Combating microbial resistance to antimicrobial agents through dosing regimen optimization

Michael Nikolaou

Chemical and Biomolecular Engineering Department

University of Houston

Houston, TX 77204-4004

nikolaou@uh.edu

Vincent H. Tam

Department of Clinical Sciences & Administration

University of Houston College of Pharmacy

Houston, TX 77030

vhtam@central.uh.edu

Abstract

Microbial resistance to antimicrobial agents has evolved to alarming proportions. To avert potentially catastrophic consequences for public health, a concerted effort is necessary. It should include, among other elements, the development of methods that can optimize the clinical use of existing agents and accelerate the development of new ones. For both tasks, the design of effective dosing regimens that suppress the emergence and proliferation of resistant microbial populations is crucial. In this chapter we provide a comprehensive presentation of our recent theoretical and experimental work on a mathematical modeling framework that can be used to optimize the design of such dosing regimens. Suggestions for future work are made.

Table of Contents

1. Antimicrobial resistance and the need to optimize dosing regimens

Microbial resistance to antimicrobial agents ("antimicrobial resistance") has reached alarming proportions. Repeated warnings are recently heard from concerned scientists about bacterial wars, new plagues, worldwide calamities, new apocalypses, and the risk of returning to the pre-antibiotics era (Cohen, 1992; Neu, 1992; Gold and Moellering, 1996; Levy, 1998; Drlica, 2001; Landman et al., 2002; Varaldo, 2002; Levy and Marshall, 2004; Morens et al., 2004). The enormity of the problem has not escaped the attention of popular press (Di Justo, 2005; Comarow, 2006; Silberman, 2007). According to the U.S. Food and Drug Administration (FDA) "addressing the issue of antimicrobial resistance is one of the most urgent priorities in the fields of public health today" (Food and Drug Administration, 2006). To avert potentially catastrophic consequences of antimicrobial resistance, a concerted effort on many fronts is necessary. It should include, among other elements, the development of methods that can (a) optimize the clinical use of existing agents and (b) accelerate the development of new agents. For both tasks, tools guiding the design of dosing regimens that suppress the emergence and/or proliferation of resistant microbial populations can make a significant impact. Such design tools should maximize the killing effect of agents (or combinations of agents) on heterogeneous microbial populations (composed of microbial subpopulations of varying susceptibility/resistance to the agent(s)) while avoiding toxicity problems for host organisms. The importance of dosing regimen design for clinical use has been emphasized repeatedly (Bonhoeffer et al., 1997; Lipsitch and Levin, 1997; Lipsitch et al., 2000; Chait et al., 2007). Beyond the obvious therapeutic benefits that better design of clinical dosing regimens would have for existing antimicrobial agents, by prolonging their efficacy through maintenance of their microbial killing

effectiveness, better dosing regimen design would also make the development of new agents more attractive, by promising a longer effective period of use for a developed agent. This promise could help make antimicrobial agent development more attractive for potential developers, hopefully contributing to a welcome reversal of the dire downward trend of newly FDA approved antimicrobial agents over the last two decades (Spellberg et al., 2004). Furthermore, tools for dosing regimen design would help to directly accelerate the antimicrobial agent development process. Indeed, when developing new agents, emphasis is traditionally placed on discovering new agent candidates. As crucial as this step may be, a long (multi-year) development period ensues, until an agent is fully developed (Drusano et al., 2006). During that period of development it is common that only a few dosing regimens are empirically tested, because of the very large number of experiments required for exhaustive testing (Sidebar 2). This practice limits our ability to realize the clinical potential of agents, either through premature abandonment of promising candidates or through inadvertent pursuit of dead ends. The critical role of selecting the right dosing regimen was dramatically exemplified in the case of daptomycin, for which selection of the right dosing regimen alone was the key

Sidebar 2 – Example of dosing regimen testing

To evaluate 6 daily doses (e.g., 0.5, 1, 2, 4, 6 and 8 g), 4 dosing frequencies (e.g., every 6, 8, 12 or 24 hours), 4 intravenous dosing administrations (e.g., intermittent infusion of 0.5, 1, 2 hours and continuous infusion over 24 hours) and 3 durations of treatment (e.g., 5, 10, 14 days) would require investigation of 288 (6×4×4×3) regimens for a single candidate. Reduction by, e.g., an order of magnitude would have obvious implications.

Sidebar 1 – Importance of dosing regimens: The daptomycin case

Daptomycin (Cubicin®, Cubist Pharmaceuticals) (UCSF, 2006) was initially under development in the 1970s with an *8-hour dosing interval*. Its development was abandoned in the early 1980s due to an intolerable adverse effect (muscle toxicity). However, after understanding its exposure-related killing properties and toxicity, redevelopment began in the 1990s, to finally reach FDA approval for clinical use in 2003. The key factor for FDA approval was use of a *once-daily and weight-based dosing regimen*. This discovery was deemed so non-obvious and important as to be awarded a patent (Oleson et al., 2005).

differentiating factor between abandoning development in early 1980s and eventually securing FDA approval in 2003 (Sidebar 1).

The preceding discussion should make clear the value of methods that can guide the design of effective dosing regimens for combating antimicrobial resistance. In this chapter we provide a comprehensive presentation of a recent mathematical modeling framework (Nikolaou and Tam, 2006; Nikolaou et al., 2007) that can be used to optimize the design of such dosing regimens. Experimental in vitro validation on *Pseudomonas aeruginosa*, an important bacterial pathogen (Sidebar 3) is presented. However, we want to emphasize at the outset that the proposed modeling approach could be extrapolated to a variety of antimicrobial agents (e.g., *antibacterials*, *antifungals* and *antivirals*) with different mechanisms of action, as well as to other pathogens (e.g., *HIV*, *tuberculosis*, *anthrax* and *avian influenza*) with different biological characteristics (Gumbo et al., 2004; Tam et al., 2005). In addition, the proposed mathematical framework could also be extended for use in *cancer chemotherapy*, by accounting for heterogeneities of cancerous cell populations (Dua et al., 2005).

In the rest of this chapter we provide a background for our work, present our findings, and conclude with suggestions for further development.

Sidebar 3 – Pseudomonas aeruginosa

P. aeruginosa is associated with serious nosocomial infections such as pneumonia and sepsis. It exploits multiple mechanisms of resistance to various antimicrobial agents (such as efflux pumps, β-lactamases production, porin channel deletion, multi-functional group transferases, and target site mutation) (Livermore, 2002). Some of the mechanisms of resistance are highly specific to one agent, while others affect a broad spectrum of antimicrobial agents, and confer different levels of resistance. Resistance to first-line agents (such as β-lactams and fluoroquinolones) has been reported and is becoming more prevalent (Landman et al., 2002; Neuhauser et al., 2003). There are very few agents in the advanced stage of development designed to target multi-drug resistant Gram-negative bacteria, and none is expected to be available for clinical use in the next decade. Therefore, the need to develop antimicrobial agents against *P. aeruginosa* is imperative (Talbot et al., 2006).

2. Background

2.1. Pharmacodynamic indices and their limitations

Because the complex pharmacodynamic interaction between an antimicrobial agent and a microbial population defies detailed first-principles modeling, surrogate pharmacodynamic indices, such as the *minimum inhibitory concentration* (MIC) (Figure 1) are used to guide empirical testing of dosing regimens (e.g., Andes and Craig, 1998; Louie et al., 1998; Nicolau et al., 2000; Louie et al., 2001; Tam et al., 2002; Dandekar et al., 2003; Andes et al., 2004; Maglio et al., 2004; Miyazaki et al., 2004). This can be problematic. For example, according to the standard definition of MIC and related surrogate pharmacodynamic indices, the two populations in Figure 1 would correspond to the same MIC (there is no visible growth at exactly 24 hours) although they are otherwise clearly different: The second population could well grow beyond 24 hours whereas the first would probably not.

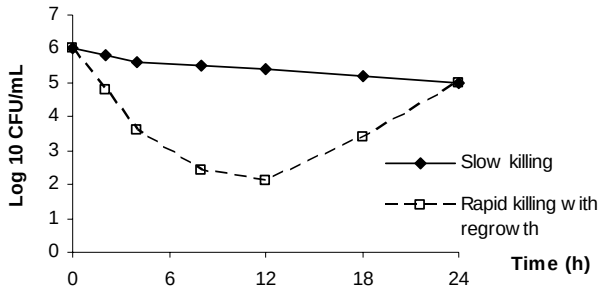


Figure 1. Two hypothetical killing profiles of microbial population size resulting in the same value at 24 hours after exposure to an antimicrobial agent at *time-invariant concentration* (Tam et al., 2005). The corresponding microbial populations would be very different in each case (slow decline (diamonds) versus rapid decline followed by regrowth (squares)).

Along the same lines, a dosing regimen maintaining agent concentration above MIC would not necessarily guarantee eventual eradication of the entire population, as argued in Figure 4. This is because inhibition of population growth at 24 hours leaves the possibility that a small resistant subpopulation of no appreciable size during the first 24 hours may well grow afterwards. MIC lumps all dynamic information of a time-kill experiment into a single point. Consequently,

methods that make use of all available (dynamic) information from time-kill experiments would be preferable. This realization, in turn, raises the question "*How is such dynamic information captured and used?*" We address this question in the next section.

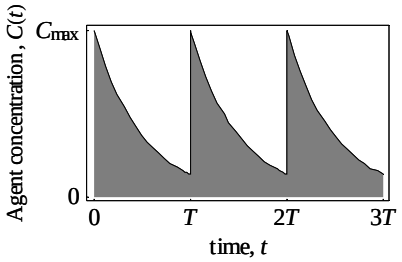


Figure 2. Example of a clinically relevant dosing regimen. *Periodic* injection of agent every T time units and its subsequent elimination result in a jump-decay periodic pattern ($C(t) = C_{\max} 2^{-t/t_{1/2}} = C_{\max} e^{-t/\tau}$, $0 \le t < T$; $C(t+T) = C(t)$) according to typical pharmacokinetics in humans.

