

Population Dynamics of Antibiotic Treatment: a Mathematical Model and Hypotheses for Time-Kill and Continuous-Culture Experiments[∇]

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The objectives of the study were to develop a quantitative framework for generating hypotheses for and interpreting the results of time-kill and continuous-culture experiments designed to evaluate the efficacy of antibiotics and to relate the results of these experiments to MIC data. A mathematical model combining the pharmacodynamics (PD) of antibiotics with the population dynamics of bacteria exposed to these drugs in batch and continuous cultures was developed, and its properties were analyzed numerically (using computer simulations). These models incorporate details of (i) the functional form of the relationship between the concentrations of the antibiotics and rates of kill, (ii) the density of the target population of bacteria, (iii) the growth rate of the bacteria, (iv) byproduct resources generated from dead bacteria, (v) antibiotic-refractory subpopulations, persistence, and wall growth (biofilms), and (vi) density-independent and -dependent decay in antibiotic concentrations. Each of the factors noted above can profoundly affect the efficacy of antibiotics. Consequently, if the traditional (CLSI) MICs represent the sole pharmacodynamic parameter, PK/PD indices can fail to predict the efficacy of antibiotic treatment protocols. More comprehensive pharmacodynamic data obtained with time-kill and continuous-culture experiments would improve the predictive value of these indices. The mathematical model developed here can facilitate the design and interpretation of these experiments. The validity of the assumptions behind the construction of these models and the predictions (hypotheses) generated from the analysis of their properties can be tested experimentally. These hypotheses are presented, suggestions are made about how they can be tested, and the existing statuses of these tests are briefly discussed.

In accordance with the rational design of protocols for antibiotic therapy, dosing regimens are based on the changes in the concentration of the antibiotic during the course of treatment (pharmacokinetics [PK]) and on the in vitro relationship between the concentration of that antibiotic and the growth or death rate of the target bacteria (pharmacodynamics [PD]). Together, these factors comprise the PK/PD indices (1, 19), which are employed as a priori estimates of the potential efficacy of antibiotic treatment regimens. At least three different measures of the PK are used for these indices: (i) peak antibiotic concentration (C_{\max}), (ii) time above the MIC, and (iii) area of the antibiotic concentration curve above the MIC (AUC/MIC) (3, 4, 15, 24, 40, 50). Although antibiotics are classified as time or concentration dependent, the only formal pharmacodynamic parameter used in these indices is the MIC (3, 9, 27), which is also the dominant parameter employed as a measure of the susceptibility (or resistance) of bacteria to antibiotics (14, 21, 39). MICs are estimated in vitro using protocols that are precisely defined for each antibiotic-bacterial species combination (14) and conditions that are optimal for the action of the drug: relatively low ($<10^6$) densities of bacteria growing exponentially in liquid media at temperatures and under ionic conditions in which the drug is most effective.

It is well established that pharmacodynamic parameters

other than MICs measured under the optimal conditions specified by the CLSI protocols are likely to affect the course of antibiotic treatment. Included among these are (i) the functional form of the relationship between the concentration of an antibiotic and the rate of growth or death of bacteria (the pharmacodynamic function) (49), (ii) the density of the bacterial population (18, 52, 57), (iii) ionic conditions (pH and cation concentrations) (2, 5, 9, 16), (iv) the presence of nondividing or slowly dividing subpopulations of bacteria (persistence) (8, 10, 32, 58), (v) the physical structure of the bacterial population, e.g., biofilms (25, 46, 55), and (vi) mortality and delayed replication of antibiotic-exposed bacteria following the elimination of the antibiotic (postantibiotic effects) (16, 34, 35).

At least two in vitro procedures have been used to address some of these limitations of MICs as measures of the in vitro efficacy of antibiotics. One is the classical time-kill experiment where, for a defined amount of time, bacteria in liquid culture are exposed to different concentrations of bactericidal antibiotics. The estimated rate of decline in density of viable cells in these experiments is used as a measure of the efficacy of the antibiotic (4, 30, 41, 49, 57). Another approach designed to more comprehensively explore the efficacy of antibiotics in vitro is to add these drugs to bacteria maintained in continuous culture devices (9, 11, 12, 31, 33, 43, 44, 48) and then follow the changes in viable-cell density. With this continuous-culture approach, it is possible to evaluate the efficacy of bacteriostatic as well as bactericidal drugs and to do so in situations in which the concentration of the drug is continually changing. These continuous-culture devices can also be used to explore the role of biofilms, such as those on the surfaces of the vessels (7, 13, 26), and postantibiotic effects on the dynamics of antibiotic action (42).

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There are vast bodies of MIC data representing different associations of antibiotics with bacteria (2, 14, 22), and it would be particularly useful to be able to compare these single-parameter estimates of the pharmacodynamics of antibiotic-bacterial species interactions with those obtained with time-kill and continuous-culture experiments. In part to achieve this end but also to provide a quantitative framework for generating testable hypotheses and interpreting the results of time-kill and continuous-culture experiments of antibiotic efficacy, we developed and numerically analyzed the properties of mathematical models describing them. These models combine the pharmacodynamics of antibiotics with the population dynamics of bacteria exposed to these drugs in batch and continuous cultures. The models take into account (i) the functional form of the relationship between the concentration of the antibiotics and rates of kill, (ii) the density of the target population of bacteria, (iii) the growth rate of the bacteria, (iv) byproduct resources generated from dead bacteria, (v) antibiotic-refractory subpopulations, wall growing bacteria (biofilm), and persistent states, and (vi) density-independent and density-dependent decay in antibiotic concentrations. On the basis of the results of our analysis of the properties of these models, we present a series of hypotheses regarding the pharmacodynamics and population dynamics of antibiotic treatment that can be tested with time-kill and continuous-culture experiments. We briefly discuss procedures to test these hypotheses and the anticipated results of these experiments.

MATERIALS AND METHODS

A model of antibiotic treatment with density-dependent and resource concentration-dependent pharmacodynamics. This model attempts to capture the relationship between the concentration of an antibiotic (A) (measured in milligrams per liter) and the rate of growth or death (negative growth) of bacteria in mass (liquid) culture in situations in which both the density (N) and physiological state of the exposed bacteria contribute to the efficacy of the antibiotic. As a surrogate for the physiological state, we use the concentration of the resource limiting the growth of the population (R) (measured in micrograms per milliliter). The idea behind this approach, which was first formally considered by Jacques Monod (37, 38), is that the rate of replication of a population of bacteria is a monotonic increasing function of the concentration of a limiting resource (R) (measured in micrograms per milliliter). We are assuming that the metabolic activity of bacteria and therefore their susceptibility to antibiotics are proportional to the availability of the limiting resource(s). For determination of these pharmacodynamics values, we use a Hill function (49), where the maximum and minimum growth (maximum kill) rates [$\psi_{\max}(R)$ and $\psi_{\min}(R)$, respectively] are monotonic increasing functions of the concentrations of the limiting resource. As with the method described in reference 57, we also assume that the realized MIC increases with density [$M(N)$]. In this model, the per-capita rate of growth or death of the bacteria is given by $\theta(A, N, R)$, so that

$$\theta(A, N, R) = \psi(R) - H(A, N, R) \tag{1}$$

$$H(A, N, R) = \left[\frac{\psi_{\max}(R) - \psi_{\min}(R) \left(\frac{A}{M(N)} \right)^\kappa}{\left[\left(\frac{A}{M(N)} \right)^\kappa - \left(\frac{\psi_{\min}(R)}{\psi_{\max}(R)} \right) \right]} \right] \tag{2}$$

$$\psi_{\max}(R) = V_{\max} \left(\frac{R}{R + km} \right) \tag{3}$$

$$\psi_{\min}(R) = V_{\min} (1 - pr) + prV_{\min} \left(\frac{R}{R + km} \right) \tag{4}$$

$$M(N) = M_{\min} + pd \left(M_{\max} \frac{N}{N + ka} \right) \tag{5}$$

The parameters of this equation are κ (the Hill coefficient), which determines the

shape of the function; $V_{\max} \text{ h}^{-1}$, the maximum growth rate of the bacteria in the absence of the antibiotic; $V_{\min} \text{ h}^{-1}$, the minimum growth (maximum kill) rate in the presence of the antibiotic (for bactericidal drugs, $V_{\min} < 0$); and km (measured in micrograms per milliliter), the concentration of the resource that results in growth or death rates that are half their maximum and minimum values. The parameter pr ($0 \leq pr \leq 1$) is a measure of the extent to which the minimum growth rate (maximum kill rate) is affected by the concentration of the resource R . When $pr = 0$, the PDs are independent of the concentration of the resource and therefore independent of the growth rate of the bacteria. As with the method described in reference 57, M_{\min} (in micrograms per milliliter) is the baseline MIC (bMIC) of the antibiotic as estimated by the CLSI protocol, pd ($0 \leq pd \leq 1$) is a scaling parameter that specifies the relationship of the sensitivity of the realized MIC to the density of the bacteria exposed, M_{\max} (in micrograms per milliliter) is the maximum MIC, and ka (in numbers of cells per milliliter) is the density N at which the realized MIC is half this maximum value ($M_{\max}/2$). When $pd = 0$, the MIC is the bMIC.

