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The Population Dynamics of Antimicrobial Chemotherapy

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We present and analyze a series of mathematical models for the emergence of resistance during antibiotic treatment of an infected host. The models consider the population dynamics of antibiotic-sensitive and -resistant bacteria during the course of treatment and addresses the following problems: (i) the probability of obtaining a resistant mutant during the course of treatment as a function of antibiotic exposure; (ii) the conditions under which high, infrequent doses of an antibiotic are predicted to succeed in preventing the emergence of resistance; (iii) the conditions for the success of multiple drug treatment in suppressing the emergence of resistance and the relationship between antibiotic synergism and suppression of resistance; and (iv) the conditions under which nonadherence to the prescribed treatment regimen is predicted to result in treatment failure due to resistance. We analyze the predictions of the model for interpreting and extrapolating existing experimental studies of treatment efficacy and for optimizing treatment protocols to prevent the emergence of resistance.

The emergence of resistance to antimicrobial agents during therapy threatens the successful treatment of several important bacterial infections and in some cases risks the further spread of resistant organisms to other patients. The growth of drug-resistant subpopulations during the treatment of initially sensitive infections is a particular problem in tuberculosis chemotherapy (37), but it also occurs in the treatment of pseudomonal (19, 22), staphylococcal (51), and other bacterial (19) infections.

A number of animal and clinical studies have tested various treatment protocols for their ability to control bacterial infections and to prevent the ascent of resistant organisms that can lead to treatment failure. Two general principles have been used with some success in designing dosing regimens that suppress resistant mutants. Combination therapy (treatment with multiple antibiotics to which bacteria do not show cross-resistance) has been used with considerable success to prevent the emergence of resistance in treating tuberculosis (37) and other infections, particularly in immunocompromised patients (9, 19, 35, 36). The rationale behind combination therapy is simple: if mutants resistant to any single drug are present at frequency ϕ, then the frequency of mutants resistant to two or three drugs will be ϕ^2 or ϕ^3, respectively. Since frequencies of mutation to resistance are often on the order of 10^{-6} to 10^{-9}, bacterial populations of reasonable size in vivo are likely to contain singly resistant mutants but not mutants resistant to two or three drugs simultaneously. Therefore, at least one drug in a combination therapy regimen should be effective against all subpopulations of bacteria. Despite this logic, combination therapy does not always succeed in preventing the emergence of resistance; cases of failure have been attributed to factors including nonadherence to the treatment regimen (50), heterogeneity in drug exposure (17), and inadequate dosing.

A second means of suppressing resistant subpopulations that has been proposed and tested in vivo is the use of large, infrequent doses in antibiotic monotherapy (3, 14). Despite the success of such regimens in some experimental tests, there has been little theoretical or experimental consideration of the circumstances under which such success is expected (for a general discussion of the question in fluoroquinolones, see reference 15).

Most of the studies examining the efficacy of various dosing regimens in preventing the emergence of resistance have been purely empirical and focused on particular systems. Virtually none have employed mathematical models for the population dynamics of sensitive and resistant organisms that provide an explicit theoretical basis for how such regimens should be developed and evaluated or for predicting the conditions under which they might be expected to work.

The tools for developing such models are available. Explicit models that combine pharmacokinetics with bacterial population dynamics have been proposed to predict and optimize the effectiveness of particular dosing regimens against bacteria sensitive to the single drug considered (2, 25, 40, 54). So far, however, this literature has given little if any consideration to the problem of resistance (40).

Here we present a simple mathematical model of pharmacokinetics and bacterial population dynamics that is designed explicitly to address the problem of suppressing the emergence of resistance during treatment. Specifically, the model is used to consider the following questions: under what circumstances will resistant mutants appear and be selected during treatment? Under what conditions will a particular single-drug treatment regimen be able to suppress subpopulations with low-level resistance? Why does combination therapy sometimes but not always prevent the ascent of resistant mutants? How will the dosage, dosing frequency, and number of drugs used in combination therapy affect the ability to prevent the emergence of resistance? How does nonadherence to treatment protocols affect the likelihood that resistance will emerge, and how can protocols be modified to minimize this likelihood? The purpose of such models is to suggest general principles that may be useful in designing treatment protocols and interpreting the results of tests of such protocols.

In the present models, we restrict our consideration to treatment with bactericidal antibiotics and the evolution of resistance by mutation (see Discussion for further consideration of this assumption). We model treatment with a single antibiotic and evaluate (i) the probability that new resistant mutants will appear and be selected in a previously sensitive population under drug treatment and (ii) the conditions under which low-level resistance can be suppressed by using higher doses and

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lower frequencies of dosing. In considering combination (multiple-drug) therapy, we evaluate the effects of two major factors on the development of resistance during the course of treatment: temporal fluctuations in the concentrations of antibiotics between regular doses and nonadherence to the prescribed treatment regimen.

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THEORY

Basic mathematical model. We model the changes of concentration C of a single antibiotic, given at dose D to a host with volume of distribution V, assuming instantaneous distribution, so that the antibiotic concentration increases by $C^0 \equiv D/V$ at the time of each dose, with an interval of τ time units between doses. Elimination follows first-order kinetics with elimination constant k_{el}. The model also considers the changes in the populations of antibiotic-sensitive (S) and -resistant (R) bacteria. Bacterial kinetics assume exponential growth at a rate as follows: $g = b - x$, where g is the net growth rate of sensitive bacteria, b is the rate of cell division, and x is the death rate due to host defenses (assumed to be constant) and any other sources except the antibiotic. The corresponding terms for resistant bacteria are marked with subscript Rs. Killing by the antibiotic occurs according to an E_{max} model, where killing effect is a saturating function of the antibiotic concentration with maximum killing rate k_k and half-maximum killing achieved at concentration C_{50}. To model intermediate-level resistance, we assume that resistant bacteria are also killed by the antibiotic at a lower rate than are sensitive bacteria; the extreme case in which an antibiotic has no effect on resistant bacteria can be accommodated in the model by suitable choices of parameters ($k_{kR} \to 0$, $C_{50R} \to \infty$). Thus, the model is as follows:

$$dC/dt = -k_{el}C$$

$$C = C + C^0 \text{ (at each dosing interval } \tau)$$

$$dS/dt = gS - f(C)S$$

$$dR/dt = g_R R - f_R(C)R$$

$$f(C) = k_k \frac{C}{C + C_{50}}$$

$$f_R(C) = k_{kR} \frac{C}{C + C_{50R}}$$

This deterministic model is supplemented with a stochastic component, representing the appearance of mutants resistant to the antibiotic. These appear as a Poisson process with rate μ when sensitive bacteria replicate. Thus, in time interval dt, R is set to $R + 1$ with a probability equal to $\mu bSdt$.

Although the models are continuous, it is necessary to define the extinction of a population. To model extinction of a particular population, computer simulations of these models set populations whose sizes drop below 1 to 0.