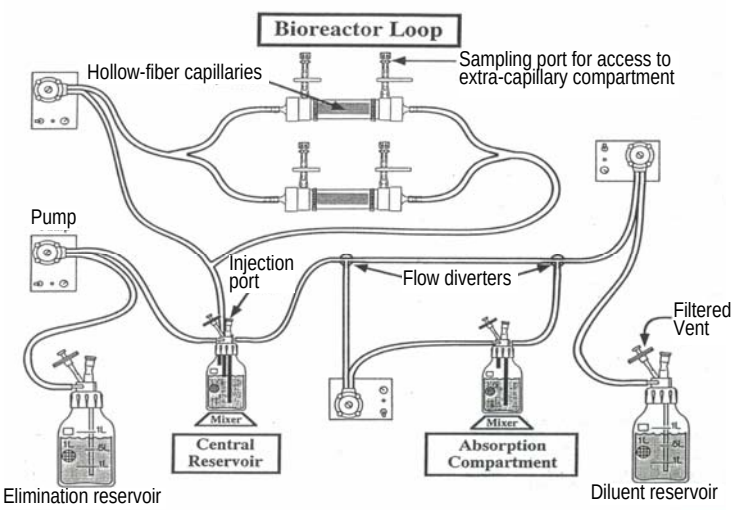


Figure 3. In vitro hollow-fiber infection model (Bilello et al., 1994) is a cell culture system in which a microbial population is exposed to fluctuating antimicrobial concentration, simulating human elimination and repeated dosing over a few days, corresponding to a clinical course of treatment. The system has been used by our group to investigate the pre-clinical potential of antimicrobial agents under development (Tam et al., 2005; Nikolaou et al., 2006).

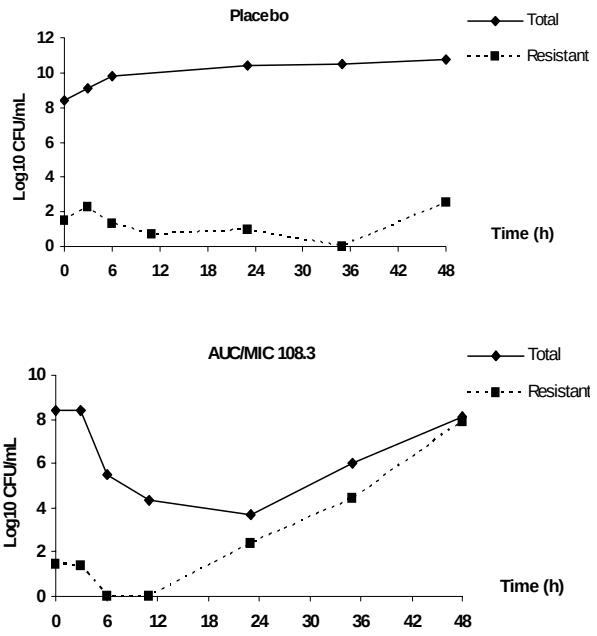


Figure 4. Selection of resistant *P. aeruginosa* population by garenoxacin in an in-vitro hollow-fiber infection model (Figure 3). A population of approximately 10^8 CFU/ml bacteria was investigated (Tam et al., 2005). In the absence of selective pressure by garenoxacin ($C(t) = 0$, top) the fraction of the resistant subpopulation remained low and relatively constant over time. In contrast, exposure to a fluctuating garenoxacin concentration $C(t)$ (bottom) as in Figure 2 selectively amplified the resistant subpopulation (MIC of the resistant subpopulation ≥ 3 × MIC of the entire population) and led to population regrowth, *despite the fact that garenoxacin concentration $C(t)$ remained well above MIC during the entire period of the experiment*.

2.2. Dynamic models of pharmacodynamic activity and their limitations for microbial populations of nonuniform susceptibility/resistance

In an effort to use the dynamic information that pharmacodynamic indices leave out, dynamic models have been formulated for homogeneous microbial populations (i.e. of uniform susceptibility or resistance) based on conservation principles and bacteria-agent Hill-like (Hill, 1910) kinetics (Wagner, 1968; Jusko, 1971; Giraldo et al., 2002) (Sidebar 4). When applied to heterogeneous microbial populations (i.e. of *non*uniform susceptibility or resistance), such

Sidebar 4 – Basic model of effect of antimicrobial agent on homogeneous microbial population

$$\frac{dN(t)}{dt} = \underbrace{G[\overbrace{N(t)}^{\text{Population size}}]}_{\text{Physiological net growth rate}} - \underbrace{K[\overbrace{C(t)}^{\text{Agent concentration}}, N(t)]}_{\text{Kill rate due to antimicrobial agent}} \quad (1)$$

Example: (Hill and Michaelis-Menten dynamics)

$$G[N(t)] = K_g\left(1-\tfrac{N(t)}{N_{\max}}\right)N(t) \approx K_g N(t) \text{ if } N(t) \ll N_{\max}$$

$$K[C(t), N(t)] = \underbrace{\frac{K_k C(t)^{H\}\text{ Hill factor}}}{C(t)^H + C_{50}{}^H}}_{\text{kill rate constant, } r} N(t)$$

The population is eradicated if and only if $r > K_g$. If C is time-invariant, eradication corresponds to the straight line $\ln\frac{N(t)}{N(0)} = \left(K_g - r(C)\right)t$.

models lump sub-populations into two distinct classes: *resistant* and *susceptible* (Lipsitch and Levin, 1997; Mouton et al., 1997; Jumbe et al., 2003; Gumbo et al., 2004; Meagher et al., 2004; Campion et al., 2005; Tam et al., 2005). Though conceptually appealing, when such models are calibrated using standard short-term data (e.g. over 24-hours (Andes and Craig, 1998; Nicolau et al., 2000; Dandekar et al., 2003; Miyazaki et al., 2004)) they may easily fail to predict the emergence of resistance – manifested as eventual population regrowth (Oliver et al., 2004) – as shown in Figure 5 (top). By lumping subpopulations of varied resistance into *two* distinct subpopulations (resistant and susceptible), this modeling approach essentially produces two asymptotes for the dependence of population size on time corresponding to short and long time, respectively. As Figure 5 (top) shows, the two asymptotes estimated by fitting 24-hour data produce overly optimistic results beyond 24 hours. For similar reasons, dynamic modeling approaches that have focused on the early time course (<60 minutes) of antimicrobial agent exposure (Regoes et al., 2004) are equally problematic.

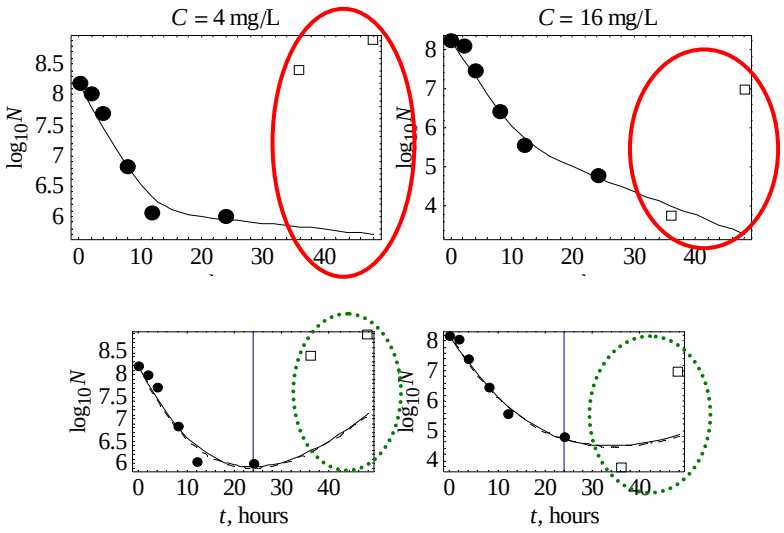


Figure 5. *In vitro* effect of the antibiotic meropenem on *Pseudomonas Aeruginosa* ATCC 27853 (Tam et al., 2005). A standard (two-subpopulation) dynamic model (*top*) built from 24-hour data (dots) fails to predict population regrowth beyond 24 hours (squares). By contrast, a model based on the approach developed by Nikolaou and Tam (2006) (*bottom*) successfully predicts regrowth *using the same 24-hour data*, thus suggesting that much higher antibiotic concentration is needed for eradication of the entire population.

3. A new approach to modeling the effect of antimicrobial agents on heterogeneous microbial populations

To capture the decline-regrowth behavior of a heterogeneous microbial population, Nikolaou and Tam (2006) developed a corresponding mathematical modeling approach (excerpted in Sidebar 5) for heterogeneous microbial populations exposed to time-invariant antimicrobial agent concentrations. Dispensing with the need to rely on the asymptotic time behavior of two distinct sub-populations, this approach considers a distribution of resistance over a microbial population and employs the *cumulants* of that distribution. Figure 5 (bottom) demonstrates that this approach can successfully make use of *standard* 24-hour time-kill data to predict regrowth beyond 24 hours and estimate the agent concentration needed to eradicate the entire microbial

Sidebar 5 – Modeling the effect of antimicrobial agents on heterogeneous microbial populations

$$\frac{dN(t)}{dt} = [K_g - \mu(t)]N(t), \quad \frac{d\mu(t)}{dt} = -\sigma(t)^2, \quad \left\{\frac{d\kappa_n(t)}{dt} = -\kappa_{n+1}(t)\right\}_{n\geq 1} \quad \left(\kappa_1 \equiv \mu,\ \kappa_2 \equiv \sigma^2\right) \tag{2}$$

$N(t)$: microbial population size at time *t*

K_g: growth rate constant

$\mu(t)$, $\sigma^2(t)$: average and variance of kill rate constant over entire microbial population

$\kappa_n(t)$: n-order cumulant (Weisstein, 2005) of kill rate constant

For a distribution $f(r_i,t)$ of the kill rate constant r (Sidebar 4) cumulants are defined as $\kappa_n(t) \triangleq \dfrac{\partial^n \Psi(s,t)}{\partial s^n}$ where $\Psi(s,t) \triangleq \ln\left[M(s,t)\right]$ $M(s,t) \triangleq \sum_i e^{sr_i} f(r_i,t)$.