To illustrate the effects of increasing density and declining resources on the efficacy of bactericidal antibiotics, we plotted the relationship between the growth or death rate of the bacteria and the concentration of the antibiotic, pharmacodynamics functions for different densities and resource concentrations, and parameter values (Fig. 1). While the parameter values used for this and the rest of the numerical solutions (computer simulations) presented in this report are in a realistic range for *Staphylococcus aureus* and *Escherichia coli* exposed to different antibiotics, they are not specific for any particular antibiotic-bacterial species combination.

As measured by the increase in the MIC and the antibiotic concentration-dependent rate of kill relative to the density- and resource concentration-independent Hill function, the efficacy of the antibiotic declines with increases in the density of the bacteria and with decreases in the concentration of the resource. Under the rich-medium conditions, $R \gg km$, and, with low densities of bacteria, $N \ll ka$, used in CLSI protocols to estimate MICs, even substantial resource and density effects on this parameter would not be noticed.

Population dynamics. One way to maintain a population of bacteria and control their rate of replication and density is by culturing them in chemostats (12, 45, 48) or hollow-fiber reactors (9, 11, 33) maintained at different dilution rates and with reservoirs containing different concentrations of the limiting resource. To model this situation, we assume a vessel of unit volume (1 ml) and a reservoir where the concentration of the limiting resource is C (measured in micrograms per milliliter). Resources enter the vessel at a rate of w per hour, which is the same as the rate at which excess resources, antibiotics, and bacteria are removed. These resources are taken up at a rate proportional to the total density of viable bacteria in the chemostat, the maximum resource-dependent growth rate, and a conversion parameter, e (measured in micrograms per milliliter), which is the amount of resource needed to produce a single bacterium (54). The bacteria grow at a rate that depends on the concentrations of the limiting resource and antibiotics and the total density, which, for the N population, is represented by $\theta(A, N, R)$. In the absence of new drug input, the concentration of the antibiotic, A (measured in milligrams per liter), declines with the dilution rate, w , and a function which has density-dependent and dependent-independent elements (57) as follows:

$$\Delta(N_T) = d_i + d_d(N_T) \tag{6}$$

where N_T is the total density of viable cells in the culture (see below), d_i (per hour) is a constant (representing density-independent decay), and d_d (per hour) is a measure of the extent to which each viable cell in the population contributes to the decay in the effective concentration of the antibiotic. We are assuming that dead cells do not contribute to the decay in the effective concentration of the antibiotic.

Antibiotic-free chemostat equilibrium. In the absence of the antibiotic, the chemostat reaches equilibrium when the rate of growth is precisely equal to the rate of washout. When sensitive cells (represented by N) are the only bacteria present, at this ‘‘chemostat equilibrium,’’ this relationship is represented by the following equations:

$$R^* = \frac{km}{(V_{\max} - w)} \tag{7}$$

$$N^* = \frac{(C - R^*)}{e} \tag{8}$$

and the generation (doubling) time is calculated as follows:

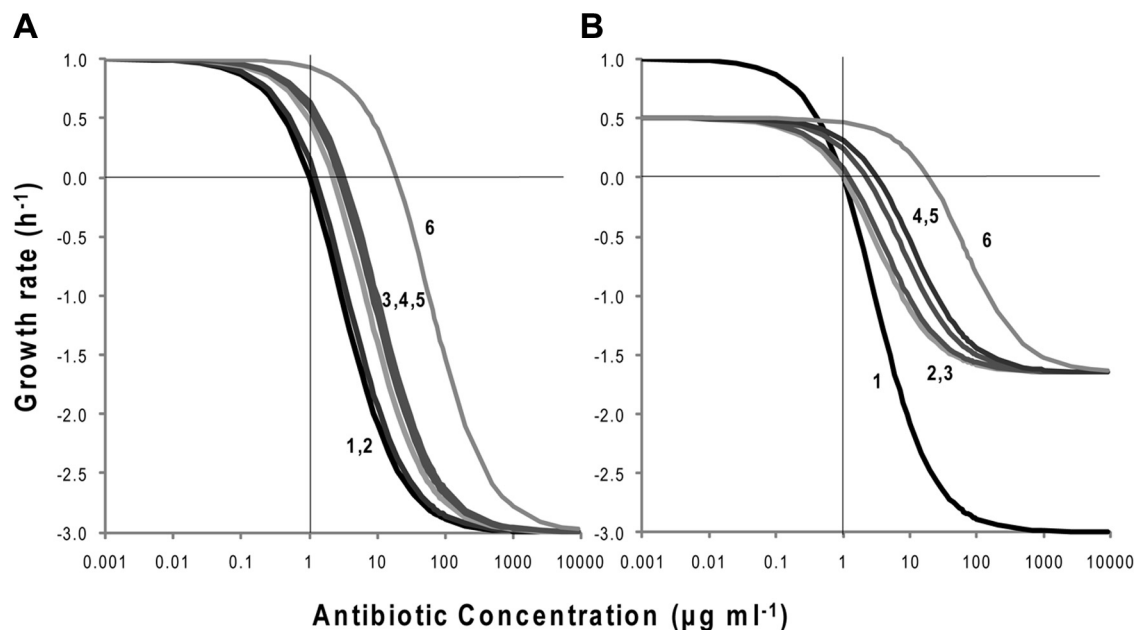


FIG. 1. Density- and resource-dependent pharmacodynamic functions for bactericidal antibiotics. Hourly rates of population growth or death as functions of the concentration of the antibiotic are presented. Common parameters: $km = 0.25$, $V_{\max} = 1.0$, and $V_{\min} = -3.0$. (A) Effects of density on the PD of antibiotics. Line 1, control, with no density or resource effects ($pd = pr = 0$). Lines 2, 3, and 4, modest density effects ($pd = 0.5$, $M_{\max} = 5.0$) (line 2, $n = 10^6$; line 3, $n = 10^7$; line 4, $n = 10^8$). Lines 5 and 6, strong density effects ($pd = 0.5$; $M_{\max} = 40.0$) (line 5, $n = 10^6$; line 6, $n = 10^8$). (B) Joint effects of resource and density levels on PD. Line 1, control, with no density or resource effect ($pd = pr = 0$). For the remaining lines, the resource concentration (R) was $0.25 \mu\text{g/ml}$. Line 2, resource effect, no density effect ($pr = 0.9$, $pd = 0$). Lines 3, 4, and 5, resource and modest density effects ($pr = 0.9$, $pd = 0.5$, $M_{\max} = 5.0$) (line 3, $n = 10^6$; line 4, $n = 10^7$; line 5, $n = 10^8$). Line 6, resource and strong density effects ($pr = 0.9$, $pd = 0.5$, $M_{\max} = 40.0$) ($n = 10^8$).

$$T = \frac{\ln(2)}{w} \quad (9)$$

For details, see reference 29 and/or www.ecf.net/chemostat.

Secondary resources. While the density that a population attains and maintains in a chemostat or batch culture depends on the concentration of the limiting resource in the reservoir, it is reasonable to expect that, as a consequence of the release of metabolic byproducts by viable cells and the decay of dead cells, additional or byproduct resources would be produced. Although the concentration of these byproduct resources might not be sufficient for a net increase in a high-density population, they could contribute to cellular metabolism and thereby to susceptibility to antibiotics. Particularly important in a culture exposed to antibiotics treatment is the use of these secondary resources to fuel cell metabolism and thereby to mediate antibiotic susceptibility. To model this situation, we separately considered populations of planktonic cells that have been killed by the antibiotic (with cell population density represented by D in cells per milliliter). At a rate of f per cell per hour, these dead bacteria are converted into byproduct resources such that each dead cell produces $e \times x$ units of byproduct resource, where e represents the conversion efficiency and x ($0 \leq x \leq 1$) represents a recycling parameter. For convenience, we are assuming that these byproduct resources become part of the primary resource pool (R) and consist solely of converted dead cells.