Multidrug treatment extension of this model. In a multidrug extension of this model, we track the sensitive population plus populations of bacteria resistant to each one of the drugs used individually and in all possible combinations (three resistant subpopulations for two drugs and seven for three drugs, etc.). In this model, variables and parameters relating to populations carrying resistance to each drug are labeled with subscripts with the number(s) of the drug(s) to which the population is resistant; for example, bacteria resistant to drugs 1 and 2 are counted by the variable R_{12}.

We make one of two assumptions about the interaction between drugs. Assumption A is that drugs 1 and 2 have the same value of k_k and combine according to Loewe additivity (34), so that the drugs substitute for one another as if they were different dilutions of the same compound. Under assumption B, the killing effects of each drug act independently. This assumption that killing effects are added together would appear synergistic according to Loewe's definition (34).

For two drugs the equations read as follows:

$$dC_1/dt = -k_{el1}C_1$$

$$dC_2/dt = -k_{el2}C_2$$

$$C_1 = C_1 + C_1^0 \text{ (at each dosing interval } \tau)$$

$$C_2 = C_2 + C_2^0 \text{ (at each dosing interval } \tau)$$

$$dS/dt = gS - f_s(C_1,C_2)S$$

$$dR_1/dt = g_1R_1 - f_1(C_2)R_1$$

$$dR_2/dt = g_2R_2 - f_2(C_1)R_2$$

$$dR_{12}/dt = g_{12}R_{12}$$

$$f_1(C_2) = k_{k2} \frac{C_2}{C_2 + C_{50-2}}$$

$$f_2(C_1) = k_{k1} \frac{C_1}{C_1 + C_{50-1}}$$

$$f(C_1,C_2) = \begin{cases} k_{k1} \dfrac{C_1/C_{50-1} + C_2/C_{50-2}}{1 + C_1/C_{50-1} + C_2/C_{50-2}} & \text{(assumption A)} \\ \text{or} & \\ k_{k1} \dfrac{C_1}{C_1 + C_{50-1}} + k_{k2} \dfrac{C_2}{C_2 + C_{50-2}} & \text{(assumption B)} \end{cases}$$

As in the single-drug case, mutations to resistance to drug i occur with probability μ_i during each cell division. The mutation process, again, is modeled stochastically. Thus, for example, the expected number of R_{12} mutants in time period dt is $(\mu_1 b_1 R_2 + \mu_2 b_2 R_1)dt$.

Low-level resistance models. We used the models above to consider the effects of different dosing schedules with the same total dose against infections containing bacterial subpopulations showing partial resistance to the drug used. Since most existing work on the effects of such schedules on the suppression of resistance has been done using fluoroquinolones, we have chosen pharmacokinetic (7) and pharmacodynamic (26) parameters in ranges consistent with the literature on ciprofloxacin at standard human doses (see legend to Fig. 2). The MIC for the resistant subpopulation is set to 1.0 or 4.0 μg/ml, which is in the range of eight times the observed MIC for a number of organisms sensitive to ciprofloxacin (26). For the purposes of the model, the MIC is defined as the concentration of the drug that produces bacterial killing at a rate equal to the net growth rate in the absence of antibiotic, yielding zero net growth: $C_{50}g/(k_k - g)$. Parameters for partially resistant strains are constructed by multiplying the approximate C_{50} measured for wild-type organisms (26) by a factor equivalent to the ratio of the strains' MICs and setting k_{kR} equal to k_k and g_R equal to g.

Nonadherence model. Two models of nonadherence to the prescribed treatment regimen are considered. Under the random nonadherence model, the patient has probability P of taking each dose of drugs ($0 \leq P \leq 1$), and the decision whether to take each dose is independent of decisions concerning each of the other doses (1). Under the thermostat model of nonadherence, the patient's decision to take drugs is determined by the level of symptoms (1, 50); using bacterial numbers as surrogates for symptoms, it is assumed that the patient adheres to the treatment regimen from the beginning of treatment until the point when the total number of bacteria in the host falls below minimum value N_{min}; the patient resumes compliance when the number of bacteria has grown back to N_{max}. In this paper, N_{min} is always set to 10^4 and N_{max} is set to 10^8. Apart from these changes, the nonadherence simulations follow the equations given above.

RESULTS

Appearance and ascent of resistance in a wholly sensitive population during treatment. We begin our analysis of these models by examining the simplest case. A host is infected with S_0 bacteria which, at the start of treatment, are all sensitive to the drug used. We wish to determine the likelihood that resistance will emerge in the course of treatment. Initially, we assume that the drug is maintained at a constant concentration, $\hat{C}$ (consequences of relaxing this assumption are addressed below). Thus, the net per capita growth rate of sensitive bacteria is $b - x - f(\hat{C})$. If the drug is given at an adequate dose, the bacterial population will continuously decline, and the opportunity for the appearance of a resistant mutant is proportional to the number of cell divisions during this period of decline, up to the extinction of the sensitive population.

Mathematically, the expected number of mutants appearing during treatment, $E(M)$, is given by

$$E(M) = \mu \int_{t_0}^{t_{ext}} bS(t)dt \quad (1)$$

where μ is the mutation rate, t_0 is the time at which treatment starts, and t_{ext} is the time at which the sensitive population is extinguished. Solving the integral gives