Simplifying assumptions (Nikolaou and Tam, 2006) yield

$$\ln\left[\frac{N(t)}{N(0)}\right] \approx \left(K_g \underbrace{-\mu(0) + \frac{\sigma(0)^2}{A}}_{-b}\right)t + \underbrace{\frac{\sigma(0)^2}{A^2}}_{R/A}\left(e^{-At}-1\right) \triangleq \left(K_g - b\right)t + \frac{R}{A}\left(e^{-At}-1\right), \tag{3}$$

$$\mu(t) \approx \underbrace{\mu(0) - \frac{\sigma(0)^2}{A}}_{b} + \underbrace{\frac{\sigma(0)^2}{A}}_{R} e^{-At} \triangleq b + Re^{-At}, \tag{4}$$

$$\sigma(t)^2 \approx \sigma(0)^2 e^{-At}. \tag{5}$$

population (Nikolaou and Tam, 2006).

Using the approach mentioned above, we can now address the following question, which is the main focus of this work: *"Given time-kill data over 24 hours at a number of time-invariant agent concentrations, what is an effective (preferably optimal) dosing regimen (daily dose and dosing interval) for time-varying agent concentration corresponding to realistic pharmacokinetics* (*Figure 2*)?". Optimal here refers to the smallest daily dose and corresponding dosing interval that can completely eradicate a microbial population. For lack of quantitative aids to answering the preceding question, it is common practice for antimicrobial killing action to be classified into two distinct categories: *peak-concentration*- or *time-of-exposure*-dependent (Vogelman and Craig, 1986; Craig, 1998) as shown in Figure 6. However, it has been widely observed that some recent antimicrobials (e.g. quinolones) do not clearly fall in either category. Therefore, a more quantitative answer to the preceding question is needed, as discussed next.

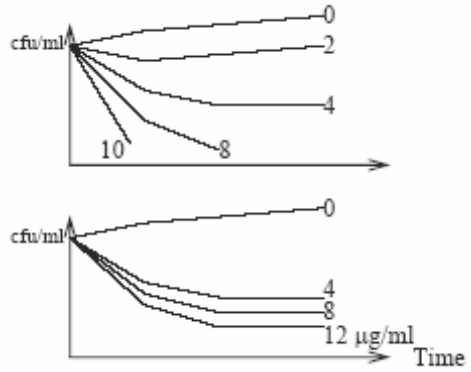


Figure 6. Concentration-dependent (upper) and time-dependent (lower) killing activity of antimicrobial agents. In the concentration-dependent case, killing activity depends on the concentration of the antimicrobial agent used, and suggests dosing regimens that achieve high concentrations at injection points. In the time-dependent case, killing activity quickly reaches a plateau as agent concentration increases, indicating that dosing regimens need to maintain a certain agent concentration most of the time. Increasing agent concentration will increase toxicity without appreciably increasing killing activity.

3.1. Homogeneous microbial population under pharmacokinetically realistic antimicrobial concentration

Sidebar 6 – Model of effect of antimicrobial agent on heterogeneous microbial population

A homogeneous population is subjected to periodically fluctuating antimicrobial agent concentration, i.e. $C(t) = C\left(t+T\right)$. Then, it can be shown (Nikolaou et al., 2007) that

$$\ln\frac{N(t)}{N(0)} = K_g t - \left[\!\left[\frac{t}{T}\right]\!\right] DT - \int_0^{t-\left[\!\left[\frac{t}{T}\right]\!\right]T} r\left(C(\eta)\right)d\eta$$

where $\left[\!\left[\frac{t}{T}\right]\!\right]$ is the integer part of the real number $\frac{t}{T}$, and $\boxed{D \triangleq \frac{1}{T}\int_0^T r\left(C(\eta)\right)d\eta}$ is the time-averaged kill rate constant.

At times $t = nT$, $n = 0,1,2,...$ the total population satisfies the equation

$$\boxed{\ln\frac{N(nT)}{N_0} = \left(K_g - D\right)nT}, \quad n = 0,1,2,...$$

Assume now that the antimicrobial agent concentration does not remain time-invariant but fluctuates periodically due to periodic injection of agent every T time units and its subsequent elimination, as shown in Figure 2. The kill rate constant $r(C(t))$ will obviously fluctuate with the same period *T*. Under these conditions, it can be shown (Nikolaou et al., 2007) that the total population $N(t)$ exhibits a periodic pattern with period *T*, and the values of $\log\left(N(nT)\right)$, $n = 0,1,2,...,$ lie on a straight line, akin to the case corresponding to time-invariant agent concentration (Sidebar 4) as summarized in Sidebar 6. In other words, the points $\frac{N(nT)}{N(0)}$ appear as if they were generated by a system under *time-invariant* agent concentration *D*, a fact that significantly simplifies the ensuing analysis (Figure 7).

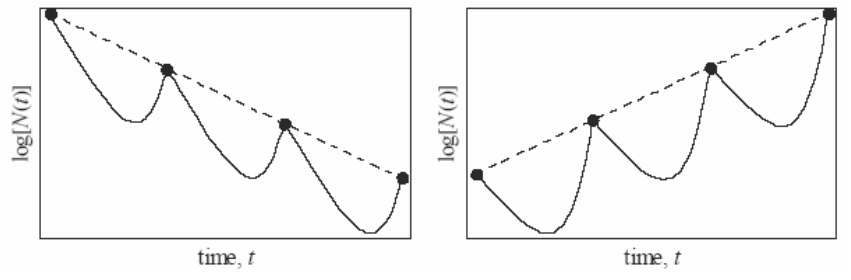


Figure 7. Eradication (left) or regrowth (right) of a microbial population in an environment of antimicrobial agent concentration following Sidebar 6.

Therefore, according to Sidebar 6, $\frac{D}{K_g} > 1$ implies eradication of the entire microbial population, whereas $\frac{D}{K_g} < 1$ implies eventual proliferation of the population, except for the case where eradication can occur during the first dosing interval. The latter case can occur if the minimum of $\ln N(t)$, $0 \le t \le T$, is at or below 0.

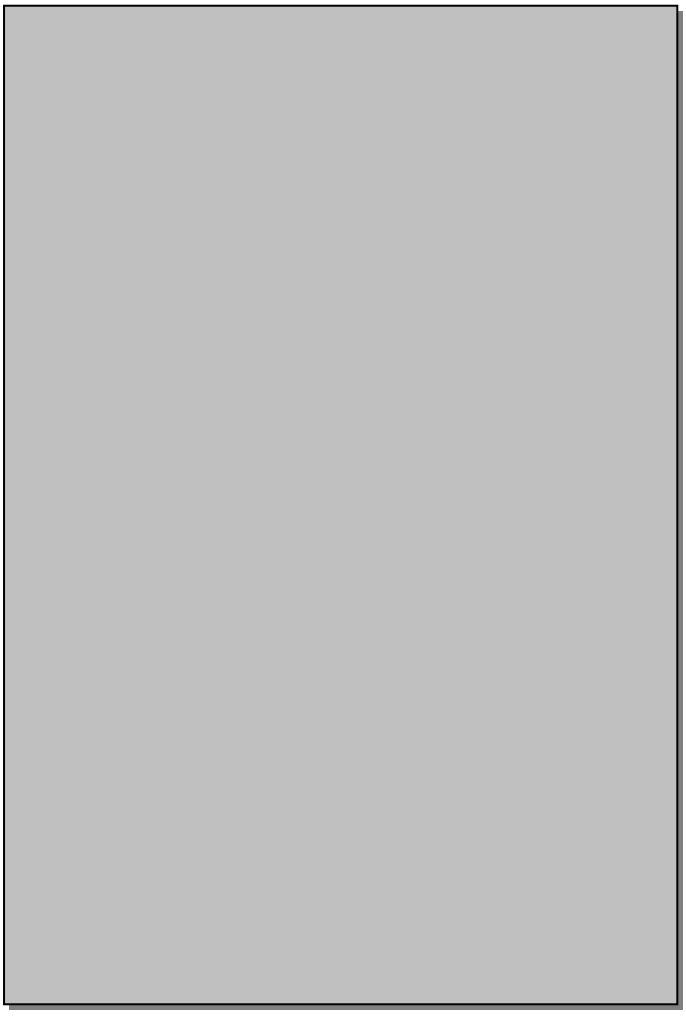
We can now ask "*For what dosing regimens is the condition* $\frac{D}{K_g} > 1$ *satisfied*?" We first provide a qualitative approximate answer, followed by a quantitative answer.