Persistent and wall subpopulations. Exponentially growing populations of *S. aureus* as well as many other species include slowly dividing or nondividing subpopulations that are more refractory to antibiotics than exponentially growing cells, the so-called persisters (8, 10, 58). In addition to a planktonic population, chemostats may include subpopulations of cells adhering to the walls and other immersed glassware. These biofilm (6, 7) and other wall-adhering populations may also be more refractory to antibiotics than the planktonic cells. To include these antibiotic-refractory subpopulations in this model, we considered a second planktonic population of bacteria (at a density of P cells per milliliter), persisters, and a nonplanktonic, wall-adhering population of density B . The rate of growth or death of this subpopulation depends on antibiotic and resource concentrations as well as on a density function similar to that of the planktonic (N) population, represented by $\theta_P(A, N, R)$, where N_T represents the total density of bacteria, i.e., $N_T = N + P + B$. The antibiotic specific growth and sensitivity parameters of the

P and B populations may differ from those of the N population; in particular, the P and B populations would have a lower maximum growth rate (i.e., $V_{\max P}$ and $V_{\max B} < V_{\max}$) and, for a bactericidal antibiotic, a lower minimum growth rate (lower kill rate) (i.e., $V_{\min P}$ and $V_{\min B} > V_{\min}$). The wall population B has a lower rate of washout than the planktonic populations ($w_B \ll w$). We assume that the P cells are generated by N at a rate of f_{NP} per hour and that P cells return to the N state at a rate of f_{PN} per hour. The B population is generated at the rate f_{NB} from the N population and returns to the planktonic N state at the f_{BN} , hourly rate. We neglect the formation of the B from the P population and the return to the N state and do not include the B or N populations in the density-dependent MIC function.

In Fig. 2, we present a conceptual diagram of this model with the different populations of bacteria. With the definitions of variables and parameters presented above, the rates of change in the densities of the bacterial populations and concentrations of the resource and antibiotics are given by the following set of six coupled differential equations:

$$\frac{dR}{dt} = w(C - R) - e \frac{R}{(R + km)} (NV_{\max} + PV_{\max P} + BV_{\max B}) + fDex \quad (10)$$

$$\frac{dN}{dt} = \theta(A, N, R)N - wN - f_{NP}N + f_{PN}P - f_{NB}N + f_{BN}B \quad (11)$$

$$\frac{dD}{dt} = H(A, N, R)N + H_P(A, P, R)P + H_B(A, B, R)B - fD(w + ex) \quad (12)$$

$$\frac{dP}{dt} = \theta_P(A, P, R)P + f_{NP}N - f_{PN}P - wP \quad (13)$$

$$\frac{dB}{dt} = \theta_B(A, B, R)B + f_{NB}N - f_{BN}B - w_B B \quad (14)$$

$$\frac{dA}{dt} = -[w + \Delta(N_T)]A \quad (15)$$

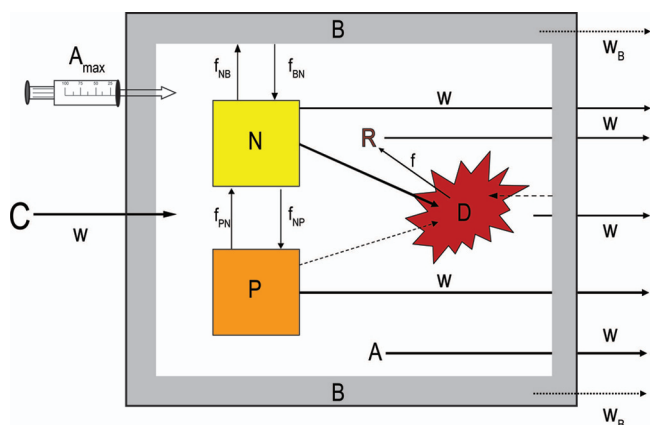


FIG. 2. Model of antibiotic treatment of bacteria in continuous culture. N, density of susceptible bacteria; P, density of persistent bacteria; B, density of wall-adhering bacteria; D, density of killed bacteria still present in the culture; R, internal concentration of the limiting resource; C, concentration of the resource in the reservoir; A, internal concentration of the antibiotic; A_{max} , concentration of the antibiotic periodically added; w , flow rate of resource into the vessel and of the removal of the resource, antibiotic, and planktonic bacteria (N, P, and D); w_B , the rate of flow of the wall population from the vessel ($w_B \ll w$).

In the equations above, N represents the planktonic rapidly growing cells and $N_T = N + P + B$. The variables and parameters of these equations are separately presented in Table 1 along with the range of values used in the numerical solutions (computer simulations) with which we explored the properties of these models. The simulation was programmed in Berkeley Madonna. Copies of the program are available at www.eclf.net/chemostat.

RESULTS

The population dynamics of antibiotic-mediated killing in batch culture. When the flow parameter is set to $w = 0$ in equations 3 to 7 above, the model becomes appropriate for a batch culture of the sort used in time-kill experiments. In this section, we explore how different pharmacodynamic and population growth factors affect the outcome of time-kill experiments.

Resource- and density-independent killing in the absence of persistence. In the absence of a resource or density effect on antibiotic-mediated killing, the decline in the density of bacteria confronted with an antibiotic is exponential, with a rate that increases monotonically with the concentration of the drug (Fig. 3A, lines 1 and 2). This is effectively the case when there

TABLE 1. Variables, parameters, and values employed for numerical solutions^a

Variable or parameter	Definition	Value or range considered
Variables		
N_T	Total density of bacteria (cells per ml)	1–10 ⁸
A	Concn of the antibiotic (mg/liter)	0–100
N	Density of susceptible bacteria (cells per ml)	1–10 ⁸
D	Density of antibiotic-killed bacteria (cells per ml)	1–10 ⁸
P	Density of persisters or wall bacteria (cells per ml)	1–10 ⁸
B	Density of wall population (cells per ml)	1–10 ⁸
R	Concn of the limiting resource ^b (μg/ml)	50
Parameters		
V_{max}	Maximum hourly growth rate of sensitive cells	(1.0)
V_{min}	Minimum hourly growth rate of sensitive cells (maximum kill)	(–3.0)
V_{maxP}	Maximum hourly growth rate of persisters	(0.001)
V_{minP}	Minimum hourly growth rate of persisters (maximum kill)	(–0.001)
V_{maxB}	Maximum hourly growth rate of wall population	(0.001)
V_{minB}	Minimum hourly growth rate of wall population (maximum kill)	(–0.001)
κ	Hill function coefficient	(1.0)
e	Efficiency of conversion of resource to cells (μg/cell)	(5 × 10 ^{–7})
km	mg of resource/ml at half of maximum or minimum growth	(0.25)
M_{min}	Minimum MIC (mg/liter)	(1.0)
M_{max}	Maximum MIC (mg/liter)	(5) or 40
pd	Density-dependent MIC coefficient (0 ≤ pd ≤ 1)	(0) or 0.50
pr	Resource concn-kill coefficient (0 ≤ pr < 1)	(0) or 0.9 ^c
ka	Density where the MIC is half the maximum value	(1 × 10 ⁷)
f	Hourly rate at which dead cells become resource	(0) or 0.1
x	Fraction of a dead cell converted into resource	(0.5)
f_{NP}	Hourly rate of conversion of N into P	10 ^{–4} or 10 ^{–5}
f_{PN}	Hourly rate of conversion of P into N	10 ^{–4} or 10 ^{–5}
f_{NB}	Hourly rate of formation of wall (conversion of N into B)	10 ^{–4} or 10 ^{–5}
f_{BN}	Hourly rate of loss of wall to planktonic cells (conversion of B into N)	10 ^{–4} or 10 ^{–5}
C	μg of resource/ml in the reservoir	(50)
w	Hourly dilution rate of the chemostat-planktonic cells	0, 0.05, 0.25, 0.5
w_B	Hourly rate of washout of the wall (biofilm) population	(0.01)
d_i	Density-independent antibiotic decay coefficient	(0) or 0.1
d_d	Density-dependent antibiotic decay coefficient	(0) or 10 ^{–9} , 5 × 10 ^{–9}
A_{max}	mg of antibiotic/liter added at each dose	0–100
Dose	Time between doses (h)	(24)

^a Unless otherwise noted, the values in parentheses (standard values) are those used for the numerical analysis of the properties of these models.

^b When the initial resource concentration is set to 10⁹ mg/ml, there is effectively no resource effect on the rates of growth or antibiotic-mediated killing.

^c When $pr = 0$, there is no resource effect on the maximum kill rate [$\psi_{min}(R) = V_{min}$].