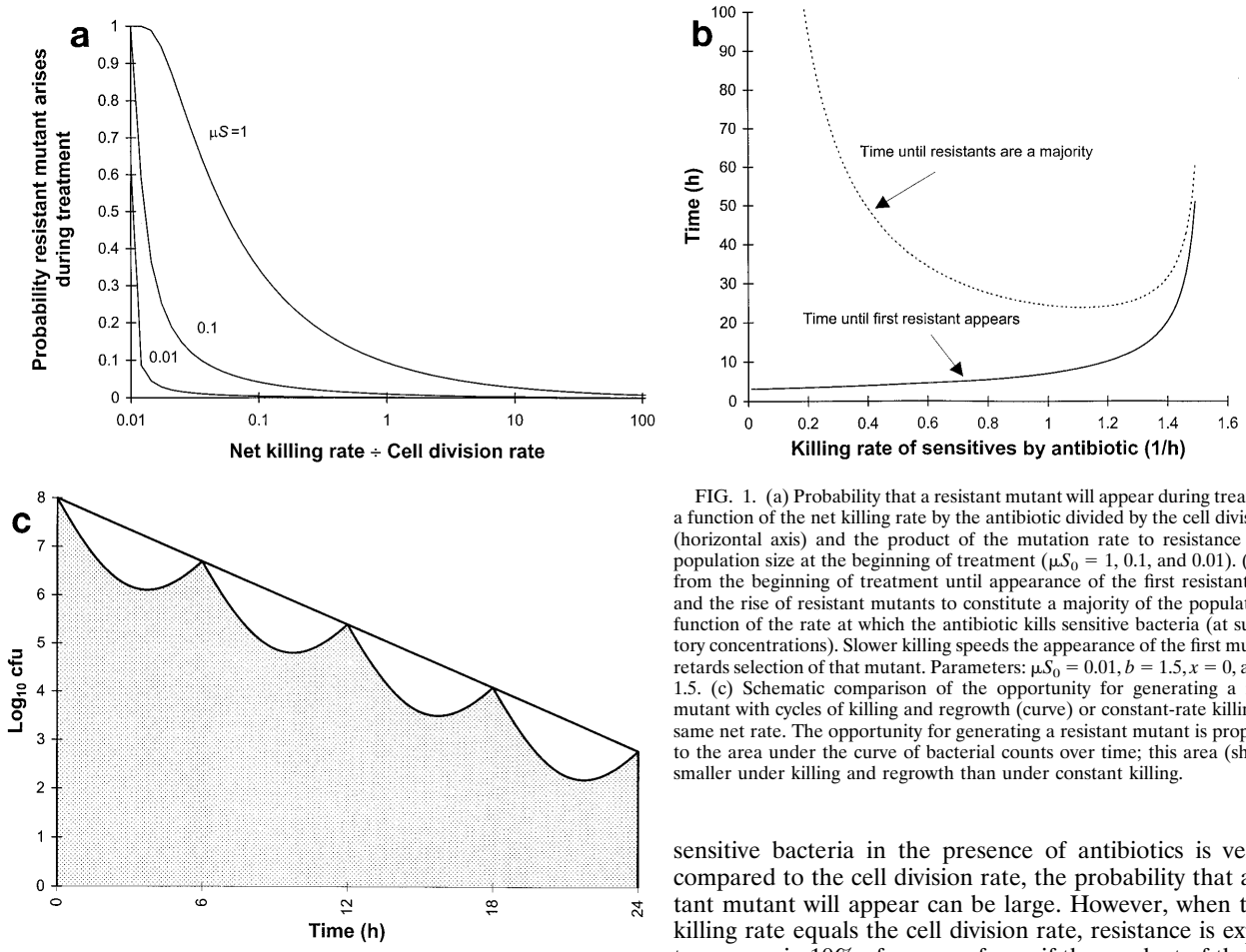

FIG. 1. (a) Probability that a resistant mutant will appear during treatment as a function of the net killing rate by the antibiotic divided by the cell division rate (horizontal axis) and the product of the mutation rate to resistance and the population size at the beginning of treatment ($\mu S_0 = 1$, 0.1, and 0.01). (b) Time from the beginning of treatment until appearance of the first resistant mutant and the rise of resistant mutants to constitute a majority of the population as a function of the rate at which the antibiotic kills sensitive bacteria (at subinhibitory concentrations). Slower killing speeds the appearance of the first mutant but retards selection of that mutant. Parameters: $\mu S_0 = 0.01, b = 1.5, x = 0$, and $b_R = 1.5$. (c) Schematic comparison of the opportunity for generating a resistant mutant with cycles of killing and regrowth (curve) or constant-rate killing at the same net rate. The opportunity for generating a resistant mutant is proportional to the area under the curve of bacterial counts over time; this area (shaded) is smaller under killing and regrowth than under constant killing.

$$E(M) \approx \mu S_0 \frac{b}{x + f(\hat{C}) - b} \quad (2)$$

the product of the mutation rate, the initial population size, and the cell division rate divided by the net rate of decline. If the rate of cell division in the absence of any host defenses or drug (b) has the same order of magnitude as the total rate of decline in the presence of host defenses and the drug, then $E(M)$ is $\approx \mu S_0$. This number is the same as the expectation of the number of mutants present in the population at the start of treatment. If the existing population contains no resistant mutants, that suggests that μS_0 is less than 1. Therefore, if no resistant mutants are present at the start of treatment and if the antibiotic (plus any host defenses) kills bacteria at a rate such that their net decline occurs at a rate comparable to their intrinsic rate of cell division, then no mutants are likely to arise during treatment. Several studies of mouse thigh infections with gram-negative bacteria and in vitro models of antibiotic killing suggest that realized killing rates can indeed be as fast as or faster than growth rates in the absence of drug or host defenses (10, 20, 22, 29).

Figure 1a shows how the probability that a resistant mutant will appear during treatment depends on the mutation rate and initial population size of sensitive mutants and on the cell division and net killing rates. This is calculated by using the Poisson assumption that the probability a mutant will appear is $P(M) = 1 - \exp[-E(M)]$. When the net rate of decline of sensitive bacteria in the presence of antibiotics is very low compared to the cell division rate, the probability that a resistant mutant will appear can be large. However, when the net killing rate equals the cell division rate, resistance is expected to emerge in 10% of cases or fewer if the product of the initial population size and the mutation rate is less than 0.1. As the figure shows, this probability falls off rapidly as killing rates increase or the product of the initial population size and the mutation rate decreases.

If the antibiotic concentration is low enough to allow net growth of the sensitive population, it will eventually be large enough to give rise to a resistant mutant. The first resistant mutant will occur, on average at a time after the start of treatment (t_1) as follows:

$$t_1 = \frac{1}{b - x - f(\hat{C})} \ln\left(\frac{b - x - f(\hat{C})}{S_0 b \mu} + 1\right) \quad (3)$$

The first appearance of a resistant mutant occurs later as the killing rate increases, since the growth of the sensitive population is thereby delayed (Fig. 1b). Once the resistant mutant has appeared, the ratio of sensitive to resistant cells will change at a rate of $b' - b + f(\hat{C})$ per unit of time, which will make resistant bacteria the majority population at a time after the first resistant mutant appears (t_2) as follows:

$$t_2 = \frac{1}{b' - b + f(\hat{C})} \ln\left(S_0 + \frac{b - x - f(\hat{C})}{b\mu}\right) \quad (4)$$

Thus, the time from the start of treatment until resistant bacteria reach the majority is $t_1 + t_2$ (Fig. 1b). Resistant mutants reach a majority of the population fastest when the killing rate $f(\hat{C})$ reaches an intermediate value. Selection is most efficient at this intermediate value because when killing is quite fast, the first resistant mutant takes a long time to appear, but when

killing is too slow, the selection in favor of the resistant mutant becomes weak. The killing rate $f(C^*)$ that minimizes the time before resistant bacteria constitute a majority of the population can be obtained by setting

$$\frac{d(t_1 + t_2)}{df(C)} \Big|_{f(C)=f(C^*)} = 0$$

C^* can be calculated numerically from the following implicit equation:

$$\ln\left[\frac{h(C^*)}{S_0\, b\mu} + 1\right]/h(C^*) - \ln\left[\frac{h(C^*)}{b\mu} + S_0\right]/[b' - h(C^*)]^2 - \frac{b'}{h(C^*)[b - h(C^*)][h(C^*) - S_0\, b'\mu]} = 0 \quad (5)$$

where $h(C)$ is defined to mean $b - x - f(C)$, and all other variables are as stated in Theory.

This prediction is consistent with the frequent observation that growth of bacteria in subinhibitory concentrations can select effectively for resistant mutants (5, 6, 16, 23, 30, 32, 52). The model predicts that such selection will be most efficient at an intermediate rate of antibiotic-mediated killing.

