward where drugs are mixed, the fractions of patients receiving drug 1 and drug 2 do not change appreciably over time. In a ward with cycling, the fractions receiving each drug change considerably over time. At any given time, however, each individual bacterial clone faces a selective regime defined by the drug used by a single patient, not by the ward average. Thus, in the course of patient-to-patient transfer, a bacterial clone actually faces more rapid environmental fluctuations in the mixing ward. In Fig. 5 *Left* (mixing), the clone tracked by the solid line faces a new drug five times during the span of the diagram. By contrast, the clone tracked in Fig. 5 *Right* (cycling) faces a new drug only once, during the mass switch-over from drug 1 to drug 2.

Thus, mixing provides a more heterogeneous environment than does cycling at the single-patient scale, despite its constancy in treatment protocol at the scale of the ward.

Emergence of New Resistance

Multiply resistant strains of bacteria often arise through lateral gene transfer of antibiotic resistance genes (36, 37). This process requires that a donor strain resistant to one drug encounter a recipient strain resistant to the other drug. Such encounters will occur at a rate proportional to the frequency with which patients carrying one resistant strain encounter bacteria of the other resistant strain. This rate can be approximated by (some constant multiplied by) the product of the fraction of patients colonized by each resistant type in the hospital. Thus, in the two-drug, two-resistance example above, the instantaneous probability (hereafter called, imprecisely, the "rate") of generating multiple resistance is proportional to $R_1(t) \times R_2(t)$, and the chance that a strain acquires a novel combination of resistance genes is to a first-order approximation proportional to the mean product $\int R_1(t) \times R_2(t)\, dt$.

When the two resistant strains are at similar frequencies in the community and have similar costs of resistance, cycling between two alternative antibiotics can reduce this mean product relative to mixing because the cycling program never positively selects on both strains at the same time. As a result, either $R_1(t)$ or $R_2(t)$ should always be at low frequency, and thus the product $R_1(t) \times R_2(t)$ should always be small. Fig. 6 *A–C* shows the time course of the product $R_1(t) \times R_2(t)$ for the parameter values given in Fig. 2. The product is always lower under cycling than under mixing. Fig. 7*A* shows the mean product as a function of cycling period for these parameter values. As cycling period increases, the rate of generating multiple resistance decreases monotonically.

When the two resistant strains differ in their community prevalence or in their resistance costs, cycling is less likely to reduce the rate of generating multiple resistance. Fig. 6 *D–F* shows the time course of the product $R_1(t) \times R_2(t)$ when the strain resistant to one drug is more common in the community than that resistant to the other. Here, multiple resistance is

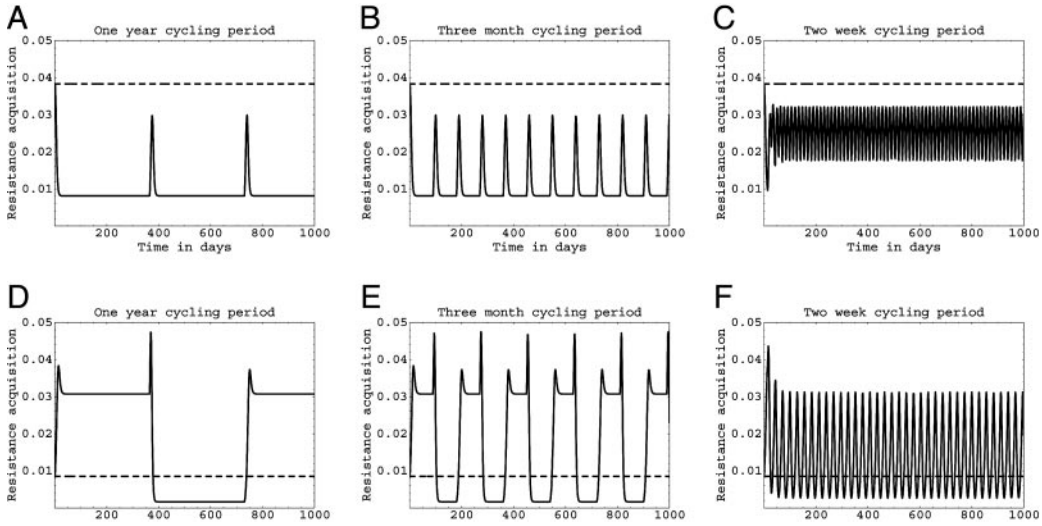

Fig. 6. Rate at which novel multiresistant strains are generated, as measured by $R_1(t) \times R_2(t)\, dt$. (*A–C*) Symmetric input of strains into the hospital. Parameters are as in Fig. 2. (*D–F*) Asymmetric input of strains into the hospital. Parameters are as before, but with $m_1 = 0.19$, $m_2 = 0.01$, and $m = 0.6$. Solid lines, cycling; dashed lines, mixing.