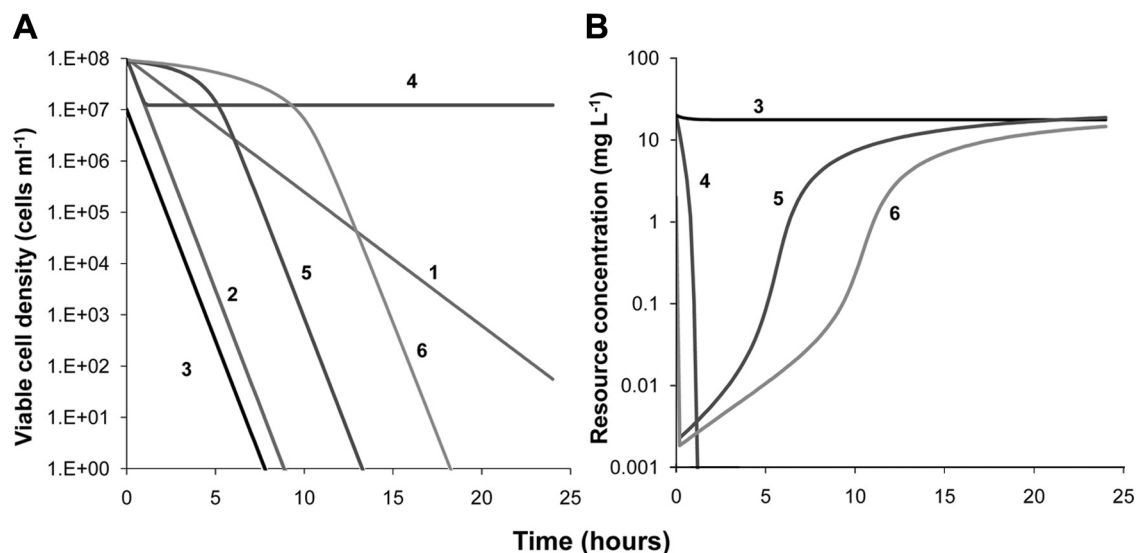


FIG. 3. Simulation of resource-concentration-dependent, density-independent antibiotic-mediated killing in batch culture. Unless otherwise noted, the parameter values are the standard values presented in Table 1. (A) Changes in the viable-cell density. Lines 1 and 2, no resource effect ($R_0 = 10^8$) and effect of two concentrations of antibiotics (line 1, $A = 2$ mg/liter; line 2, $A = 10$ mg/liter). Lines 3 and 4, resource effect on maximum growth with no byproduct resource ($R_0 = 20$, $A = 10$). Line 5, resource effect on maximum growth with byproduct resource ($R_0 = 2$, $f = 0.1$, $A = 10$). Line 6, resource effect on maximum and minimum growth rates with byproduct resource ($R_0 = 2$, $f = 0.1$, $A = 10$, $pr = 0.9$). (B) Change in resource concentration for resource-limited pharmacodynamics. The population numbers are the same as for the corresponding lines in Fig. 3A.

is a resource effect but the initial cell density is low such that the per-cell concentration of the resource is great enough for the bacteria to replicate at nearly their maximum rate (Fig. 3A, line 3). At these lower densities, the available resources do not become significantly depleted through their consumption by the bacteria. If, however, the initial density of bacteria is higher and the initial concentration of resource is modest, antibiotic-mediated killing proceeds until the resources are consumed, at which time the population density no longer declines (Fig. 3A, line 4). This situation is remedied if the bacteria that are killed become recycled and generate byproduct resource (Fig. 3A and B, lines 5 and 6). The difference between the simulations represented by lines 5 and 6 is that, in line 5, the only effect of the resource is on the maximum growth rates, as represented by $\psi_{\max}(R)$, whereas in line 6, there is a resource concentration effect on $\psi_{\min}(R)$ ($pr = 0.9$). In both of these situations, the initial killing effect is relatively modest but then accelerates as increasing numbers of dead cells are converted into resources.

Resource- and density-dependent killing in the absence of persistence. In Fig. 4A, we consider those dynamics with a modest density effect; the maximum MIC is five times the minimum, and at any given time, 50% of the MIC used for the Hill function $M(N)$ is determined by the minimum MIC ($pd=0.5$). In other words, at an infinite density, the MIC of the culture is 3.5 times the minimum MIC and, with 10^7 cells per ml (the ka), the realized MIC is 2.25. At lower densities (10^5 and 10^6 with the parameters used), the density and resource effects on killing are imperceptible (lines 1 and 2). When the initial density is 10^7 , the density effects can be seen; antibiotic-mediated killing is initially slow, but its pace picks up as the density falls. When the initial density is 10^8 and the initial concentration of the antibiotic is $10\times$ the MIC, there is virtually no antibiotic-mediated killing (Fig. 4A, line 4). This is in part because of the high density but also because of the low

initial concentration of the resource, which is nearly completely consumed in short order. As a result of an increase in the concentration of the antibiotic, killing occurs and does so at an increasing rate as the density of viable cells declines and dead cells are converted into resources (Fig. 4A, lines 5 and 6).

As shown in Fig. 4B, the density effect is substantial ($M_{\max} = 40$); i.e., at an infinite density the realized MIC is 21 times the basal (CLSI) MIC. While this may seem exceptionally high, it is less than that observed for *S. aureus* with oxacillin (57). Although 10 times the MIC is not sufficient to induce antibiotic-mediated killing with an inoculum of 10^8 (data not shown), at higher concentrations the antibiotic is effective with 10^7 cells per ml (line 3), albeit with slightly delayed action. However, even with 75 or 100 times the MIC, the delay can be substantial when the cell density is 10^8 (Fig. 4B, lines 4 and 5).

Persistence. For the simulations discussed thus far, we assumed that all the bacteria in a population are equally susceptible to antibiotic-mediated killing. In the simulations that follow, we allow for a minority population that is refractory to the antibiotic, the so-called persister population that divides at a very low rate (8, 10, 28, 58). We also assume that the maximum and minimum growth rates of the persister population are 0.001 and -0.001 per h, respectively, and that the rate at which persisters (P) are produced from the rapidly growing population (N) is the same as the rate at which P is converted into N ($f_{PN} = f_{NP}$).

In Fig. 5A, we follow the changes in the persistent (P), susceptible (N), and total (N_T) populations for two initial densities in the absence of any effect of density or resource concentrations on the pharmacodynamics of the antibiotics. At both densities, the viable-cell density initially declines exponentially at a rate proportional to the concentration of the antibiotic. While the density of the N population continues to decline, that of the P population increases, and there is a

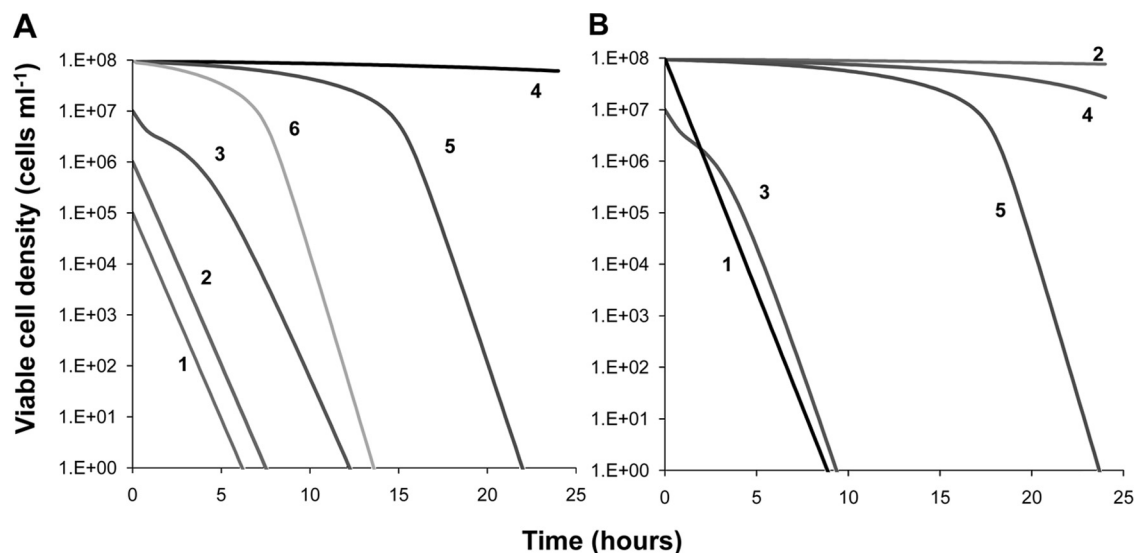


FIG. 4. Simulation of resource- and density-dependent antibiotic-mediated killing in batch culture: Unless otherwise noted, the parameter values are the standard values presented in Table 1, with $pd = 0.5$, $pr = 0.9$, $R_0 = 2.0$, and $f = 0.1$. (A) Change in viable-cell density with a modest density effect ($M_{\max} = 5$). Lines 1, 2, 3, and 4, $A = 10$ mg/liter; line 5, $A = 20$ mg/ml; line 6, $A = 50$ mg/liter. (B) Change in viable-cell density with a strong density effect ($M_{\max} = 40$). Line 1, density- and resource-independent control ($A = 10$ mg/ml). Lines 2 and 3, $A = 50$ mg/liter; line 4, $A = 75$ mg/liter; line 5, $A = 100$ mg/liter.

leveling off of the total density, at which time persistent cells dominate the population. With the parameters used, this leveling off is only an artifact of the calculation method; there is in fact a continuous but slow decline in the total cell density. The persistent cells continue to produce susceptible bacteria, which are killed off by the drug. The level of the persistent population after the killing off of the dominant susceptible population is proportional to the initial density of the population and declines somewhat but not dramatically with increase in concentration of antibiotic (Fig. 5B). The density of the persister population is also proportional to the rate of conversion of sensitive cells to persistent cells.