Qualitatively, the value of $\frac{D}{K_g}$, to first-order approximation, can be shown to be

$$\frac{D}{K_g} \approx 1 + \frac{r'(C_{\text{cr}})}{K_g}\left(C_{\text{avg}} - C_{\text{cr}}\right) = 1 + \frac{r'(C_{\text{cr}})}{K_g} C_{\text{cr}} \left(\frac{\text{AUC}/T}{C_{\text{cr}}} - 1 \right)$$

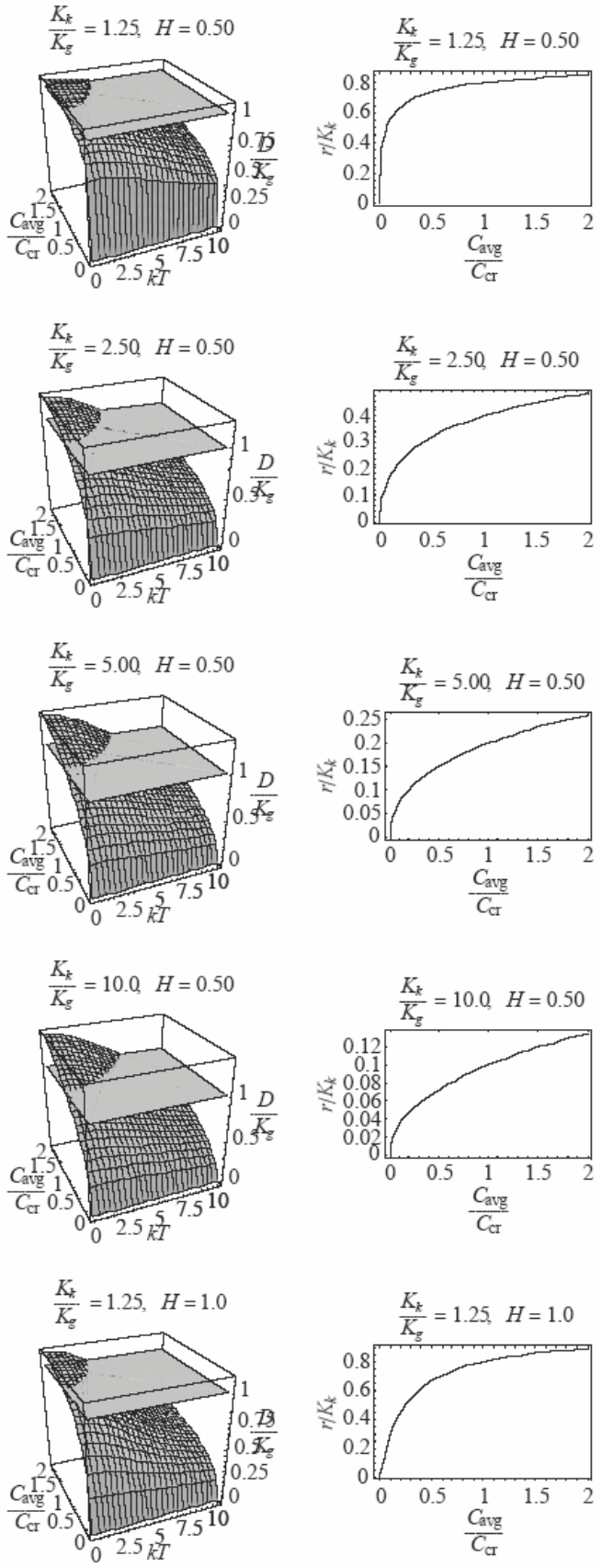
where the area under the concentration curve (AUC) is defined as $\text{AUC} \hat{=} \int_0^T C(t)dt$. The above approximation of $\frac{D}{K_g}$ indicates that in order to design a dosing regimen resulting in eradication of a microbial population, the average concentration of the agent, $C_{\text{avg}} \hat{=} \frac{1}{T}\int_0^T C(\eta)d\eta$, must be above the critical concentration C_{cr}, defined as the concentration at which the kill rate constant $r\left(C_{\text{cr}}\right)$ is equal to the growth rate constant K_g. It follows that the effectiveness of an agent is *approximately* related to the well known pharmacokinetic/pharmacodynamic parameter $\text{AUC/MIC} \approx \text{AUC}/C_{\text{cr}}$. However, it should be stressed that the dependence of an agent's effectiveness on AUC/MIC is only approximate. A more accurate index would have to be used to account for strong effects of higher-order derivatives in the above series expansion of $\frac{D}{K_g}$. This motivates the quantitative results presented next.

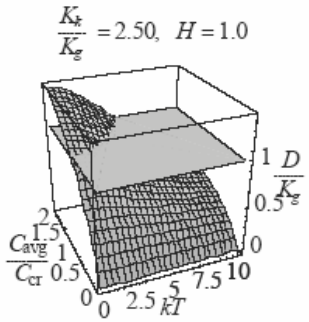
From a quantitative viewpoint, let the agent concentration follow the realistic pharmacokinetic pattern $C(t) = C_{\max} e^{-kt}, \quad 0 \le t < T$ (Figure 2) where $k = \frac{\ln 2}{t_{1/2}}$ is the agent elimination rate constant (reciprocally proportional to the half-time $t_{1/2}$) and T is the dosing interval; and let the kill rate constant follow the Michaelis-Menten/Hill kinetics in Sidebar 4. Then it can be shown (Nikolaou et al., 2007) that the value of $\frac{D}{K_g}$ can be influenced by selecting *two dimensionless variables* associated with the *dose* and *dosing interval* of a dosing regimen, namely the scaled average concentration $z \hat{=} \frac{C_{\text{avg}}}{C_{\text{cr}}}$ (or, equivalently, $y \hat{=} \frac{C_{\text{avg}}}{C_{50}}$) and the scaled dosing interval $x \hat{=} kT$, where C_{avg} is proportional to the administered dose (mass of agent over 24 hour period). The functional dependence of $\frac{D}{K_g}$ on x, z depends on *two pharmacodynamic parameters*: H and $\frac{K_k}{K_g}$.

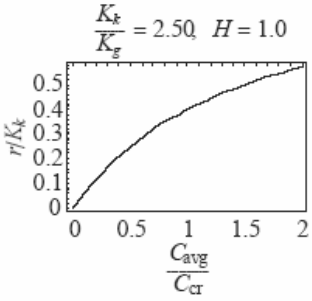


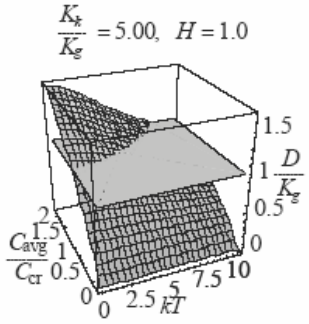
Thus, if the values of H and $\frac{K_k}{K_g} = 1 + \left(\frac{C_{50}}{C_{\text{cr}}}\right)^H$ have been estimated from experimental time-kill data, one can visualize the agent effectiveness, i.e. value of $\frac{D}{K_g}$ in comparison to 1, as a function of the two variables that characterize a dosing regimen, namely $z \hat{=} \frac{C_{\text{avg}}}{C_{\text{cr}}}$ and $x \hat{=} kT$ (Sidebar 7).

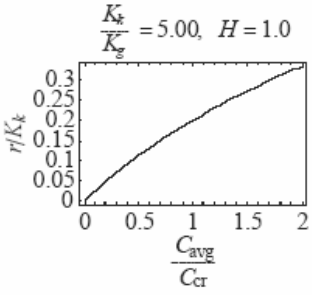
Figure 8 shows a small library of such patterns for different values of H and $\frac{K_k}{K_g}$, along with associated plots of the scaled kill rate constant $\frac{r(C)}{K_k}$ as a function of $\frac{C}{C_{\text{cr}}}$. A careful examination of these patterns for $\frac{D}{K_g}$ (lines corresponding to $\frac{D}{K_g}=1$) reveals qualitatively different behaviors for different values of H and $\frac{K_k}{K_g}$, suggesting different designs for optimal dosing regimens. For example, for $H=1$ and $\frac{K_k}{K_g}=5$ it is clear that the shorter the dosing interval T (Figure 2), the lower the dose that can be used. Consequently, the optimal dosing regimen would be continuous infusion. This is due to the dependence of the kill rate constant *r* on *C*: A relative increase in *C* is associated with a lower relative increase in *r*. Therefore, for a periodically fluctuating profile of *C* around an average value C_{avg} a lot more killing power *r* would be lost while $C(t)<C_{\text{avg}}$ than would be gained while $C(t)>C_{\text{avg}}$. By contrast, $H=4$ and $\frac{K_k}{K_g}=5$ in Figure 8 indicates that there is an optimal value (around $kT=5$) for the dosing interval *T* at the cut-off point $\frac{D}{K_g}=1$, corresponding to the minimum dose $C_{\text{avg}}/C_{\text{cr}}\approx 1$. This is due to the presence of an inflection point in the curve corresponding to the dependence of the kill rate constant *r* on *C*: Around the inflection point, a relative increase in *C* is associated with a lower relative increase in *r*. However, a relative decline in *C* is also associated with a lower relative decline in *r*. Therefore, in balance, for a periodically fluctuating profile of *C* around the optimal average value C_{avg} a lot less killing power *r* is lost while $C(t)<C_{\text{avg}}$ than is gained while $C(t)>C_{\text{avg}}$. While Figure 8 may be sensitive to experimental errors in the estimates of *H* and $\frac{K_k}{K_g}$, it establishes a continuum for the model of action of antimicrobial agents, at the two ends of which are the two well known categories, namely *peak-concentration*- or *time-of-exposure*-dependent, established by Vogelman and Graig (1986).