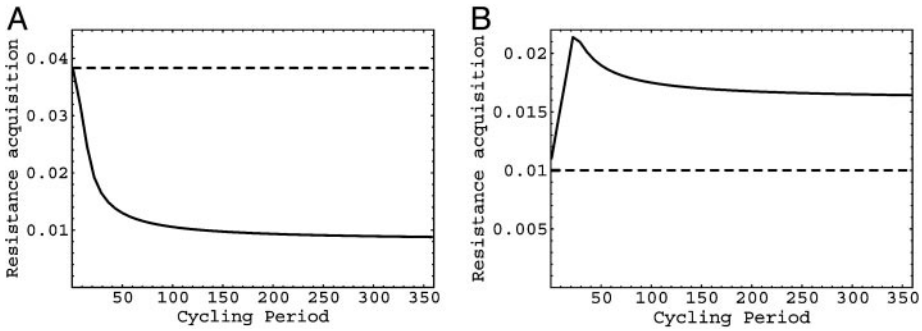

Fig. 7. Average rate of dual resistance acquisition as function of cycle period. (*A*) Symmetric input of strains into the hospital. (*B*) Asymmetric input of strains into the hospital. Solid lines, cycling; dashed lines, 50–50 mixing regime. Parameters are as in Fig. 6.

expected to arise more quickly under cycling than under mixing. Fig. 7*B* shows the mean product as a function of cycling period for these asymmetric parameter values. The relation between cycling period is no longer monotone, but here cycling always performs worse than mixing.

To assess the overall efficacy of cycling as a strategy for slowing the emergence of multiple resistance, we derived parametric plots analogous to those in Fig. 7 for each of the 100 random parameter sets described in *Results*. For only 4 of these 100 parameter sets did cycling periods of 2 weeks or longer reduce the rate at which multiple resistance emerged, relative to mixing. Furthermore, systematic numerical exploration revealed that, relative to mixing, a cycling program is more likely to slow multiple resistance evolution as (*i*) the fraction of incoming strains resistant to drug 1 and drug 2 becomes more symmetric, (*ii*) the fraction of incoming patients carrying resistance decreases, (*iii*) the total drug use increases, and (*iv*) the resistance costs decrease.

Why does cycling fail to slow the generation of multiple resistance when strain frequencies are asymmetric in the community? Cycling alternates between two treatment regimes: one in which the common strain is positively selected and the rare one is unselected and one in which the converse occurs. During the former, the common strain reaches high frequency, but the rare strain stays rare in the hospital. The result is a low product $R_1(t) \times R_2(t)$. During the latter, the rare strain is positively selected and thus reaches high frequency in the hospital, whereas the common strain, although unselected, enters at a relatively high rate. The result is a high product $R_1(t) \times R_2(t)$. Relative to mixing, the advantages gained by reducing the product during the former regime are outweighed by the costs of increasing the product during the latter, for a higher mean rate of multiple resistance evolution.

Although cycling may reduce the rate of multiple resistance evolution under certain restricted circumstances, this advantage is unlikely to be compelling in practice. Normally, the frequencies of the different resistant strains will be asymmetric; furthermore, we will rarely have sufficient information about these frequencies and other relevant parameters to determine that we are in a range of parameter space for which cycling is advantageous. Finally, even when cycling can slow the emergence of multiple resistance, our model predicts that any cycling program will reap this benefit at the expense of an increased average incidence of single resistance. This latter measure is likely to be a greater concern in most hospitals, most of the time.

Conclusions

Systematic programs of antimicrobial cycling are touted as likely strategies for reducing the spread of antibiotic-resistant bacteria in hospitals. Here, we have shown that the proposed mechanism by which cycling would have this effect is untenable on theoretical grounds. Although the models here treat bacterial transmission within the hospital as a deterministic process, our results are echoed by Monte Carlo simulations of stochastic transmission, even for small hospital wards (unpublished results).

Models alone are necessarily simplifications of reality, and, therefore, our work here cannot rule out the possibility that cycling could be beneficial in resistance control. Clinical trials will be needed to address that matter definitively. If those trials do indicate a real benefit to cycling, however, the present model suggests that advocates of antimicrobial cycling will need to revisit the matter of why such benefits accrue.

Appendix

Here, we explore a simple model originally studied by Lachmann and Jablonka (38). Our analysis differs from theirs in that we fix mutation and migration and treat the rate of environmental change as our control variable; they do the converse. Our aim differs as well. We seek to illustrate the intuition that rapid environmental fluctuations reduce the ability of a population to track its environment by adaptive change in allele frequencies.

As a simplest possible case, we consider an asexual population with two genotypes, 1 and 2, undergoing unconstrained exponential growth. Growth rate is determined by the environment, which cycles symmetrically between two possible states A and B. In state A, genotype 1 grows at rate $r + d$ and genotype 2 grows at rate $r - d$. In state B, growth rates are reversed: genotype 1 grows at $r - d$ and 2 grows at $r + d$. Individuals leave the population at rate 2μ and are replaced by emigrants from an outside pool composed of 50% genotype 1 individuals and 50% genotype 2 individuals. (This migration process could alternatively be interpreted as a mutation process with rate μ.)