In Fig. 5C, we present the results of simulations that allow for density and resource concentration effects on the PD of the antibiotics and persistence. Because of the density and resource effects, the dynamic of killing is no longer simply exponential; the pace of killing is initially low and then increases as the density declines and byproduct resources become available. The term before killing commences, as seen in Fig. 5, is greater when the density effect is more profound (compare lines 1 and 3 for $M_{\max} = 5$ and 40, respectively). At that point, however, the population does not continue to decline but rather levels off due to persistence, with the level of persistence declining with the rate of killing. However, even with a substantial density effect, at lower initial densities the rate of killing is little affected by the density dependence of the PD.

Antibiotic decay. In Fig. 6A, we consider decay in the effective concentration of the antibiotic occurring at a rate that is independent of the density of the bacteria. The antibiotic concentration declines exponentially in the absence of a density- and resource-dependent effect on the PD (line 1). At higher densities and even without persistence, however, as the antibiotic concentration wanes, the bacterial population recovers after an initial decline (Fig. 6A, line 2). With the same param-

eters and a lower initial density, the bacterial population is effectively cleared (line 3). Allowing for persistence, at this higher initial density the bacterial population is maintained (line 4) but the total density does not increase perceptibly after the concentration of the antibiotic reaches a level much less than its MIC.

In Fig. 6B, we consider density-dependent declines in the antibiotic concentration. With a density-dependent decay of $d_d = 10^{-9}$ and a modest density effect ($M_{\max} = 5$), the decline in the effective concentration of the antibiotic has little perceptible effect on the dynamics or rate of decline in viable-cell density (compare line N1 with N2). The reason for this is that, because of antibiotic-mediated killing reducing the density of viable cells, the change in the antibiotic concentration is small (compare lines A1 and A2). With a greater antibiotic decay function ($d_d = 10^{-8}$) and the same parameters, the decline in the antibiotic concentration is greater (line A3) and there is a lag before the rate of antibiotic-mediated killing reaches its maximum value (line N3). With a more intense density effect ($M_{\max} = 40$), the antibiotic decays (line A4), and there is little antibiotic-mediated killing (line N4).

The population dynamics of antibiotic treatment in continuous culture. In the following simulations of antibiotic treatment in continuous culture, with the concentration of the limiting resource (C) at 50 μ g/ml, in the absence of antibiotics, and with the same parameters used, the density of the population at equilibrium is $N = \sim 108$ cells per ml. Antibiotics are added at a concentration of A_{\max} (in milligrams per liter) at the end of the first day, when the bacterial population is at the density (chemostat) equilibrium, and, following that, every 24 h.

Dynamics in the absence of persistence and wall growth. In Fig. 7A and B, we display the changes in the concentration of the antibiotic (pharmacokinetics) for 10 and 50 mg/liter of the

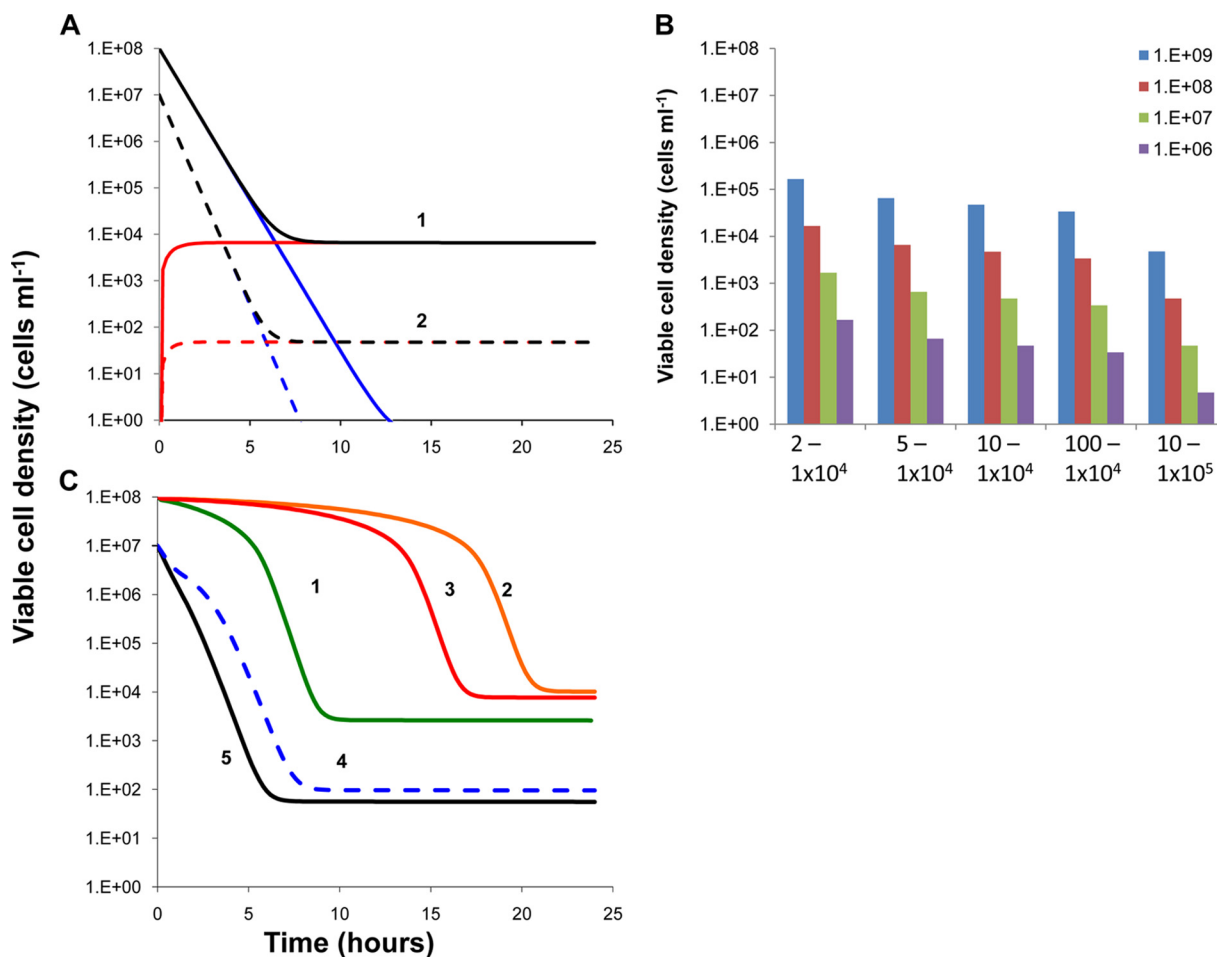


FIG. 5. Antibiotic-mediated killing in batch culture with persistence considered. Unless otherwise noted, the parameter values are the standard values presented in Table 1. (A) Changes in viable-cell density in the absence of resource or density effects ($R_0 = 10^9$, $pd = pr = 0$, $f_{NP} = f_{PN} = 0.0001$). The total cell population (N_T) is represented by a bold line, susceptible cells (N) are represented by a descending line, and persistent cells (P) are represented by a leveling-off line. Line 1 represents a 5 mg/liter concentration of the antibiotic; line 2 represents a 10 mg/liter concentration. (B) Total viable-cell density at 24 h with different initial cell densities, antibiotic concentrations, and persistence levels (x axis). No density or resource concentration effects were seen. (C) Change in the total cell density with persistence ($f_{NP} = f_{PN} = 0.0001$) and resource- and density-dependent pharmacodynamics ($R_0 = 10$, $pr = 0.9$, $pd = 0.5$, $ka = 1E7$) and different concentrations of antibiotics. Unless otherwise noted, $A = 100$ and $M_{max} = 40$. Line 1, $M_{max} = 5$; line 2, $M_{max} = 40$; line 3, $A = 150$; line 4, $A = 100$; line 5, $A = 50$.

antibiotic introduced every 24 h with three different rates of flow. At the lowest rate ($w = 0.05$ per h), the antibiotic concentration never falls below the baseline MIC of 1 mg/liter. For the higher flow rates (when $A_{max} = 10$ mg/liter), the times above the baseline MIC are 9.2 and 4.6 h for the 0.25 and 0.50 flow rates, respectively. For $A_{max} = 50$ mg/liter, the corresponding times above the baseline MIC are 15.6 and 7.8 h.