The results in this section assume that the drug concentration is constant and that bacterial cell division continues at its normal pace in the presence of antibiotic; all effects of the antibiotic are attributed to replication-independent killing of cells. Relaxing either assumption decreases the probability that new resistant bacteria will appear.

If the bacterial cell division rate is a decreasing function of drug concentration, then the expected number of resistant mutants is proportional to $S_0\ b(\hat{C})/[x + f(\hat{C}) - b(\hat{C})]$, which is less than the probability predicted above.

In a treated host, drug concentrations will fluctuate between doses, possibly permitting regrowth of the drug-sensitive bacterial population (10); a mathematical description of this process has been published for the first-order kinetics/E_{max} model used here (2, 54). As shown in Fig. 1c, the number of cell divisions when concentrations fluctuate between doses will be less than the number observed if the net rate of decline remained constant at its average value over the entire dosing interval. The expected number of new mutants arising during treatment is equal to cell division rate b times mutation rate μ times the area under the curve of bacterial numbers (from equation 1). As Fig. 1c demonstrates, the area is less in the case of killing and regrowth than under constant killing at the average rate; therefore, the probability of emergence is correspondingly less.

Suppression of mutant subpopulations with low-level resistance. For a number of drug-organism combinations, resistant subpopulations are already present at the start of treatment. In many cases, these initially resistant subpopulations are still partially sensitive to the drug used, with MICs 4 to 16 times those for the majority population (14, 22–24, 28, 39, 44, 47). High peak concentrations of antibiotics have been proposed and tested in vitro and in animal models as a way of eliminating these minority populations of intermediate resistance (3, 14). We have modeled the treatment of infections with small, partially resistant subpopulations to study the predicted effects of such strategies.

The E_{max} model implies that the marginal effectiveness of a given quantity of antibiotic decreases as concentration increases—that is, the effects of antibiotic increase less than linearly with concentration. Under this model, increasing the peak concentration by giving the same total amount of drug per unit of time in less frequent, larger doses will always decrease the total effect over the dosing interval (25). Therefore, if the dose-response function of partially resistant bacteria follows an E_{max} model, the use of high peak concentrations should be less effective against this population over the course of a whole dosing interval than the same total amount of antibiotic given more frequently in smaller doses.

However, high peak concentrations may prevent the outgrowth of low-level resistant strains in another way: by driving the resistant subpopulation to extinction with an early, high dose, before the concentration drops low enough to allow regrowth of the subpopulation. This strategy takes advantage of the fact that resistant subpopulations will initially be small, so sustained killing over a whole dosing interval may not be necessary to extinguish them. Not surprisingly, the E_{max} model predicts that a large dose will have a greater maximum killing effect than a small one, although the total reduction in the bacterial population over a 24-h period will be less for large, infrequent doses than for the same total dose given in smaller, more frequent administrations.

For an E_{max} model with first-order pharmacokinetics the number of bacteria (B) remaining after time t from the administration of dose $C(0)$ is given (2, 54) by

$$\ln\frac{B(t)}{B(0)} = gt + \frac{k_k}{k_{el}} \ln \frac{C_{50} + C(0)\exp(-k_{el}t)}{C_{50} + C(0)} \quad (6)$$

and the bacterial population reaches its minimum value at a time (T_{min}) as follows:

$$T_{min} = \frac{1}{k_{el}} \ln\left[\frac{C(0)}{C_{50}} \cdot \frac{k_k - g}{g}\right] \quad (7)$$

By using equations 6 and 7, the minimum number of bacteria during the dosing interval is as follows:

$$\ln B_{min} = \ln B(T_{min}) = \ln B(0) + \frac{g}{k_{el}} \ln\left(\frac{C(0)}{C_{50}} \cdot \frac{k_k - g}{g}\right) - \frac{k_k}{k_{el}} \ln\left(\frac{C_{50} + C(0)}{C_{50}} \cdot \frac{k_k - g}{g}\right) \quad (8)$$

By applying this equation to the subpopulation with low-level resistance (with appropriate values of k_k, g, and C_{50}), it is possible to calculate the maximum decrease in the (logarithmic) population size during the first dosing interval, as well as the change in the bacterial population over an entire dosing interval, for a range of dosing schedules. Figure 2 shows the predicted effects of different dose fractionation regimens using the same total dose over 24 h. As described above, the overall effect in 24 h is greatest for small, frequent doses, while the maximum kill is greatest for large, infrequent doses. The value of the overall decline is given assuming that the peak concentration has reached its steady-state value (due to pharmacokinetic accumulation), while the value for the maximum drop is given for the first dose. Values are shown assuming MICs of 1 μg/ml (panel a) and 4 μg/ml (panel b).

Figure 2 shows that the efficacy of large, infrequent doses against resistant subpopulations depends on the parameters of dose-effect relations and drug elimination. Under certain circumstances, such as those in Fig. 2b, the high-dosing strategy is predicted to be effective in extinguishing a small population of partially resistant organisms. The equivalent of a single intravenous dose of 400 mg (see figure legend for details) produces a maximum decline of about 1 $\log_{10}$ in the population of bacteria for which the MIC is 4 μg/ml. This is the best outcome of any dosing regimen in Fig. 2b, since none of the dosing