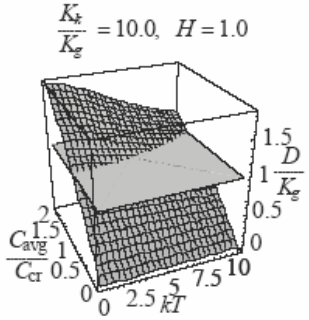

$\frac{K_k}{K_g} = 1.25,\ H = 0.50$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT
$\frac{r}{K_k}$
$\frac{K_k}{K_g} = 2.50,\ H = 0.50$
$\frac{K_k}{K_g} = 5.00,\ H = 0.50$
$\frac{K_k}{K_g} = 10.0,\ H = 0.50$
$\frac{K_k}{K_g} = 1.25,\ H = 1.0$

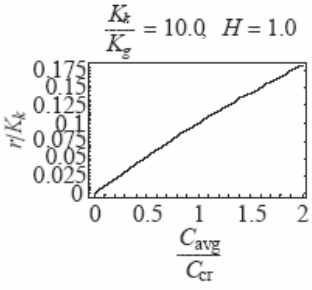

$\frac{K_k}{K_g} = 2.50,\ H = 1.0$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT

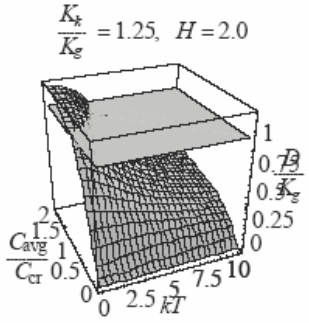

$\frac{K_k}{K_g} = 2.50,\ H = 1.0$
r/K_k
$\frac{C_{avg}}{C_{cr}}$

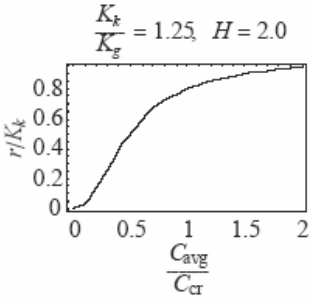

$\frac{K_k}{K_g} = 5.00,\ H = 1.0$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT

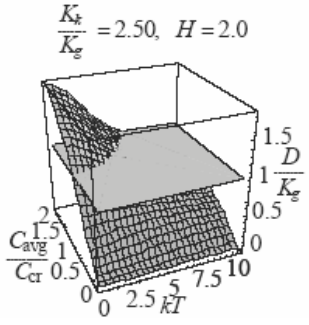

$\frac{K_k}{K_g} = 5.00,\ H = 1.0$
r/K_k
$\frac{C_{avg}}{C_{cr}}$

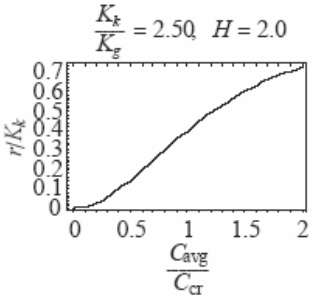

$\frac{K_k}{K_g} = 10.0,\ H = 1.0$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT

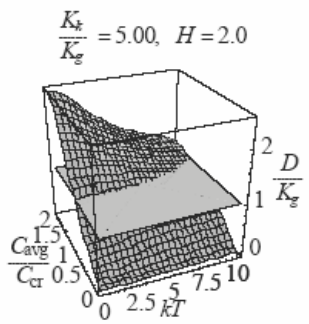

$\frac{K_k}{K_g} = 10.0,\ H = 1.0$
r/K_k
$\frac{C_{avg}}{C_{cr}}$

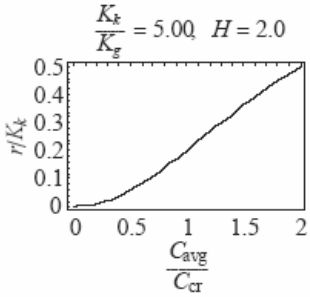

$\frac{K_k}{K_g} = 1.25,\ H = 2.0$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT

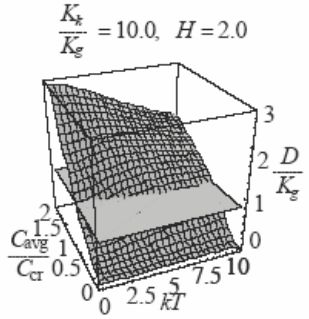

$\frac{K_k}{K_g} = 1.25,\ H = 2.0$
r/K_k
$\frac{C_{avg}}{C_{cr}}$

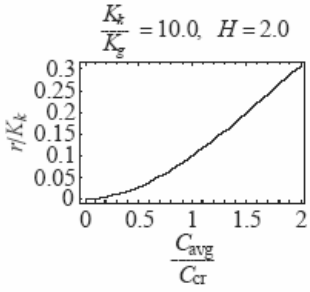

$\frac{K_k}{K_g} = 2.50,\ H = 2.0$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT

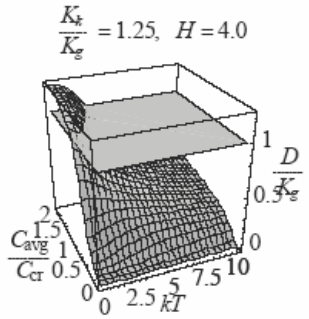

$\frac{K_k}{K_g} = 2.50,\ H = 2.0$
r/K_k
$\frac{C_{avg}}{C_{cr}}$

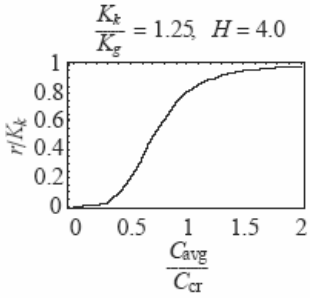

$\frac{K_k}{K_g} = 5.00,\ H = 2.0$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT

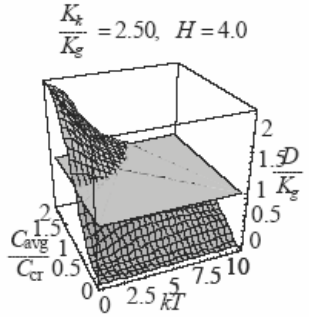

$\frac{K_k}{K_g} = 5.00,\ H = 2.0$
r/K_k
$\frac{C_{avg}}{C_{cr}}$

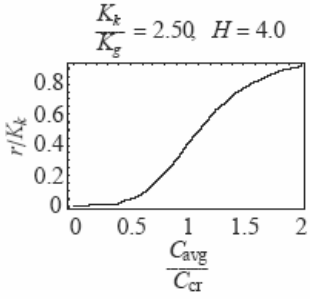

$\frac{K_k}{K_g} = 10.0,\ H = 2.0$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT

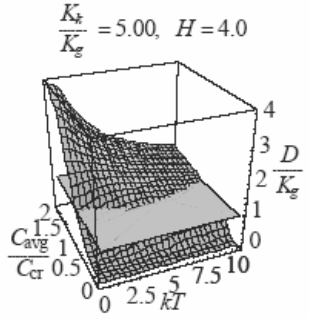

$\frac{K_k}{K_g} = 10.0,\ H = 2.0$
r/K_k
$\frac{C_{avg}}{C_{cr}}$

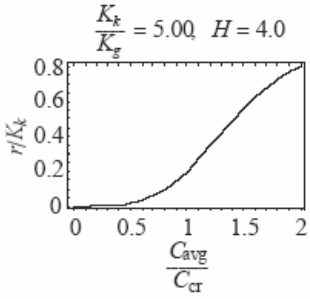

$\frac{K_k}{K_g} = 1.25,\ H = 4.0$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT

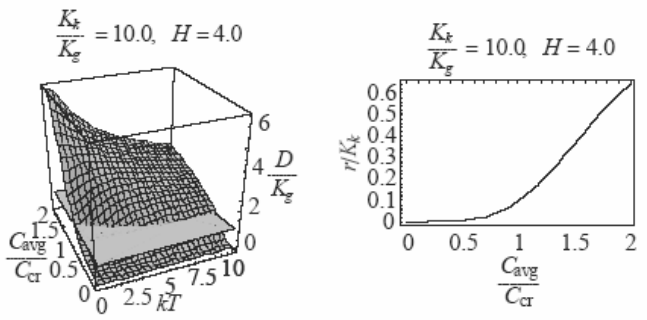

$\frac{K_k}{K_g} = 1.25,\ H = 4.0$
r/K_k
$\frac{C_{avg}}{C_{cr}}$


$\frac{K_k}{K_g} = 2.50,\ H = 4.0$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT

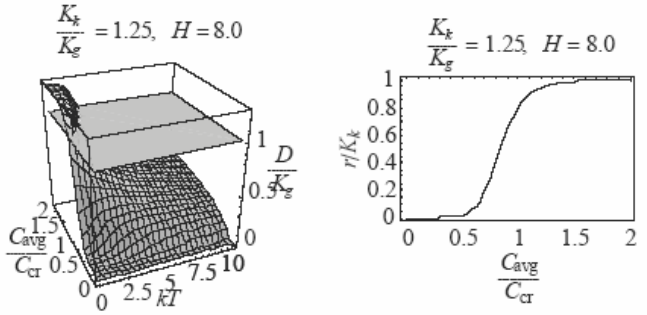

$\frac{K_k}{K_g} = 2.50,\ H = 4.0$
r/K_k
$\frac{C_{avg}}{C_{cr}}$


$\frac{K_k}{K_g} = 5.00,\ H = 4.0$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT

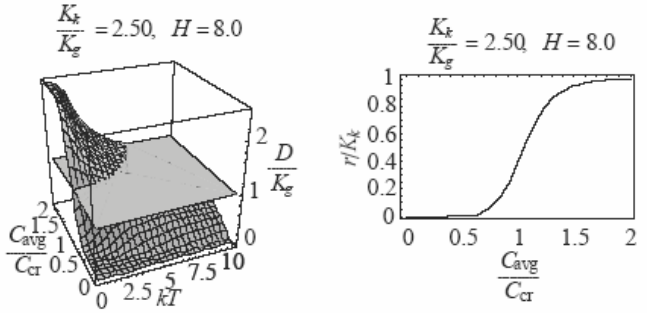

$\frac{K_k}{K_g} = 5.00,\ H = 4.0$
r/K_k
$\frac{C_{avg}}{C_{cr}}$


$\frac{K_k}{K_g} = 10.0,\ H = 4.0$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT

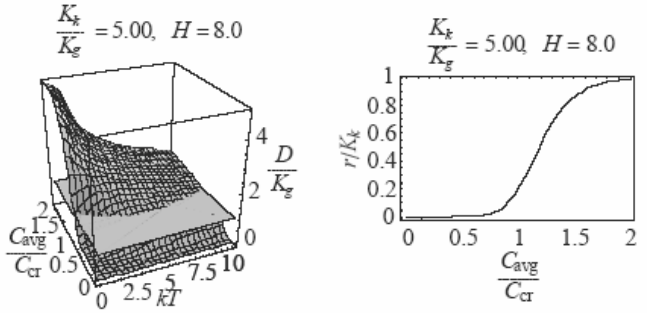
$\frac{K_k}{K_g} = 10.0,\ H = 4.0$
r/K_k
$\frac{C_{avg}}{C_{cr}}$