In the absence of a density- and resource-related effect on the PD, the rate of killing is proportional to the amount of time the concentration of the antibiotic exceeds the MIC (Fig. 7C). However, even with 10 times the MIC of antibiotic added every 24 h at the highest dilution rate of $w = 0.50$ ml/h, the bacteria are not cleared, whereas they are cleared at the lower dilution rates of 0.05 and 0.25 ml/h, with the rate of clearance being greatest at the lowest rate. When we allow for a density and resource effect with $A_{max} = 10$ times the MIC, there is little change in the density of the bacteria over the period considered (data not shown). With 20 times the MIC, clearance

occurs only at the lowest dilution rate, but not until more than 60 h after the first dose is administered, rather than at 10 h (Fig. 7C and data not shown). With 50 times the MIC (Fig. 7D), clearance occurs at both the lower dilution rates but not at the higher dilution rate (0.5). Under these circumstances, unlike the situation in which there are no resource effects on the kill rate of $\psi_{min}(R) = V_{min}$ but there are density effects (Fig. 7C), there is not much difference in the times before the population approaches extinction (1 cell per ml) for the 0.05 and 0.25 dilution rate simulations (Fig. 7D).

In Fig. 7E, we consider these same pharmacokinetics but with an antibiotic that kills at a very low rate of $V_{min} = -0.01$ and is therefore effectively bacteriostatic. In these simulations, we are assuming density- and resource-dependent MICs ($pd = 0.5$, $pr = 0.9$, and $M_{max} = 40$). In contrast to what obtains with the bactericidal antibiotics represented in Fig. 7D, the rate of decline in the viable-cell density is least at the lowest dilution (0.05). The reason for this is that, with bacteriostatic antibiot-

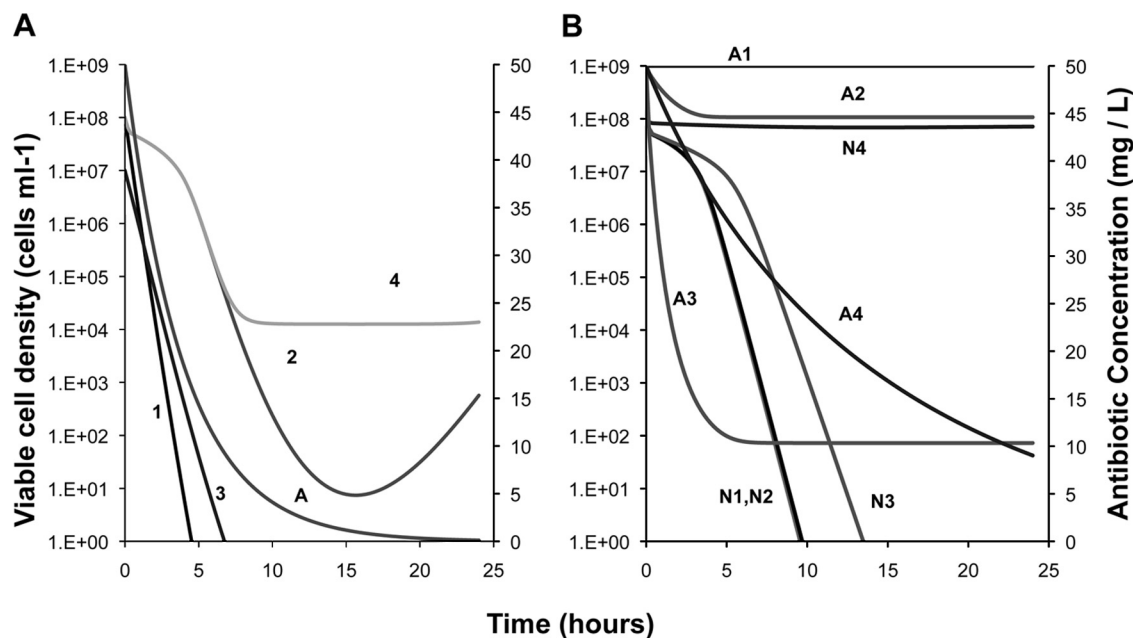


FIG. 6. Changes in antibiotic concentrations during time-kill experiments. Unless otherwise noted, the parameter values used are the standard values presented in Table 1, with the initial concentration of resource (R_0) = 10 $\mu\text{g/ml}$ and A_0 = 50 mg/liter. (A) Change in antibiotic concentration (A) and viable-cell density with density-independent antibiotic decay ($d_x = 0.25$, $d_d = 0$). Line 1, control (no density or resource concentration effect). Lines 2, 3, and 4, $pr = 0.5$, $pd = 0.9$, $M_{\text{max}} = 5$; line 4, persistence ($f_{dp} = f_{pd} = 0.0001$). (B) Change in viable-cell density and antibiotic concentrations with density-dependent antibiotic decay. Lines A1 and N1, no antibiotic decay. Lines N2 and A2, modest density effect ($M_{\text{max}} = 5$) and density-dependent decay ($d_d = 10^{-9}$). Lines N3 and A3, a greater density-dependent decay coefficient ($d_d = 10^{-8}$) and modest density effect ($M_{\text{max}} = 4$). Lines A4 and N4, $d_d = 10^{-9}$, $M_{\text{max}} = 40$.

ics, the decline in the density of the bacteria occurs through flow rather than through killing. Although the total reductions in the density of viable cells for $w = 0.25$ and $w = 0.50$ are similar over the same period, the dynamics are different. The reason for this is that, with the higher dilution rate, the time during which the concentration of the antibiotic exceeds the MIC is less than it is at the lower dilution rate.

Population dynamics with persister and wall populations. We assume that the physiologically persistent subpopulation (P) is planktonic and is washed out at the same rate as the N population w rate whereas the wall population (B) is washed out at a rate of $w_B = 0.01$ per h independently of the value of w for the planktonic (N) population. In these simulations, we assume the same values for the maximum growth and death rates of the P and B populations, i.e., $V_{\text{maxP}} = 0.001$ and $V_{\text{minP}} = -0.001$ per h for the P population and $V_{\text{maxB}} = 0.001$ and $V_{\text{minB}} = -0.001$ per h for the B population. For the persister and wall populations, we assume that the Hill function is density and resource concentration independent ($pr = 0.9$, $pd = 0.5$, and $M_{\text{max}} = 40$; for line 4, $w = 0.05$; for line 5, $w = 0.25$; and for line 6, $w = 0.5$).

In Fig. 8A, we follow the changes in density of bacteria in a chemostat treated every 24 h with 50 mg/liter of a bactericidal antibiotic, with persistence where $f_{NP} = f_{PN} = 10^{-5}$ per cell per h. With these parameters, without a density effect or resource effect on the maximum kill rate, bacteria are rapidly cleared at both the intermediate and higher dilution rates of $w = 0.25$ and 0.50 ml h^{-1} (lines 2, 3, and 5). With the resource and density effects on the PD, at the highest dilution rate, clearance does not occur. Although at the lowest dilution rate of $w = 0.05 \text{ h}^{-1}$

the pharmacokinetics are more favorable for clearance than at the higher dilution rates, because of the low rate of washout the persister cells remain for an extended period. Save for when there is a density and resource effect on the PD and a high dilution rate line (Fig. 8B, line 6), the planktonic population of bacteria is killed off by the antibiotic. On the other hand, the rate at which the total bacterial population approaches clearance is greatly diminished because of the formation of an antibiotic-refractory wall population Fig. 8B, lines 1 to 5).

DISCUSSION

“All models are wrong, some are useful” (12a).