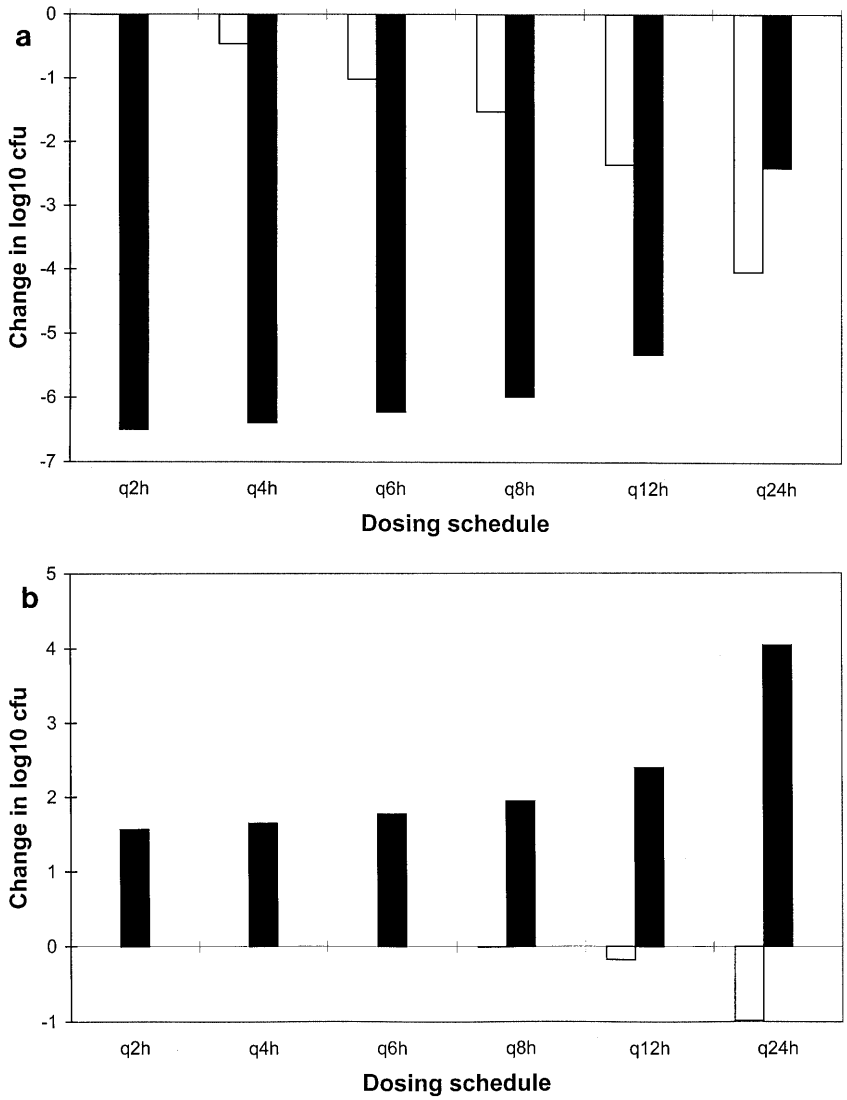

FIG. 2. Model's predicted effects of giving the same total daily dose fractionated every 2, 4, 6, 8, 12, and 24 h (qh2 and qh4, etc.) on the killing of partially resistant bacteria. White bars show maximum decline in the partially resistant subpopulation obtained during the first dosing interval. Black bars show change in the partially resistant population over 24 h. Shown are predictions for partially resistant strains for which the MIC is 1 μg/ml (a) and 4 μg/ml (b). Parameters are specific for human dosing of ciprofloxacin: half-life, 4 h; C^0 = 6.7 μg/ml for a 400-mg intravenous dose (7) (assumed to be linearly related to dose for other doses); total dose = 800 mg (intravenous)/day; k_k = 2.4/h; g = 1.2/h (26). Total effect over 24 h declines with less frequent doses in both cases, while maximum killing improves with less frequent doses. For the subpopulation for which the MIC = 4 μg/ml (b), decreased frequency is beneficial, but decreased frequency is less effective for the subpopulation for which the MIC = 1.0 μg/ml.

schedules is capable of producing a net reduction over the whole 24-h period. In Fig. 2a, however, a subpopulation with low-level resistance (MIC = 1 μg/ml) is better controlled by more frequent dosing, since all schedules considered can produce net killing over the full 24 h. While increasing the size of and interval between doses increases the maximum effect, it decreases the total effect over the dosing interval.

Multiple-drug treatment: conditions for suppressing resistant mutants and necessity of suppressing singly resistant subpopulations. Treatment failure with multidrug regimens occurs as a result of the outgrowth of singly resistant subpopulations, which may then give rise to doubly or multiply resistant mutants. In multidrug therapy of an infection that includes subpopulations resistant to either of the drugs individually, the fate of these singly resistant populations is likely to be important to treatment success.

Under combination antibiotic therapy, subpopulations resistant to one of the drugs effectively experience single-drug treatment. Therefore, the results given above for the probability of single-drug resistance arising in a sensitive population under single-drug therapy are applicable directly to the probability that resistance to a second drug will arise in a singly resistant subpopulation. If a subpopulation resistant to one of the drugs in a two-drug regimen is present at the start of treatment, then rapid killing of this subpopulation (relative to the rate of cell division) will minimize the likelihood that multiple resistance will emerge.

Since the successful suppression of resistance depends on a treatment regimen's effect on singly resistant subpopulations, combination therapy regimens designed to maximize killing of fully sensitive organisms may not be optimal for preventing the growth of resistant subpopulations. Figure 3 shows the simu-

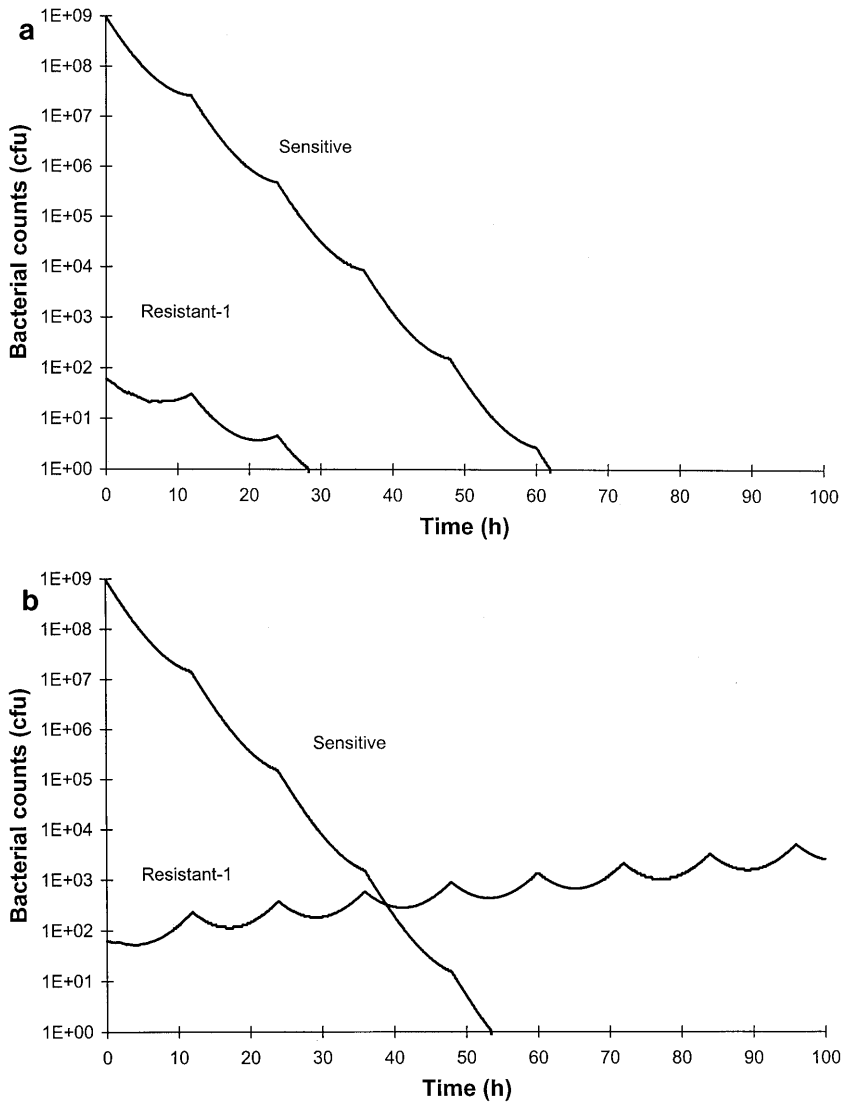

FIG. 3. Comparison of two combination dosing regimens for their ability to kill the majority, sensitive population and a subpopulation resistant to antibiotic 1. Peak concentrations used are 4 μg of both drugs per ml (a) and 8 μg of drug 1 per ml plus 2 μg of drug 2 per ml (b). Drugs are assumed to act according to Loewe additivity, so the larger total dose in panel b results in faster clearance of the susceptible population. However, the dose of drug 2 in panel b is inadequate to suppress mutants resistant to drug 1, resulting in the outgrowth of drug 1-resistant mutants. Parameters: $g = g_R = 0.6$/h; $k_k = 1.25$; $C_{50} = 1.0$; half-life, 4 h. All parameters (except dose) are the same for both drugs.

