ANTIBIOTICS AND RESISTANT BACTERIAL POPULATIONS: A SYSTEMS APPROACH

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Abstract.

Bacterial resistance to antibiotics has reached alarming proportions. There is a dire need for drastic improvements in the development and clinical use of antibiotics, in terms of both experimental and computational advances. In this publication we present a systems approach to modeling the effect of antibiotics on bacterial populations. The proposed framework can be used to design better clinical practices and help improve the development of new antibiotics. Preliminary validation on in vitro experimental data is presented. A number of suggestions for future work are given.

Keywords

Antibiotics, resistant bacteria, bacterial populations, modeling, pharmacodynamics, pharmacokinetics

Introduction

Often hailed as therapeutic "magic bullets" for bacterial infections (Mann, 1999) antibiotics have inadvertently created a serious problem of alarming proportions in the form of resistant bacteria (U.S. Food and Drug Administration; Chadwick and Goode, 1997; Centers for Disease Control and Prevention; Infectious Diseases Society of America, 2004). In recent years one hears warnings of bacterial wars, new plagues, worldwide calamities, and new apocalypses (Cohen, 1992; Neu, 1992; Gold and Moellering, 1996; Levy, 1998; Radetsky, 1998; Drlica, 2001; Varaldo, 2002; Morens et al., 2004) as well as of the risk of going back to the pre-antibiotic era (Landman et al., 2002). Broad-spectrum antibiotic resistance in bacteria implicated in bioterrorism (e.g. anthrax) is especially worrisome.

As widely appreciated as the magnitude of this problem may be, the development of new antibiotics using traditional methods is not capable of and is not keeping up with the emergence of resistant pathogenic bacteria (Projan, 2003; Infectious Diseases Society of America, 2004; Wenzel, 2004). Therefore, we must (a) preserve the efficacy of available antibiotics through judicious use, and (b) find ways to accelerate development of new antibiotics.

With regard to task (a) there are, among others, important issues related to public health policies and practices for resistance suppression and infection control (Centers for Disease Control and Prevention, 2000; Laxminarayan, 2001; World Health Organization, 2001) that go beyond the scope of this publication and will not be discussed. Rather, we will be concerned with methodologies that can help improve clinical practices. This would be of great importance, given that hospitals, and particularly intensive care units, are an important breeding ground for the development and spread of resistant bacteria (Struelens, 1998). Current clinical practices rely on short-term in vitro tests for selection of an appropriate antibiotic and heuristic design of a dosing regimen. This practice can lead to ineffective dosing regimens that may amplify resistant bacterial populations, even though the selected antibiotic could be effective if a proper dosing regimen were selected.

With regard to task (b) there are, among others, important issues related to the economics of drug discovery and development (Coates et al., 2002; Miesel et al., 2003; Walsh, 2003; Service, 2004) that will not be discussed here. Rather, we will be concerned with methodologies that can be used in drug development. Even though biotechnology has created tremendous possibilities for initial discovery of an antibiotic molecule (Chemical Heritage Foundation, 2002; Nathan, 2004), the subsequent development process involves pre-clinical testing of dosing regimens that is poorly guided, is lengthy, has limited ability to identify the antibiotic's clinical potential, and may lead to unnecessary trials or premature abandonment of good candidates. The implications can be as dramatic as in the case of daptomycin (Cubicin®,

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Cubist Pharmaceuticals) whose development was abandoned by its original developer (Lilly) in the early 1980's because of toxicity concerns in clinical trials. Yet, after rekindled interest by its new developer in the 1990's, the exact same antibiotic was approved for clinical use by the FDA in 2003 using *only a different dosing regimen* (Tally and DeBruin, 2000; Tedesco and Rybak, 2004).

In both antibiotic development and use, guidance in terms of relevant models built from limited data would help (a) design clinical dosing regimens that make effective use of available antibiotics while suppressing the emergence of resistance, and (b) identify the clinical potential of new antibiotics at early pre-clinical development stages. In this publication we present an overview of some new mathematical modeling tools that we have developed for these tasks, hoping to stir interest in both the systems and pharmacology communities.