Although the models developed here are more complex than we would like in terms of aesthetics as well as mathematical tractability, they are only simplistic (minimalist) caricatures of the in vitro pharmacodynamics and population dynamics of antibiotic treatment. They are certainly not quantitatively precise analogues of these dynamics. Nevertheless, we believe that, in a general and realistic way, these models capture the dominant factors that contribute to the pharmacodynamics of antibiotic and bacteria in vitro and how these PDs would be manifested as changes in the densities of populations exposed to these drugs in time-kill and continuous-liquid-culture experiments. The utility of these models and our analysis of their properties are intended to allow the generation of testable (and rejectable) hypotheses about these dynamics and of a framework for interpreting the results of time-kill and continuous-culture experiments. In the following paragraphs we list

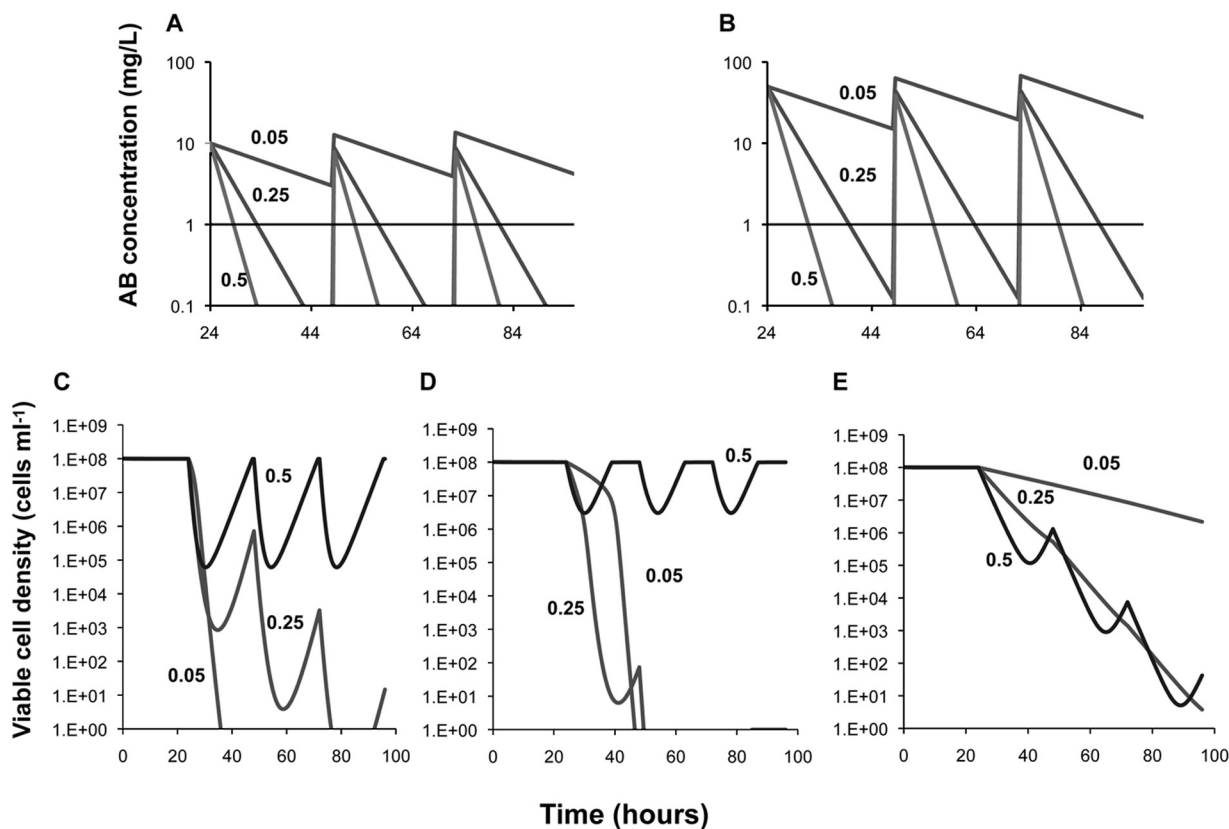


FIG. 7. Simulation of the population dynamics of antibiotic treatment in chemostat culture. Unless specifically noted, the parameter values used for this simulation are the standard values presented in Table 1. (A) Change in the antibiotic concentration for different dilution rates ($A_{\max} = 10$ mg/liter). (B) Change in the antibiotic concentration for different dilution rates ($A_{\max} = 50$ mg/liter). (C) Change in viable-cell density for a bactericidal antibiotic with no density effect ($pd = 0$) or resource effect on the maximum kill rate, with $pr = 0$ and $\psi_{\min}(R) = V_{\min}$. The pharmacokinetics is the same as that described for panel 7A. (D) Change in the viable-cell density for a bactericidal antibiotic with density- and resource-dependent PD, with $pd = 0.5$ and $pr = 0.9$. The pharmacokinetics is the same as that described for panel 7B. (E) Change in the viable-cell density for a bacteriostatic antibiotic ($V_{\min} = -0.01$), with density and resource effects on efficacy ($pd = 0.5$, $pr = 0.9$). The pharmacokinetics is the same as that described for panel 7B.

these hypotheses. We also briefly describe ways they can be tested and the status of existing evidence arguing for or against their validity.

Pharmacodynamics and time-kill experiments. The pharmacodynamic elements of this model of antibiotic treatment are dependent on three assumptions, and the validity of each of these assumptions can be and, to some extent, has been tested.

(i) **A Hill function of the sort employed here provides a reasonable fit for the antibiotic concentration-dependent rates of growth or mortality during the initial phase of time-kill experiments.** The procedures to test this hypothesis and evidence in support of it can be found in reference 49. While reference 49 provides evidence that a Hill function provides a good fit for the relationship between the concentration of the antibiotic and the rate of growth or killing of bacteria, that evidence is restricted to relatively low densities of a single strain of *E. coli* and antibiotics of only five different classes.

(ii) **The efficacy of antibiotics as measured by time-kill experiments and MICs declines with the increasing density of the bacteria exposed.** For a number of bacterial strain and antibiotic combinations, killing efficacy declines with increasing densities of bacteria exposed; this is the so-called inoculum effect (47, 51, 53, 57). There is also support for the logistic form

of the MIC-density relationship used in this model for experiments with *S. aureus* and antibiotics of six different classes, with the MIC increasing and leveling off at higher densities (57). How general this functional relationship is remains to be seen, but it can be explored by applying the methods described in reference 57 to different bacterial species and antibiotics.

(iii) **The rate of killing by a given concentration of a bactericidal antibiotic is directly proportional to the rate of cell division of the bacteria.** One way to test this hypothesis is to perform time-kill experiments with bacteria removed from chemostats maintained at different dilution rates. As long as the reservoir concentration is the same, the dilution rate would have little effect on the density of the bacteria at the chemostat equilibrium. This can be seen from equations 1 and 2. For example, with the standard parameters in Table 1 at the chemostat equilibrium, the densities would be 9.95×10^7 , 9.93×10^7 , and 9.90×10^7 for dilution rates of 0.05, 0.25, and 0.50 h^{-1} , respectively. Consequently, by the use of cells derived from chemostats with the same medium and different dilution rates, the effects of density on antibiotic efficacy can be controlled for. The model predicts that as long as there are by-product resources, it should be possible to detect differences in the rate of antibiotic-mediated killing when batch cultures

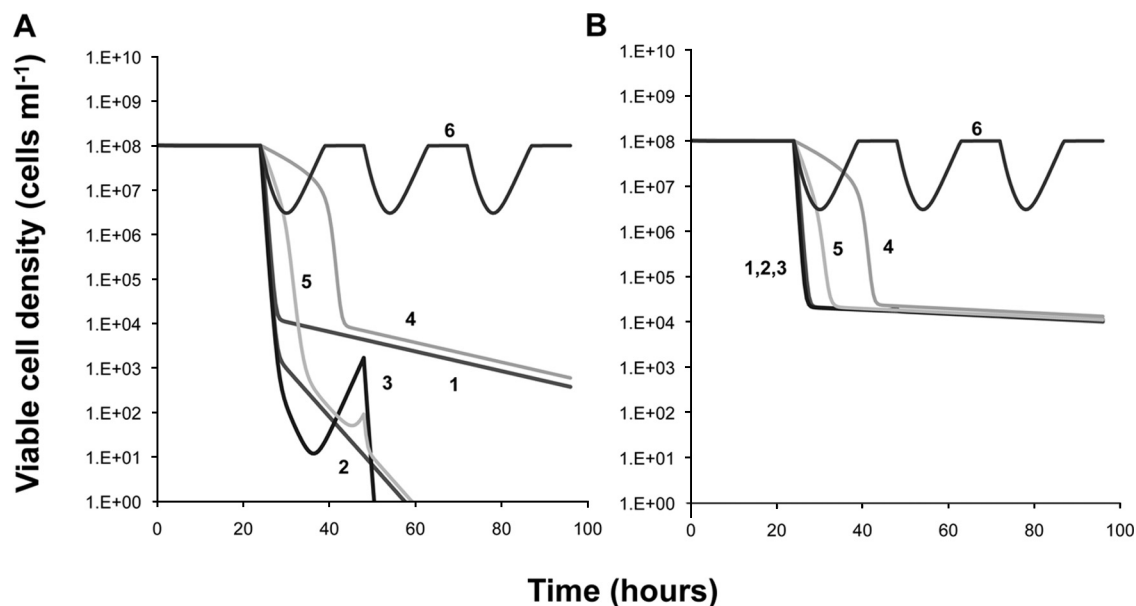


FIG. 8. Simulation of the population dynamics of antibiotic treatment in continuous culture with persistence or wall growth. Unless otherwise noted, the parameters are the standard values in Table 1 and pharmacokinetics as in figure 7A. (A) Changes in the viable cell densities in continuous cultures with persistence ($f_{NP} = f_{PN} = 0.00001$). Lines 1, 2, and 3, no resource concentration or density effects on the PD of the N population ($pr = pd = 0$) (line 1, $w = 0.05$; line 2, $w = 0.25$; line 3, $w = 0.50$). Lines 4, 5, and 6, resource- and density-dependent PD for the N population ($pr = 0.9$, $pd = 0.5$, $M_{\max} = 40$) (line 4, $w = 0.05$; line 5, $w = 0.25$; line 6, $w = 0.5$). (B) Changes in viable-cell density in continuous culture with wall growth ($f_{BN} = f_{NB} = 0.00001$, $w_p = 0.01$). Lines 1, 2, and 3, no resource concentration or density effects on the PD of the N population ($pr = pd = 0$) (line 1, $w = 0.05$; line 2, $w = 0.25$; line 3, $w = 0.50$). Lines 4, 5, and 6, resource- and density-dependent PD of the N population.