$\frac{K_k}{K_g} = 1.25,\ H = 8.0$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT

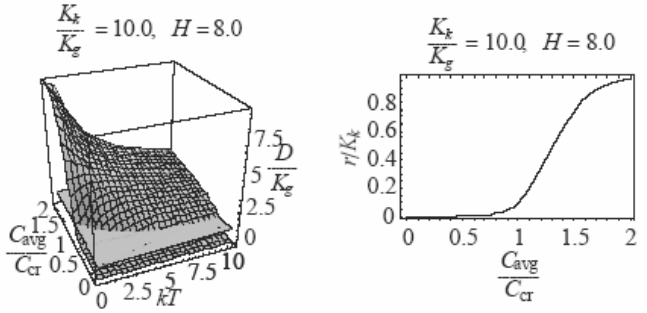
$\frac{K_k}{K_g} = 1.25,\ H = 8.0$
r/K_k
$\frac{C_{avg}}{C_{cr}}$


$\frac{K_k}{K_g} = 2.50,\ H = 8.0$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT

$\frac{K_k}{K_g} = 2.50,\ H = 8.0$
r/K_k
$\frac{C_{avg}}{C_{cr}}$


$\frac{K_k}{K_g} = 5.00,\ H = 8.0$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT

$\frac{K_k}{K_g} = 5.00,\ H = 8.0$
r/K_k
$\frac{C_{avg}}{C_{cr}}$


$\frac{K_k}{K_g} = 10.0,\ H = 8.0$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT

$\frac{K_k}{K_g} = 10.0,\ H = 8.0$
r/K_k
$\frac{C_{avg}}{C_{cr}}$

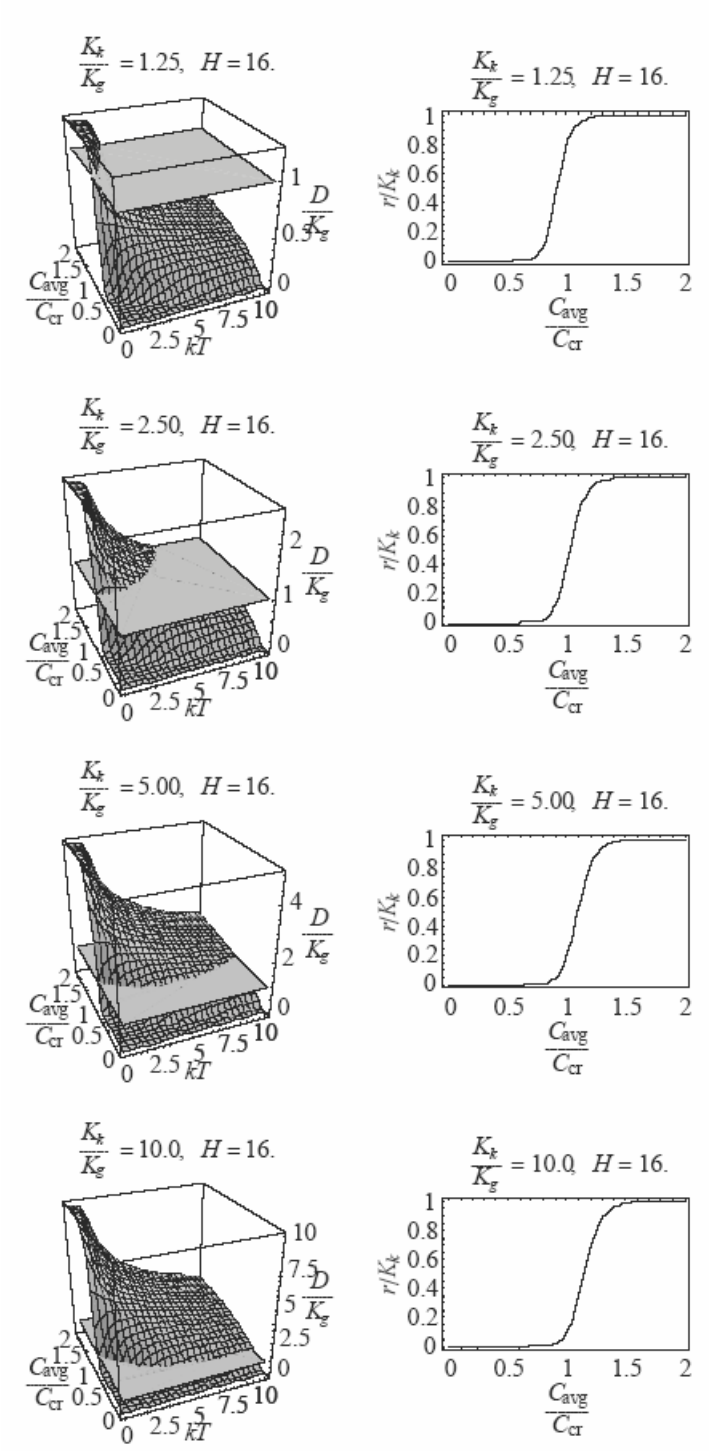

$\frac{K_k}{K_g} = 1.25,\ H = 16.$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT
$\frac{K_k}{K_g} = 1.25,\ H = 16.$
r/K_k
$\frac{C_{avg}}{C_{cr}}$
$\frac{K_k}{K_g} = 2.50,\ H = 16.$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT
$\frac{K_k}{K_g} = 2.50,\ H = 16.$
r/K_k
$\frac{C_{avg}}{C_{cr}}$
$\frac{K_k}{K_g} = 5.00,\ H = 16.$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT
$\frac{K_k}{K_g} = 5.00,\ H = 16.$
r/K_k
$\frac{C_{avg}}{C_{cr}}$
$\frac{K_k}{K_g} = 10.0,\ H = 16.$
$\frac{D}{K_g}$
$\frac{C_{avg}}{C_{cr}}$
kT
$\frac{K_k}{K_g} = 10.0,\ H = 16.$
r/K_k
$\frac{C_{avg}}{C_{cr}}$

Figure 8. A library of behaviors of $\frac{D}{K_g}$ as a function of kT and $\frac{C_{\text{avg}}}{C_{\text{cr}}}$. The optimal dosing regimen corresponds to the smallest possible value of C_{avg} that results in eradication of a microbial population, namely $\frac{D}{K_g} > 1$. The dependence of optimal C_{avg} on $\frac{K_k}{K_g}$ and H is qualitatively different for different values of $\frac{K_k}{K_g}$ and H.

3.2. Heterogeneous microbial population under pharmacokinetically realistic antimicrobial concentration

We are not going to use the results of the preceding sections to develop a method for designing optimal dosing regimens for heterogeneous microbial populations (i.e. of *non*uniform susceptibility or resistance). From a theoretical viewpoint, it would be interesting to develop an equation for $\ln\frac{N(t)}{N(0)}$ analogous to that in Sidebar 6. However, the following reasoning makes this requirement unnecessary:

To design an optimal dosing regimen it is required to find the minimum of the time-averaged agent concentration C_{avg} and corresponding dosing interval T that will eradicate a microbial population entirely. To accomplish this, it is necessary and sufficient to eradicate the most resistant subpopulation of the microbial population, by finding the minimum of the time-averaged agent concentration C_{avg} and corresponding dosing interval T *for that subpopulation*. According to the analysis in section 3.1, eradication of the most resistant subpopulation means that $\frac{D}{K_g} > 1$ *for that subpopulation*, as suggested in Figure 8. Hence, the dependence of $\frac{D}{K_g}$ on

dosing regimens (namely C_{avg} and T) *for that subpopulation* must be estimated from experimental data. Now, the analysis in section 3.1 indicates that standard time-kill experiments can be used for that purpose. Indeed, for a heterogeneous population subjected to a number of time-invariant agent concentrations *C*, eqn. (4) in Sidebar 5 indicates that the population-average kill rate constant will eventually reach a value *b* for each time-invariant agent concentration *C*. This *C*-dependent kill rate constant, *b*, corresponds to the most resistant subpopulation, which will eventually dominate the entire population, and which is homogeneous, as suggested by eqn. (5) when $t \to \infty$. Therefore, it is reasonable to assume that the functional dependence of *b* on *C* follows the kinetics discussed in Sidebar 4, namely

$$b(C) = K_b \frac{C^{H_b}}{C^{H_b} + C_{50b}^{H_b}}$$

Similarly, it can be argued (Nikolaou and Tam, 2006) that it is reasonable to postulate that

$$R(C) = K_k \frac{C^{H}}{C^{H} + C_{50}^{H}} - K_b \frac{C^{H_b}}{C^{H_b} + C_{50b}^{H_b}}$$

and

$$A(C) = K_A \frac{C^{H_A}}{C^{H_A} + C_{50A}^{H_A}}$$

Therefore, if experimental data are available from time-kill studies (measurements of population size at various sampling points in time, for a number of time-invariant concentrations *C*), then the parameters involved in the above expressions for $b(C)$, $R(C)$, and $A(C)$ can be estimated. Then, using the identified expression for $b(C)$ in place of $r(C)$ in the analysis of

Sidebar 7, one can construct a surface showing the dependence of $\frac{D}{K_g}$ on C_{avg} and T, as in Figure 8.

3.3. Experimental verification

We discuss an example of our approach (Nikolaou and Tam, 2006; Nikolaou et al., 2007) where effective dosing regimens (dose and dosing intervals) are characterized for levofloxacin against *P. aeruginosa*. Time-kill data are collected over 24 hours at various time-invariant concentrations of levofloxacin, and curve-fit using the approach discussed in section 3.2, as shown in Figure 9.