Pharmacodynamic of bacterial populations

Modeling the effect of an antibiotic on a bacterial population in a host organism is extremely complex, because of the many interactions and uncertainties involved with pharmacodynamics, pharmacokinetics, toxicity, and mechanisms of resistance (Schentag et al., 1985; Yacobi et al., 1993; Cutler et al., 1994). Modeling information is often captured in terms of parameters that are critical for the antibiotic bactericidal effect such as minimum inhibitory concentration (MIC) (Craig, 1998; Mueller et al., 2004) area under the curve (AUC) of antibiotic concentration vs. time, and others (Firsov et al., 1997; Corvaisier et al., 1998; Firsov et al., 2001). However, these parameters discard a lot of the *dynamic* information contained in in vitro time-kill curves. This is particularly problematic for newer antibiotics such as quinolones (e.g. Cipro®) or azolides, for which no parameters have been found to be unequivocally connected with bactericidal activity. Dynamic models can be used to address this issue, such as (Wagner, 1968; Jusko, 1971)

$$\frac{dN}{dt} = \underbrace{K_g N(t)}_{\text{physiological growth rate}} - \underbrace{\overbrace{\frac{K_k C(t)^H}{C(t)^H + C_{50}^H}}^{r(C(t))} N(t)}_{\text{kill rate due to antibiotic}} \qquad (1)$$

where N = bacterial population size, with $N(0) = N_0$; K_g = growth rate constant (Robertson, 1923); $N_{\max}$ = maximum population size under physiological growth conditions; K_k = maximum kill rate achieved as the antibiotic concentration $C \to \infty$; C_{50} = constant, $r(C_{50}) = 0.50 r(\infty)$; and H = Hill exponent (Hill, 1910) determining how inflected r is as a function of C. The kill rate expression $r(C)$ has been derived from mass action theory and the Langmuir equation (Clark, 1933) and has been linked to Michaelis-Menten enzyme kinetics and protein binding theory (Ariens and Simonis, 1964). Reviews of these and other pharmacodynamic models can be found in (Holford and Sheiner, 1981; Grevel, 1987; Schwinghammer and Kroboth, 1988)

The model of Eq. (1) predicts a straight line in a log plot of $N(t)$ vs. t for in vitro experiments at constant C. This is hardly ever observed in practice, when, for example, in vitro data are collected over a short-time (24-hour) period, to predict the longer-term efficacy of an antibiotic on a bacterial population. To remedy this situation, propositions have been made to split the bacterial population into two subpopulations, a *resistant* and a *susceptible* one. While qualitatively appealing, this approach can fail to make useful predictions for the time course of a bacterial population subjected to antibiotic concentration C, as shown in Figure 1. To address this issue, we developed a population-based approached, the crux of which can be summarized by the following Theorem (Nikolaou and Tam, 2005):

> The dynamics of a bacterial population of distributed $r(C)$ subjected to constant antibiotic concentration C are
>
> $$\frac{dN}{dt} = \left(K_g - \kappa_{r,1}(t)\right) N(t); \quad \frac{d\kappa_{r,n}}{dt} = -\kappa_{r,n+1}(t) \qquad (2)$$
>
> where $\kappa_{r,n}(t)$, $n \geq 1$ are the cumulants (Weisstein, 2004) of the kill rate distribution $f(r,t)$ at time t.

Note that the first four cumulants are directly related to the average, variance, skewness, and kurtosis of $f(r,t)$. For $n = 1$ and $n = 2$ Eq. (2) yields

$$\frac{d\mu_r}{dt} = -\sigma_r(t)^2; \quad \frac{d\sigma_r^2}{dt} = -\kappa_{r3}(t). \qquad (3)$$

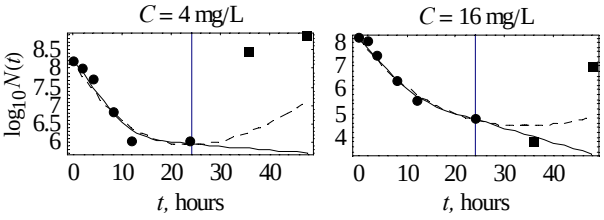

Figure 1. Modeling of meropenem effect on P. aeruginosa. (–) Resistant/susceptible subpopulations model; (---) distributed $r(C)$ model. ●: fitting data; ■: validation data.