from equilibrium chemostats with different dilution rates are exposed to the same concentration of a bactericidal antibiotic. If we use the standard parameters in Table 1 and assume that byproduct resources are produced ($f = 0.1$), after 8 h of exposure to 50 mg/liter (50 times the MIC) of the antibiotic, the densities of viable cells derived from an equilibrium chemostat (initial density, 10^8) would be 6.6×10^7 , 3.1×10^7 , and 7.7×10^6 for dilution rates of 0.05, 0.25, and 0.50, respectively. There is evidence from studies performed with bacteria maintained in chemostats that the rate of antibiotic-mediated killing is proportional to the bacterial growth rate (13, 20, 23, 36, 56, 59), although for only a few combinations of bacterial species and antibiotics.

Our analysis of the properties of this model leads to a number of additional predictions about the anticipated results of time-kill experiments:

(iv) **Byproduct resources from non- or slow-growing cultures allow replication and antibiotic-mediated killing.** Findings consistent with this hypothesis would be substantial rates of growth and antibiotic-mediated killing when low densities of bacteria are introduced into sterile filtrates from cultures from equilibrium chemostats. The relative amount of available resource in this spent medium can be estimated from the extent of residual growth, as exemplified by the stationary-phase densities resulting from low densities of bacteria in sterile filtrates of equilibrium chemostats. When the bacteria used to determine the extent of residual growth are resistant to the antibiotic used in the treatment experiments, it is possible to estimate the extent to which killing by specific antibiotics contributes to the concentration of byproduct resources.

(v) **The rate of killing of bacteria by bactericidal antibiotics accelerates as the density of viable cells declines.** This can be tested by following the changes in the density of viable cells in populations of cells removed from equilibrium chemostats at full density and in reduced-density populations produced by diluting the culture with filtered supernatants of the full-density culture. If there are density and resource effects, the trajectory of the change in numbers of viable cells of the higher-density culture should be different from that of the lower-density culture. While the density of the population is high, the rate of decline would be low, but as its density declines, the slope of the change in viable-cell density would become increasingly similar to that of the lower-density culture. If there are no density or resource effects, the initial rates of decline should be similar for the high- and low-density cultures.

(vi) **The level of persistence is proportional to the density of bacteria exposed to the antibiotic and is only modestly affected by the antibiotic concentration.** Persistence is observed in time-kill experiments as a leveling off in the viable-cell density (58). Although not precise, the density of cells at this apparent asymptote can be used as a measure of the level of persistence. If this hypothesis were supported, for any given concentration of an antibiotic the level of persistence would be directly proportional to the initial density of bacteria. Moreover, increasing or decreasing the dose of the antibiotic would have little effect on the level of persistence. Although the results of a study by Wiuff and colleagues suggest that the level of persistence depends on the nature of the antibiotic as well as on its concentration (see Fig. 1 in reference 58), they did not explore

the effects of initial cell density on the persistence level. In a recent article, it was also observed that persister cells are selected for during the exponential phase of growth and that there is induction of additional persistence mechanisms upon antibiotic exposure, at least in the case of fluoroquinolones (17).

(vi) There is density-dependent and/or density-independent antibiotic decay. One way to estimate the rate of density-independent or density-dependent decay when using the effective concentration of the antibiotic is to incubate media containing the antibiotic in the absence of bacteria and in the presence of high densities of viable bacteria. When there is a density-independent decay, the effective concentration of the antibiotic declines in the cell-free culture. When there is a density-dependent decay, there is an additional decline in the effective concentration of the antibiotic in filtrates of the culture with high densities of cells (57).

Continuous-culture experiments. (i) As a consequence of a density effect on the efficacy of the antibiotic, dosing with low but above-MIC concentrations of bactericidal antibiotics does not have a perceptible effect on the density of viable cells. If this hypothesis is valid, the amount of antibiotic needed to produce substantial changes in the viable-cell density of an equilibrium chemostat would be substantially greater than the MIC. Presumably, with estimates of the relationship between the inoculum density and MIC obtained using the procedures outlined in references 52 and 57, it should be possible to predict the minimum concentration of the antibiotic needed for antibiotic-mediated killing in chemostats or hollow-fiber reactors.

(ii) Because the decline in an antibiotic concentration depends on the flow rate, under most conditions the extent of the reduction in viable-cell density between doses of a bactericidal antibiotic would be greater in chemostats with a lower flow rate than in those with high flow rates. This could be readily demonstrated by introducing the same concentration of the antibiotic into chemostats with low and high dilution rates.

(iii) Because the decline in the density of bacterial populations confronted with bacteriostatic antibiotics is primarily a function of the flow rate, the rate of decline in viable-cell density would be greater in fast than in slow chemostats. This hypothesis can be tested as described above (i) but with a bacteriostatic antibiotic employed.

(iv) Because of persistence and/or wall growth, as time proceeds the rate of decline of the viable-cell density following a pulse of antibiotics will either diminish or become zero. This would be shown by following the changes in viable cell densities of chemostat-grown populations treated with antibiotics. While wall growth (biofilms) and persistence may be similar in their dynamics early in a chemostat treatment experiment, their contributions can be distinguished in two ways. First, with persistence, but not wall growth, the rate of decline in viable-cell density should be proportional to the flow rate. Second, and more definitively, if an antibiotic-refractory wall population is responsible for slowing the pace of antibiotic treatment, that pace should increase when the liquid portion of the chemostat is moved to a new vessel.

(v) The concentration of the antibiotic is decaying at a rate greater than that anticipated by flow alone. By separately estimating the concentration of the antibiotic, i.e., by testing

the MICs of cell-free extracts taken at different times after dosing (57), it would be possible to determine whether the rate of decline in the concentration of the antibiotic was different from that anticipated by flow alone. While we prefer a bioassay, the concentration of antibiotics in samples can also be estimated chemically using high performance liquid chromatography (HPLC).

(vi) There are postantibiotic effects. To reduce the complexity of our model, we elected not to formally consider death (or cessation of growth) of bacteria exposed to antibiotics following the elimination of those drugs (postantibiotic effects) (16, 34, 35). If these effects exist, however, they would be characterized by a continued decline in viable-cell density when the concentration of the antibiotic fell below the MIC.

A couple of caveats and a bit of modeling philosophy. We want to emphasize that, although these models are quantitative, they are not anticipated to be numerically precise. As a result, interpretations of the results of tests of the hypotheses should be based on qualitative than rigorous quantitative criteria. For example, if hypothesis iii is valid, for any given bactericidal antibiotic concentration the rate of kill of bacteria in a culture derived from a high-dilution-rate chemostat should be significantly greater than that seen with a low-dilution-rate chemostat.

Except for persister and wall (biofilm) subpopulations, the model developed here and the hypotheses generated assume homogenous replicating populations. This model and the proposed tests of the hypotheses do not consider other physiologically distinct states where bacteria are refractory to antibiotics, e.g., conditions involving the stationary phase or spores.

By increasing their complexity and using fitting procedures to estimate their parameters, models can be used for numerically accurate predictions, but we do not see this as a necessary or even useful goal for in vitro studies of pharmacodynamics and population dynamics of antibiotic treatment. We are convinced that data from time-kill experiments and antibiotic-treated continuous cultures will provide more robust and realistic predictors of the efficacy of treatment than MICs alone. Nevertheless, no matter how precise in vitro estimates of the PD of antibiotics may be, they would almost certainly be different from the PD of those antibiotics and bacterial species for treated patients. The utility of these in vitro data, along with those obtained from animal model studies, is that they provide a rational basis for the development of antibiotic treatment protocols and for interpretation of the outcomes of clinical studies.

We hope that the results of this theoretical study stimulate as well as guide experimental investigations of the in vitro pharmacodynamics of antibiotics and bacterial species under conditions that are more realistic than those employed currently. They have certainly had that effect on us; our chemostats are bubbling away. Stay tuned to this (or a related) channel for some tests of these hypotheses.

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