lated dynamics of a bacterial population that is majority sensitive but contains a subpopulation resistant to one antibiotic, drug 1. The infection is treated with two drugs (1 and 2) that have identical dose-response functions and combine according to Loewe additivity; however, they do not show cross-resistance (such a relationship is unlikely in practice but serves as an illustration of the principle). Figure 4a shows treatment with equal doses of both drugs, while Fig. 4b shows the same infection treated with twice as much drug 1 and half as much drug 2 as in Fig. 4a.

Because the drugs are interchangeable (show Loewe additivity) and more total drug is used in Fig. 4b, the sensitive population is cleared more quickly there. However, only the regimen in Fig. 4a is capable of suppressing the subpopulation resistant to drug 1. This occurs because the prevention of resistance depends on effective killing by a drug alone, while the rapid clearance of the majority population depends on the combined effect of the drugs given. Simulations using other functions for drug interactions, including the simple form of synergism given by assumption B in Theory, can produce the same effect.

Nonadherence as a form of inadequate dosing. Nonadherence to the treatment regimen may also permit net growth of singly resistant subpopulations. The model defines conditions under which this will occur.

(i) Random nonadherence. Under random nonadherence, a patient takes each dose of combined antimicrobial treatment with a probability P between 0 and 1 and does not compensate afterwards for the failure to take a particular dose. This form of nonadherence will favor the growth of singly resistant strains in two ways. First, each missed dose will permit bacteria to grow under conditions of reduced or effectively zero antibiotic concentrations. Second, in frequent-dosing regimens, a missed dose may decrease the effective concentration of antibiotics

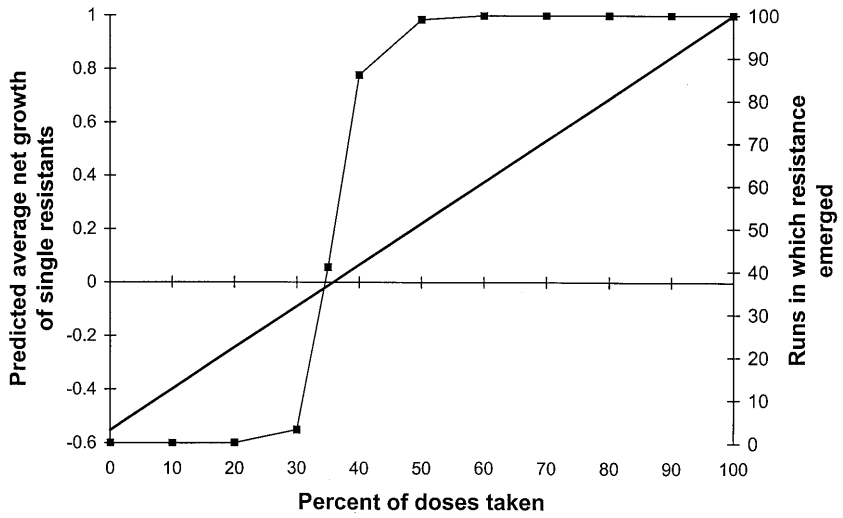

FIG. 4. Emergence of multiple resistance under random nonadherence to the treatment regimen is related to growth of singly resistant subpopulations. Predicted net growth of singly resistant subpopulations during a treatment interval (straight line) and number of runs (out of 100 in a Monte Carlo simulation) in which multiple resistance emerged (squares) increases with the probability of nonadherence to each dose; the threshold for the emergence of resistance is approximately zero net growth of singly resistant bacteria. Parameters: $\mu_1 = 2 \times 10^{-8}$/cell division; $\mu_2 = 2 \times 10^{-10}$/cell division (13); g = 1/day; k_k = 2.5/day for drugs 1 and 2; half-life, ≈2 h; C^0 = 15 μg/ml; C_{50} = 0.1 μg/ml. Drug interaction: assumption A. See Theory for other parameters.

during the next treatment interval by reducing pharmacokinetic accumulation (43). Although the effects on accumulation theoretically last into all future dosing intervals, in practice, the effects will be negligible in all dosing intervals after the next. Hence, we model only these first- and second-order effects and ignore all higher-order effects of missed doses—effects on the growth of the bacteria in dosing intervals after the one immediately following the missed dose. This assumption introduces a less than 1% error into concentrations for treatment regimens in which the peak/trough ratio of concentrations exceeds 10:1 (equivalently, for which the time between doses is longer than approximately 3.3 drug half-lives).

With these assumptions, equation 6 can be modified to show the effects of partial compliance with the treatment regimen on the growth of singly resistant subpopulations. The population resistant to drug 1 (susceptible only to drug 2) will grow according to the following pharmacodynamic equation:

$$\ln \frac{B(t)}{B(0)} \approx g_1 t + \frac{k_{k2} t}{k_{el2}\tau}\left\{P^2 \ln \frac{C_{50-2} + \alpha(1+\alpha)C_2^{\,0}}{C_{50-2} + (1+\alpha)C_2^{\,0}} + P(1-P)\left[\ln \frac{C_{50-2} + \alpha C_2^{\,0}}{C_{50-2} + C_2^{\,0}} + \ln \frac{C_{50} + \alpha^2 C_2^{\,0}}{C_{50} + \alpha C_{50}^{\,0}}\right]\right\} \quad (9)$$

Here, t is the total time since the start of treatment and τ is the time between doses; thus, there have been t/τ dosing intervals and the patient has taken his drugs, on average, Pt/τ times. The fraction of a given dose that is left at the time of the next dose is given as $\alpha = \exp(-k_{el}\tau)$.

The first term of equation 9 refers to the effect of the drug during dosing intervals in which the patient has taken the prescribed dose and did so the previous dosing interval, as well. The second term considers dosing intervals where the patient takes the dose but did not in the previous interval (thus, there is no accumulation factor), and the third considers the residual effects of the previous dose in cases where it was taken but the present dose was not. These situations occur with probabilities P^2, $P(1 - P)$, and $P(1 - P)$, respectively. Figure 4 shows the expected growth rate of singly resistant populations in a two-drug treatment regimen under different levels of random nonadherence and the number of runs (out of 100) in which multiple resistance emerged in a Monte Carlo simulation using these parameters and treatment with two drugs. As the figure demonstrates, the average growth of singly resistant populations, as specified by equation 9, is a good predictor of the outcome of stochastic simulations; when it is positive, double resistance tends to appear, and when it is negative, treatment nearly always succeeds in extinguishing the bacterial population.