Let n_0 be the vector (x_1, x_2) representing the number of individuals of genotype 1 and genotype 2 at time t_0. After a single cycle of time t in state A followed by an equal time t in state B, the new population vector n_{2t} will be $n_{2t} = e^{M_2 t} e^{M_1 t} n_0$, where M_1 and M_2 are the matrices

$$M_1 = \begin{bmatrix} (1-\mu)(r+d) & \mu(r-d) \\ \mu(r+d) & (1-\mu)(r-d) \end{bmatrix}, \quad [1]$$

$$M_2 = \begin{bmatrix} (1-\mu)(r-d) & \mu(r+d) \\ \mu(r-d) & (1-\mu)(r+d) \end{bmatrix}. \quad [2]$$

For this model, the average rate of population growth gives us an excellent indication of how well the population is able to adaptively track its environment. We measure average population growth by the log asymptotic growth rate:

$$\bar{f} = \frac{1}{t} \ln(\rho[\Psi]), \quad [3]$$

where $\rho[\Psi]$ is the dominant eigenvalue of the full-cycle growth matrix [the monodromy matrix in Floquet theory (39)] given by $\Psi = e^{M_2 t} e^{M_1 t}$. To compute $\bar{f}$, we define P as the permutation matrix

$$P = \begin{bmatrix} 0 & 1 \\ 1 & 0 \end{bmatrix} \quad [4]$$

so that $M_2 = PM_1P$. This result allows us to rewrite the full-cycle growth matrix as $\Psi = e^{PM_1Pt} e^{M_1 t} = Pe^{M_1 t} Pe^{M_1 t} = (Pe^{M_1 t})^2$ and then find the dominant eigenvalue of this simpler matrix. First,

$$e^{M_1 t} = \frac{1}{\phi} e^{(1-\mu)rt} \sinh(\phi t)[M_1 - (1-\mu)rI] + e^{(1-\mu)rt} \cosh(\phi t) I, \quad [5]$$

where I is the identity matrix and $\phi^2 = \mu^2 r^2 + (1-2\mu)d^2$. With the notational simplifications $a = e^{(1-\mu)rt}$, $s = \sinh(\phi t)$, and $c = \cosh(\phi t)$, the characteristic equation

$$\phi^2 \det(Pe^{M_1 t} - \lambda I) = 0 \quad [6]$$

becomes

$$\lambda^2\phi^2 - 2\lambda\phi as\mu r + a^2 s^2 \mu^2 r^2 + a^2 s^2 d^2 - 2a^2 s^2 d^2 \mu - a^2 c^2 \phi^2 = 0. \quad [7]$$

To simplify this equation, we first eliminate d by observing that $(1-2\mu)d^2 = \phi^2 - \mu^2 r^2$. Second, because $\cosh^2 t - \sinh^2 t = 1$ always, $c^2 = 1 + s^2$. Applying these, we get

$$\phi\lambda^2 - 2as\mu r\lambda - a^2\phi = 0. \quad [8]$$

This simplification gives us the characteristic equation

$$\lambda^2 - 2\lambda \frac{\mu r \sinh \phi t}{\phi} e^{(1-\mu)rt} - e^{2(1-\mu)rt} = 0, \quad [9]$$

$$\lambda = e^{\bar{f}t}. \quad [10]$$

Finally,

$$\bar{f} = (1-\mu)r + \frac{1}{t} \ln\left[\frac{\mu r}{\phi} \sinh(\phi t) + \sqrt{1 + \frac{\mu^2 r^2}{\phi^2} \sinh^2(\phi t)} \right]$$

$$= (1-\mu)r + \frac{1}{t} \sinh^{-1}\left[\frac{\mu r}{\phi} \sinh(\phi t) \right]. \quad [11]$$

It now remains to show that $\bar{f}$ is monotone increasing in t for all $t > 0$. To do so, we appeal to the following theorem (T. C. Reluga, personal communication):

If $Q(x)$ is strictly convex and $Q(0) \leq 0$, then $Q(x)/x$ is strictly monotone increasing.

We define $Q(t) = \sinh^{-1}[(\mu r/\phi)\sinh(\phi t)]$ so that $\bar{f} = (1-\mu)r + Q(t)/t$ and note that, if $Q(t)/t$ is monotone increasing in t, so is $\bar{f}$. Differentiating $Q(t)$ twice with respect to t, we get:

$$\frac{d^2Q}{dt^2} = \frac{r\mu\phi d^2(1-2\mu)\sinh(t\phi)}{(\phi^2 + r^2u^2\sinh^2(t\phi))\sqrt{1 + \frac{r^2u^2\sinh^2(t\phi)}{\phi^2}}}. \quad [12]$$

This quantity is positive for $t > 0$ and $0 < \mu < (1/2)$, so $Q(t)$ is convex in t for $t > 0$. Because $Q(0) = 0$, application of the theorem above implies that $Q(t)$ is monotone increasing in t for $t > 0$. Thus, $\bar{f}$ is monotone increasing with t for positive t and $0 < \mu < (1/2)$. That is, whenever the mutation rate at this locus is $<50\%$, decreasing the rate of environmental fluctuation increases the population's average fit to the current selective conditions.

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