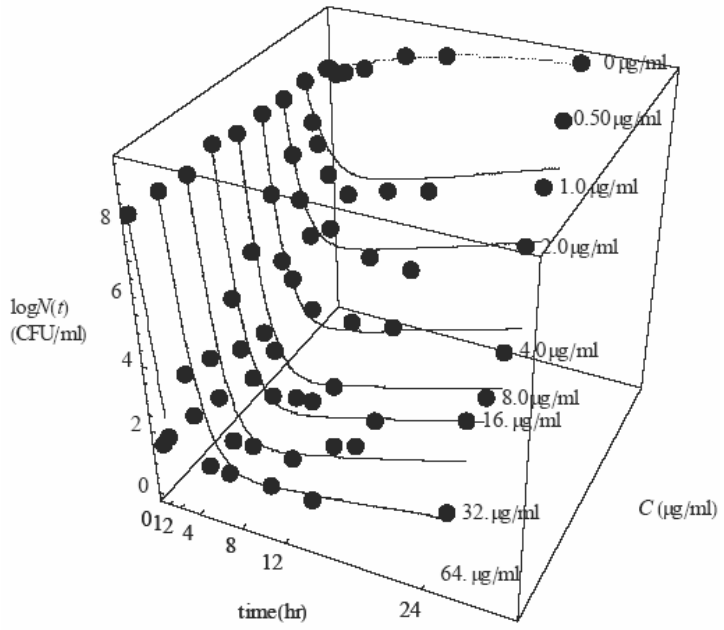


Figure 9. Time-kill studies of levofloxacin against *P. aeruginosa* ATCC 27853 (MIC = 2 μg/ml). For $C = 32 \times \text{MIC} = 64$ μg/ml there are no points beyond 1 hour, since all bacteria appear to have been eradicated beyond that point in time. Fit of the experimental data shown is done using equation (3) in Sidebar 5, with dependence of $b(C)$, $R(C)$, and $A(C)$ as discussed above in the text.

Subsequent to that, the equations of Sidebar 7 are use to construct the D/K_g surface as a function of dosing regimen, namely daily dose and dosing interval for given pharmacokinetics (Figure 10). According to Figure 10, daily doses of 750 and 3000 mg daily are predicted to be ineffective and completely effective, respectively.

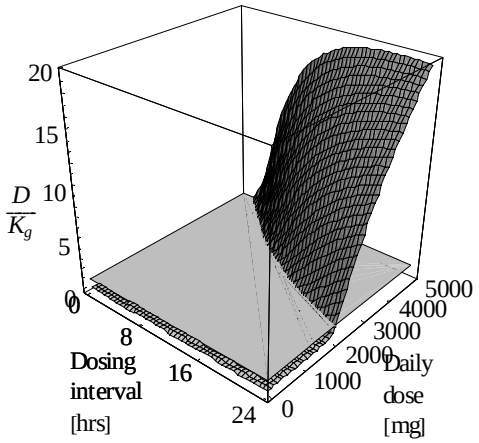


Figure 10. Model prediction of bactericidal effect of levofloxacin on *P. aeruginosa* for dosing regimens as in Figure 2 ($t_{1/2} = 6$ hrs, $T = 24$ hrs). Dosing regimens (combinations of daily dose and dosing interval) associated with resistance suppression correspond to $D/K_g > 1$ where $D \hat{=} \frac{1}{T}\int_0^T r\left(C(t)\right)dt$ is the average kill rate over T. Periodic agent injection every $T =$ 24 hours requires above 2200 mg of levofloxacin for complete eradication of the entire bacterial population. This prediction was verified both in a hollow-fiber in vitro model (Figure 3) and in a murine-thigh in vivo model (Jumbe et al., 2003) and *is significantly higher than the standard dosing recommended for levofloxacin*.

This was verified experimentally in a hollow-fiber in vitro infection model (Figure 3), as shown in Figure 11.

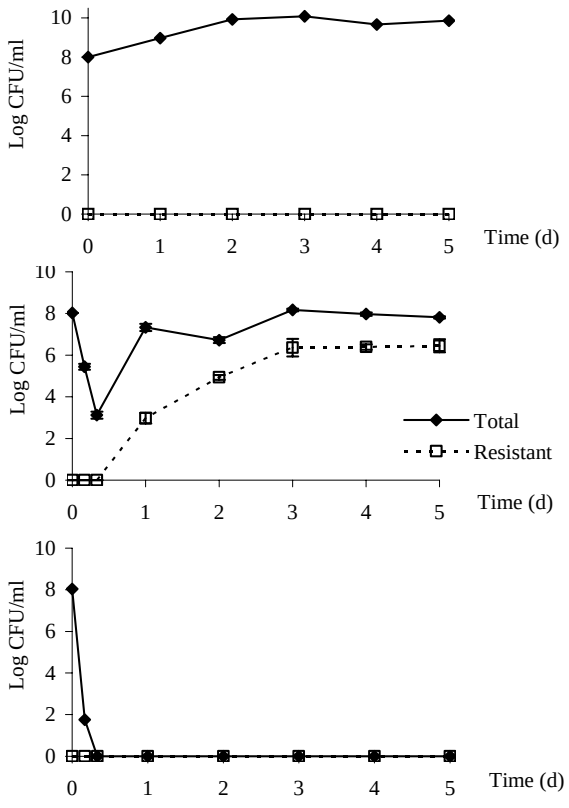


Figure 11. Prospective validation of the mathematical model in the hollow-fiber infection model for placebo (a), levofloxacin 750mg (b), levofloxacin 3000 mg (c) given every 24 hours. Data presented as mean and standard deviation of duplicate samples.

Dosing regimens predicted to be effective (corresponding to values of the index D/K_g greater than 1) or ineffective ($D/K_g < 1$) were compared to published data regarding the threshold quinolone exposure necessary to suppress resistance development of *P. aeruginosa* in a murine thigh infection model (Jumbe et al., 2003). Despite the differences between the two modeling approaches, the estimates of the levofloxacin exposure necessary for resistance suppression were consistent [approximately 2900 mg daily (total AUC/MIC = 157, free AUC/MIC = 110) demonstrated previously in the murine thigh infection model versus 2200 mg daily predicted by our model]. The closeness of our mathematical model predictions to the murine thigh infection model data exemplifies the usefulness of the proposed approach as a tool offering guidance to optimal design of dosing regimens.

4. Summary and Future Work

We have presented a mathematical modeling framework to design optimal dosing regimens of antimicrobial agents for complete eradication of microbial populations comprising subpopulations of varying degrees of susceptibility/resistance. Preliminary experimental verification of the proposed framework was presented. Further work is needed to identify the limits of the mathematical modeling framework for various combinations of microbial populations and antimicrobial agents, identify its sensitivity to available data, develop experimental protocols for collection of better experimental data, and potentially extend the framework to other related cases such as cancer chemotherapy.

Acknowledgement: Partial support from the University of Houston through a GEAR grant and from the Johns Hopkins Center for Alternatives to Animal Testing is gratefully acknowledged.

5. References

Andes, D. and W. A. Craig (1998). "In vivo activities of amoxicillin and amoxicillin-clavulanate against Streptococcus pneumoniae: application to breakpoint determinations." *Antimicrob Agents Chemother* **42**(9): 2375-9.

Andes, D., K. Marchillo, R. Conklin, G. Krishna, F. Ezzet, A. Cacciapuoti and D. Loebenberg (2004). "Pharmacodynamics of a new triazole, posaconazole, in a murine model of disseminated candidiasis." *Antimicrob Agents Chemother* **48**(1): 137-42.

Bilello, J. A., G. Bauer, M. N. Dudley, G. A. Cole and G. L. Drusano (1994). "Effect of 2',3'-didehydro-3'-deoxythymidine in an in vitro hollow-fiber pharmacodynamic model system correlates with results of dose-ranging clinical studies." *Antimicrob Agents Chemother* **38**(6): 1386-91.

Bonhoeffer, S., M. Lipsitch and B. R. Levin (1997). "Evaluating treatment protocols to prevent antibiotic resistance." *Proc Natl Acad Sci U S A* **94**(22): 12106-11.

Campion, J. J., P. J. McNamara and M. E. Evans (2005). "Pharmacodynamic modeling of ciprofloxacin resistance in Staphylococcus aureus." *Antimicrob Agents Chemother* **49**(1): 209-19.

Chait, R., A. Craney and R. Kishony (2007). "Antibiotic interactions that select against resistance." *Nature* **446**(7136): 668-671.

Cohen, M. L. (1992). "Epidemiology Of Drug-Resistance - Implications For A Postantimicrobial Era." *Science* **257**(5073): 1050-1055.

Comarow, A. (2006). "Bugs Behaving Badly." *U.S. News & World Report* **140**(1).

Craig, W. A. (1998). "Pharmacokinetic/Pharmacodynamic Parameters: Rationale for Antibacterial Dosing of Mice and Men." *Clinical Infectious Diseases* **26**: 1-12.

Dandekar, P. K., P. R. Tessier, P. Williams, C. H. Nightingale and D. P. Nicolau (2003). "Pharmacodynamic profile of daptomycin against Enterococcus species and methicillin-resistant Staphylococcus aureus in a murine thigh infection model." *J Antimicrob Chemother* **52**(3): 405-11.

Di Justo, P. (2005). "The Bug Wars." *Wired*(October): 52-53.

Drlica, K. A. (2001). "Strategy for Fighting Antibiotic Resistance." *ASM News* **67**(1): 27-33.

Drusano, G. L., A. Louie, M. Deziel and T. Gumbo (2006). "The Crisis of Resistance: Identifying Drug Exposures to Suppress Amplification of Resistant Mutant Subpopulations." *Clinical Infectious Diseases*
42(4): 525–532.

Dua, P., V. Dua and E. N. Pistikopoulos (2005). *Optimal Control of Cancer by Delivery of Chemotherapeutic Agents*. AIChE Annual Meeting, Cincinnati, OH.

Food and Drug Administration. (2006). "Antimicrobial Resistance." 2007, from www.fda.gov/cvm/antiresistvideo.htm.