(ii) Thermostat nonadherence. Under the thermostat scheme of nonadherence, selection of singly resistant mutants occurs because they are killed more slowly during periods of drug taking and then grow back during periods of nonadherence to form a larger fraction of the population. This process of enrichment is shown in Fig. 5a. Once the singly resistant population becomes sufficiently large, it may give rise to a doubly resistant mutant, setting the stage for treatment failure (in the case of treatment with two drugs to which the infecting organism is sensitive) or stepwise evolution of more resistance (in the case of treatment with more than two such drugs).

There are two necessary conditions for the selection of a singly resistant subpopulation by thermostat nonadherence. The first is that the singly resistant subpopulation must not go extinct during the period of compliance with the treatment regimen. Although singly resistant bacteria are (presumably) killed more slowly than the majority, sensitive population during periods of drug taking, the population of these bacteria is also smaller and therefore more likely to be extinguished during periods of compliance. Mathematically, the condition for a singly resistant population to persist during the first period of compliance is

$$\frac{\delta_S}{\delta_R} \ln (\phi N_{\max}) > \ln \frac{N_{\max}}{N_{\min}} \quad (10)$$

where δ_S and δ_R are the average rates of killing of sensitive and singly resistant bacteria, respectively; ϕ is the frequency of single-drug-resistant mutants in the population at the beginning of treatment; and $N_{\max}$ and $N_{\min}$ are the bacterial population sizes at which the patient begins treatment and stops

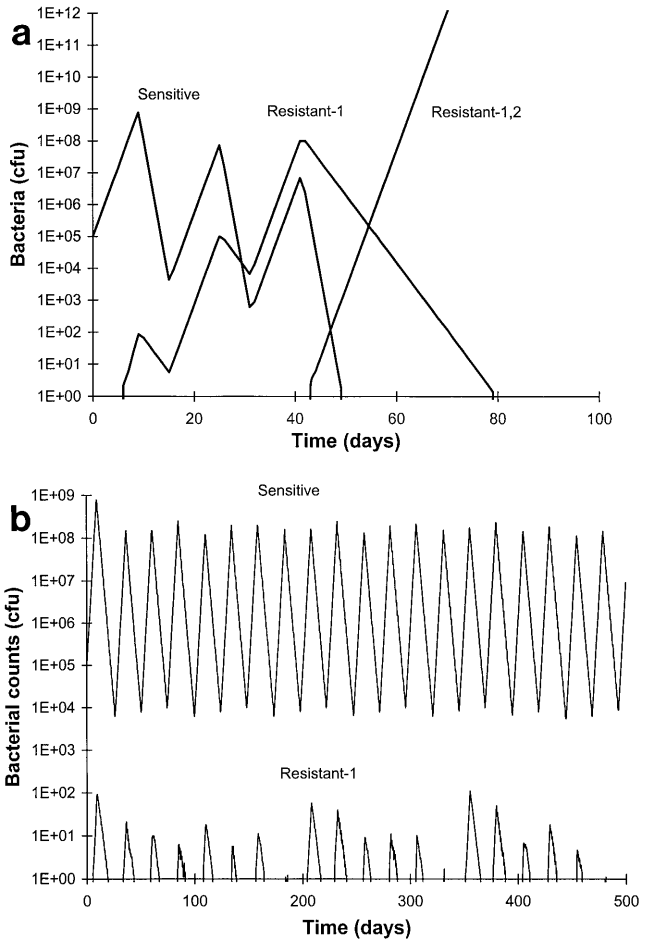

FIG. 5. Enrichment of singly resistant bacteria during thermostat noncompliance requires survival of the singly resistant subpopulation during periods of adherence to the treatment regimen, which in turn is most likely when killing rates of sensitive and singly resistant bacteria are similar. Shown are two simulations of treatment with thermostat noncompliance, in which enrichment occurs (a) or does not occur (b). (a) The interaction between drugs is synergistic; (b) drug interaction is Loewe additive. Parameters are as described in the legend to Fig. 4.

taking the prescribed antibiotics, respectively. Figure 5b shows the extinction of resistant subpopulations during periods of compliance; despite repeated periods of noncompliance, singly resistant populations are not enriched and therefore never become sufficiently large to give rise to doubly resistant mutants.

The second condition is that the effect of the cycle of adherence (as the bacterial population drops from N_{max} to N_{min} and then regrows to N_{max}) must be an increase in the proportion of the population that is singly resistant. This condition is simply

$$\frac{\delta_S}{\delta_R} > \frac{g_S}{g_R} \quad (11)$$

The resistant strain's advantage in net killing rate in the presence of drug is greater than its disadvantage in growth rate in the absence of drug.

Both conditions for the emergence of resistance require that δ_S/δ_R be sufficiently large. This ratio of the effect of treatment on sensitive organisms to that on singly resistant organisms is one measurement of synergism, since sensitive organisms experience the combined effects of all drugs, while singly resistant bacteria avoid the effects of one drug. This indicates that under thermostat nonadherence, highly synergistic drug combinations may not be optimal for the prevention of resistance. In fact, the only difference between Fig. 5a, where resistance emerges due to nonadherence, and Fig. 5b, where it does not, is that the former assumes that two drugs interact according to Loewe additivity (assumption A [see Theory]) while the latter assumes a greater degree of synergism—in which killing effects are additive (assumption B [see Theory])—between the two drugs (so that the overall interaction between the drugs is synergistic by Loewe's definition [34]). More generally, any regimen that kills singly resistant bacteria at a rate more similar to that of fully sensitive bacteria is less likely to result in enrichment of resistant strains under thermostat noncompliance.

DISCUSSION

We have analyzed a quantitative pharmacokinetic/pharmacodynamic model of the population dynamics of bacteria under single- and multiple-antimicrobial treatment. The analysis was performed with a view toward providing testable hypotheses about the mechanisms behind the emergence and prevention of resistance and the design of dosing regimens to avoid treatment failure resulting from resistance.

Our model predicts that resistance will rarely emerge in a population of antibiotic-sensitive bacteria under treatment with a given antibiotic when (i) resistant mutants are not present at the start of treatment and (ii) the average net rate of decline in the bacterial population during treatment is comparable to the rate of cell division. We have derived mathematical expressions to predict the time until the emergence of resistance when these conditions are not met. The model predicts that sustained exposure to subinhibitory concentrations should effectively select for resistance and that the effectiveness of selection, as measured by the time until resistant mutants form a majority of the population, is maximized at an intermediate rate of antibiotic-mediated killing. The theoretical treatment represents an advance over previous treatments in that (i) it incorporates the stochastic and discrete nature of resistance mutations and (ii) it predicts that an intermediate concentration provides the maximum rate of selection. For an earlier quantitative treatment of the emergence of resistance at various antibiotic concentrations, see reference 32.