Giraldo, J., N. M. Vivas, E. Vila and A. Badia (2002). "Assessing the (a)symmetry of concentration-effect curves: empirical versus mechanistic models." *Pharmacology & Therapeutics* **95**: 21-45.

Gold, H. S. and R. C. Moellering (1996). "Antimicrobial-drug resistance." *N. Engl. J. Med.* **335**: 1444-1453.

Gumbo, T., A. Louie, M. R. Deziel, L. M. Parsons, M. Salfinger and G. L. Drusano (2004). "Selection of a moxifloxacin dose that suppresses drug resistance in Mycobacterium tuberculosis, by use of an in vitro pharmacodynamic infection model and mathematical modeling." *J Infect Dis* **190**(9): 1642-51.

Hill, A. V. (1910). "The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves." *J. Physiol.* **40**: iv-vii.

Jumbe, N., A. Louie, R. Leary, W. Liu, M. R. Deziel, V. H. Tam, R. Bachhawat, C. Freeman, J. B. Kahn, K. Bush, M. N. Dudley, M. H. Miller and G. L. Drusano (2003). "Application of a mathematical model to prevent in vivo amplification of antibiotic-resistant bacterial populations during therapy." *J Clin Invest* **112**(2): 275-85.

Jusko, W. (1971). "Pharmacodynamics of chemotherapeutic effects: dose-time-response relationships for phase-nonspecific agents." *J Pharm Sci* **60**: 892-895.

Landman, D., J. M. Quale, D. Mayorga, A. Adedeji, K. Vangala, J. Ravishankar, C. Flores and S. Brooks (2002). "Citywide clonal outbreak of multiresistant Acinetobacter baumannii and Pseudomonas aeruginosa in Brooklyn, NY: the preantibiotic era has returned." *Arch Intern Med* **162**: 1515-1520.

Landman, D., J. M. Quale, D. Mayorga, A. Adedeji, K. Vangala, J. Ravishankar, C. Flores and S. Brooks (2002). "Citywide clonal outbreak of multiresistant Acinetobacter baumannii and Pseudomonas aeruginosa in Brooklyn, NY: the preantibiotic era has returned." *Arch Intern Med* **162**(13): 1515-20.

Levy, S. B. (1998). "The Challenge of Antibiotic Resistance." *Scientific American.*

Levy, S. B. and B. Marshall (2004). "Antibacterial resistance worldwide: causes, challenges and responses." *Nature Medicine* **10**(12): S122-S129.

Lipsitch, M., T. H. Bacon, J. J. Leary, R. Antia and B. R. Levin (2000). "Effects of antiviral usage on transmission dynamics of herpes simplex virus type 1 and on antiviral resistance: predictions of mathematical models." *Antimicrob Agents Chemother* **44**(10): 2824-35.

Lipsitch, M. and B. R. Levin (1997). "The population dynamics of antimicrobial chemotherapy." *Antimicrob Agents Chemother* **41**(2): 363-73.

Lipsitch, M. and B. R. Levin (1997). "The Population Dynamics of Antimicrobial Chemotherapy." *Antimicrobial Agents and Chemotherapy* **41**(2): 363-373.

Livermore, D. M. (2002). "Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nightmare?" *Clin Infect Dis* **34**(5): 634-40.

Louie, A., G. L. Drusano, P. Banerjee, Q. F. Liu, W. Liu, P. Kaw, M. Shayegani, H. Taber and M. H. Miller (1998). "Pharmacodynamics of fluconazole in a murine model of systemic candidiasis." *Antimicrob Agents Chemother* **42**(5): 1105-9.

Louie, A., P. Kaw, W. Liu, N. Jumbe, M. H. Miller and G. L. Drusano (2001). "Pharmacodynamics of daptomycin in a murine thigh model of Staphylococcus aureus infection." *Antimicrob Agents Chemother* **45**(3): 845-51.

Maglio, D., C. Ong, M. A. Banevicius, Q. Geng, C. H. Nightingale and D. P. Nicolau (2004). "Determination of the in vivo pharmacodynamic profile of cefepime against extended-spectrum-beta-lactamase-producing Escherichia coli at various inocula." *Antimicrob Agents Chemother* **48**(6): 1941-7.

Meagher, A. K., A. Forrest, A. Dalhoff, H. Stass and J. J. Schentag (2004). "Novel pharmacokinetic-pharmacodynamic model for prediction of outcomes with an extended-release formulation of ciprofloxacin." *Antimicrob Agents Chemother* **48**(6): 2061-8.

Miyazaki, S., K. Okazaki, M. Tsuji and K. Yamaguchi (2004). "Pharmacodynamics of S-3578, a novel cephem, in murine lung and systemic infection models." *Antimicrob Agents Chemother* **48**(2): 378-83.

Morens, D. M., G. K. Folkers and A. S. Fauci (2004). "The challenge of emerging and re-emerging infectious diseases." *Nature* **430**(08 July): 242 - 249.

Mouton, J. W., A. A. Vinks and N. C. Punt (1997). "Pharmacokinetic-pharmacodynamic modeling of activity of ceftazidime during continuous and intermittent infusion." *Antimicrob Agents Chemother* **41**(4): 733-8.

Neu, H. C. (1992). "The crisis in antibiotic resistance." *Science* **257**: 1064-1073.

Neuhauser, M. M., R. A. Weinstein, R. Rydman, L. H. Danziger, G. Karam and J. P. Quinn (2003). "Antibiotic resistance among gram-negative bacilli in US intensive care units: implications for fluoroquinolone use." *Jama* **289**(7): 885-8.

Nicolau, D. P., C. O. Onyeji, M. Zhong, P. R. Tessier, M. A. Banevicius and C. H. Nightingale (2000). "Pharmacodynamic assessment of cefprozil against Streptococcus pneumoniae: implications for breakpoint determinations." *Antimicrob Agents Chemother* **44**(5): 1291-5.

Nikolaou, M., A. N. Schilling, G. Vo, K.-t. Chang and V. H. Tam (2006). "Modeling of Microbial Population Responses to Time-Periodic Concentrations of Antimicrobial Agents." *Annals of Biomedical Engineering* **accepted**.

Nikolaou, M., A. N. Schilling, G. Vo, K.-t. Chang and V. H. Tam (2007). "Modeling of Microbial Population Responses to Time-Periodic Concentrations of Antimicrobial Agents." *Annals of Biomedical Engineering* **DOI: 10.1007/s10439-007-9306-x**.

Nikolaou, M. and V. H. Tam (2006). "A New Modeling Approach to the Effect of Antimicrobial Agents on Heterogeneous Microbial Populations." *Journal of Mathematical Biology* **52**(2): 154-182.

Oleson, J., B. T. Frederick and P. Francis (2005). Methods for administration of antibiotics. U. S. P. 6852689, Cubist Pharmaceuticals, Inc.

Oliver, A., B. R. Levin, C. Juan, F. Baquero and J. Blazquez (2004). "Hypermutation and the preexistence of antibiotic-resistant Pseudomonas aeruginosa mutants: implications for susceptibility testing and treatment of chronic infections." *Antimicrob Agents Chemother* **48**(11): 4226-33.

Regoes, R. R., C. Wiuff, R. M. Zappala, K. N. Garner, F. Baquero and B. R. Levin (2004). "Pharmacodynamic functions: a multiparameter approach to the design of antibiotic treatment regimens." *Antimicrob Agents Chemother* **48**(10): 3670-6.

Silberman, S. (2007). "Requiem for the Magic Bullets." *Wired*(January 22).

Spellberg, B., J. H. Powers, E. P. Brass, L. G. Miller and J. E. Edwards, Jr. (2004). "Trends in antimicrobial drug development: implications for the future.(Major Article)." *(Major Article)* **38**(9): 1279(8).

Talbot, G. H., J. Bradley, J. E. Edwards, Jr., D. Gilbert, M. Scheld and J. G. Bartlett (2006). "Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America." *Clin Infect Dis* **42**(5): 657-68.

Tam, V., A. Schilling, D. Melnick and E. Coyle (2005). "Comparison of beta-lactams in counter-selecting resistance of Pseudomonas aeruginosa." *Diagn Microbiol Infect Dis* **52**(2): 145-151.

Tam, V. H., A. Louie, M. R. Deziel, W. Liu, R. Bachhawat, D. Gajjar, D. Grasela and G. L. Drusano (2002). *Pharmacodynamics of garenoxacin in a murine thigh infection model of Staphylococcus aureus*. Annual meeting of the Infectious Diseases Society of America, Chicago, Illinois.

Tam, V. H., A. Louie, M. R. Deziel, W. Liu, R. Leary and G. L. Drusano (2005). "Bacterial-Population Responses to Drug-Selective Pressure: Examination of Garenoxacin's Effect on Pseudomonas aeruginosa." *J Infect Dis* **192**(3): 420-8.

Tam, V. H., A. N. Schilling and M. Nikolaou (2005). "Modelling time-kill studies to discern the pharmacodynamics of meropenem." *J Antimicrob Chemother* **55**(5): 699-706.

UCSF. (2006). "Daptomycin." from http://www.ucsf.edu/idmp/whatsnew/dapto_monograph.htm.

Varaldo, P. E. (2002). "Antimicrobial resistance and susceptibility testing: an evergreen topic." *Journal of Antimicrobial Chemotherapy* **50**: 1-4.

Vogelman, B. and W. Craig (1986). "Kinetics of Antimicrobial Activity." *The Journal of Pediatrics* **108**(2): 835-840.

Wagner, J. (1968). "Kinetics of Pharmacologic Response I. Proposed Relationships between Response and Drug Concentration in the Intact Animal and Man." *J. Theoret. Biol.* **20**: 173-201.

Weisstein, E. W. (2005). "Cumulant." *MathWorld--A Wolfram Web Resource*, 2005, from http://mathworld.wolfram.com/Cumulant.html.