The model can also account for the efficacy of large, infrequent doses of a single drug against bacterial populations containing a small minority of partially resistant bacteria, but only in a limited range of parameters; for other parameters, more frequent, smaller doses are predicted to be more effective. The models therefore indicate that promising results with high-dose chemotherapy as a means of suppressing resistance (3, 14) may not generalize to other drug-organism combinations. In addition, the models suggest how dose-kill rate functions for sensitive and resistant bacteria can be used to predict circumstances where such regimens may be useful.

In the multiple-drug version of the model, treatment failure (due to the outgrowth of singly resistant bacteria or the appearance of multiply resistant variants) occurs when, for any reason, singly resistant subpopulations experience net growth. This prediction is consistent with experimental results (22, 27, 37, 46), and the model emphasizes the similarities between inadequate dosing and various forms of nonadherence to the treatment regimen in the mechanism of selecting resistance. Monte Carlo simulations of random nonadherence, for exam-

ple, show that net growth or net decline of singly resistant mutants under the realized treatment regimen predicts well whether multiple resistance will arise. The importance of suppressing singly resistant populations points out that a regimen optimized for its synergistic effect against the sensitive majority population may or may not be optimal for suppressing resistance.

The purpose of the models considered here is not (at least initially) to fit curves through points but rather to suggest (i) predictions that may be tested in vitro and in animal models for how dosing regimens may be optimized and (ii) interpretations of existing data and how such data may or may not be extrapolated. Like all such models, this model makes a number of simplifying assumptions. Before considering the predictions and interpretations stemming from the model, we briefly review some of its most limiting assumptions.

First, the model makes simplistic assumptions about the immune system: (i) the death rate of bacteria from all immune mechanisms is constant and the same for sensitive and resistant populations, and (ii) the immune system is not saturable; that is, small populations and large populations of bacteria are subject to the same degree of inhibition by the immune system. Both assumptions may be well approximated in severely immunocompromised hosts (3), who form one of the major populations for whom emergence of antibiotic resistance during treatment is a major problem. For example, experiments with *Pseudomonas aeruginosa* in neutropenic mice appear to conform roughly to these assumptions (22). In the case of tuberculosis, there are clearly major changes in the antimycobacterial immune response over the course of infection, but during the course of antimicrobial treatment of frank tuberculosis, it may again be reasonable to assume that immune-mediated killing of mycobacteria remains at a constant level. In acute infections of immunocompetent hosts, these assumptions may not be met. Testing the assumptions is an important avenue for future experimental work.

In the model of dose-response relationship, we assume that killing follows a standard E_{max} model, which implies strictly decreasing marginal effects of antibiotics at higher doses, and we make no provision for a postantibiotic effect. Both of these assumptions tend to increase the effectiveness of low, sustained doses relative to that of high, infrequent doses (if the total amount of a drug given per unit of time is the same). A more complex model, such as a sigmoid E_{max} dose-response curve, may be more favorable to high, infrequent doses, because at low concentrations in such a model, the response increases more than linearly with increases in the dose. Likewise, incorporation of a postantibiotic effect increases the efficacy of fractionated dosing regimens (11, 15).

Furthermore, we assume that the exposure of the bacterial population to antibiotic action is homogeneous. Heterogeneities in antibiotic exposure have been identified in tuberculosis (17, 38) and other infections (12, 41), and we are in the process of modeling the effects of such heterogeneity (33), which is thought to contribute to the emergence of resistance (17, 37).

Finally, the model is based on the assumption that resistance occurs by mutation, which is, of course, only one of the mechanisms by which bacteria become resistant to antibiotic action. Therefore, the model is directly applicable principally to the treatment of mycobacterial and pseudomonal infections and to the use of fluoroquinolones and, for some bacteria, beta-lactams, for which mutational resistance is known. In certain cases, however, recombinational events, including plasmid transfer, can be modeled like mutation, since the frequency of such events is expected to be low and proportional to the number of sensitive bacteria present (31, 49). Such recombinational events probably occur in vivo during antibiotic treatment (45), although their frequency is unknown.

Despite these limitations and assumptions, the model makes a number of robust predictions that can be tested in dosing regimen studies. Among these predictions are the following.

(i) The elimination of partially resistant subpopulations during the first dosing interval is likely to be the key to the success of dosing regimens using large, infrequent doses. This is because the model predicts that for many realistic parameter combinations, the resistant subpopulations would experience net growth over an entire dosing interval if they survive the initial peak concentrations. Therefore, the benefits garnered from such regimens should be obtainable by a single (first) high dose, regardless of whether the succeeding doses are large and infrequent or smaller and more frequent. The only empirical study of which we are aware that has tested dosing regimens for their ability to suppress populations with low-level resistance while monitoring bacterial numbers indeed found that with a high dose, the bacterial population (both sensitive and resistant) was extinguished in the first dosing interval, before it had a chance to regrow (3).

(ii) Studies of synergism (18), while important in themselves, will not be predictive of the ability of combination therapy regimens to suppress the emergence of resistance. Rather, suppression of resistance is likely to correlate with the activity of each antibiotic alone against mutants resistant to the other antibiotic. For further discussion of the relationship between synergism and efficacy, see the work of Zinner et al. (55).

(iii) The effects of random nonadherence to the treatment regimen (for which directly observed therapy is presently recommended as a preventive measure [8]) may be lessened by more potent combinations of drugs. This is because regimens that kill rapidly will be able to accommodate more regrowth during intervals of nonadherence and still maintain a net decline in all subpopulations. This possibility, of course, should be weighed against the possibility that more rigorous courses of treatment increase the patient's inclination not to take his or her prescribed medicines.

Another interesting prediction or interpretation offered by the model, particularly for tuberculosis, is that nonadherence should be especially favorable to the development of resistance in immunocompromised patients. Under random nonadherence, this occurs because of the same mechanism just discussed; a greater fraction of doses must be taken to maintain net killing of the bacterial population if the immune system is not contributing to the suppression of bacterial growth. Under the thermostat model of nonadherence, addition of an immune killing term for both sensitive and resistant populations will decrease the ratio of killing rates between the two strains, making it less likely that inequalities 10 and 11 will be satisfied. Epidemiological studies of the emergence of drug resistance in human immunodeficiency virus-infected patients are beginning to appear (4), and such data should provide tests of the model's prediction; if it is correct, compromised immunity and nonadherence should have a more than additive effect in increasing the likelihood that resistance will emerge during treatment.

The development of theoretical frameworks for understanding antibiotic dose-effect relations (21), kinetics of populations of tumor cells (48) and infectious agents (53) in response to antimicrobial treatment, and the interactions between drugs (34, 42) has yielded important insights into the biology underlying these processes and the optimization of therapy. Continued development and testing of mathematical models of the population dynamics of drug resistance during treatment of bacterial infections will be important in the effort to evaluate

and improve treatment courses with existing and new antibacterial agents.

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