Because Eq. (2) is in Jordan form, it is straightforward to derive infinite series expressions for $N(t)$, $\mu_r(t)$, and $\sigma_r^2(t)$. In addition, one can derive easily usable closed-form expressions for $N(t)$, $\mu_r(t)$, and $\sigma_r^2(t)$ in realistic special cases (Nikolaou and Tam, 2005). For example, if the kill rate $r(C)$ of the initial population is *approximately* normally distributed, it can be shown that the distribution of $r(C)$ will remain *approximately* normal, its variance will remain *approximately* constant, and

$$\ln\left[N(t)/N_0\right] \approx \left(K_g - \mu_r(0)\right)t + \tfrac{1}{2}\sigma_r^2 t^2 . \qquad (4)$$

As another example, if $\kappa_{n,0} = \sigma_r^2(0)A^{n-2}$, $n = 2,3,\ldots$, then

$$\ln\left[\frac{N(t)}{N_0}\right] \approx \left(K_g - \mu_r(0) + \frac{\sigma_r^2(0)}{A}\right)t + \frac{\sigma_r^2(0)}{A^2}\left(e^{-At} - 1\right).$$

Figure 1 shows that the above equation can use 24-hour data to predict regrowth due to resistant bacteria beyond 24 hours. Additional results and pertinent discussion can be found in (Nikolaou and Tam, 2005).

Connecting pharmacodynamics to pharmacokinetics

While in vitro experiments test the bactericidal effect of an antibiotic at constant concentration C, in vivo situations involve either constant or fluctuating C with period T ($C(t) = C\left(t - nT\right)$), depending on the antibiotic administration mode and related pharmacokinetics. What administration mode should one select to maximize bactericidal activity with minimal toxicity? Heuristic analysis of experimental data has long suggested (Shah et al., 1976; Vogelman and Graig, 1986) that there are two classes of antibiotics, as shown in Figure 2. Figure 2a shows bactericidal activity depending on C and suggests dosing regimens that achieve high concentrations at injection points. In Figure 2b bactericidal activity quickly reaches a plateau, indicating that dosing regimens need to maintain a certain C most of the time. Increasing C will increase toxicity without increasing bactericidal activity.

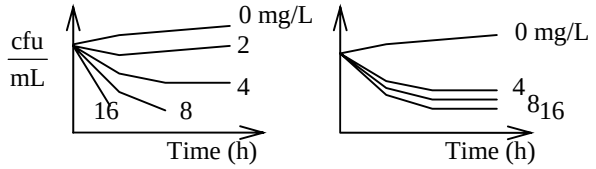

Figure 2. Concentration- (left) and time-dependent (right) antibiotic bactericidal rate.

The above classification of antibiotic bactericidal activity as C-dependent or t-dependent is often ambiguous. It can be shown (Nikolaou and Tam, 2005) that this is due to the fact that there is a continuum, rather than only two extremes of bactericidal activity, characterized by the sign and value of $K_g - D$, where K_g is as in Eq. (1) and

$$D \triangleq \left(1/T\right)\int_0^T r\left(C(\eta)\right)d\eta \qquad (5)$$

For typical pharmacokinetics captured by the equation $C(t) = C_{\max}e^{-(t-nT)/\tau}$ where $n = \text{IntegerPart}\left[t/T\right]$ and pharmacodynamics as in Eq. (1), Figure 3 shows the minimum value of $C_{\max}/C_{50}$ for which $K_g - D < 0$, to ensure bacterial killing. It is evident that for low values of MIC/C_{50} (particularly $\text{MIC}/C_{50} < 1$) increasing the injection period T does necessitate concomitant increase in $C_{\max}$, suggesting t-dependent activity. In contrast, $\text{MIC}/C_{50} >> 1$ suggests C-dependent activity.

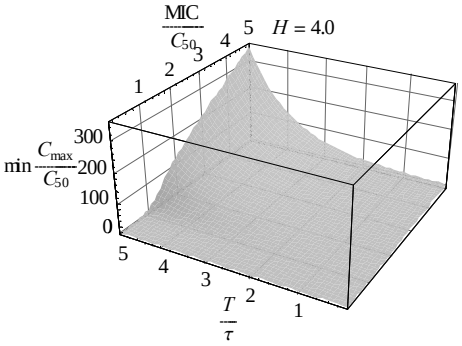

Figure 3. Minimum bactericidal $C_{\max}/C_{50}$ vs. dimensionless injection time T/τ & MIC/C_{50}.

Classification of bactericidal activity is further captured by (Nikolaou and Tam, 2005)

$$\frac{D}{K_g} = \frac{1+z^{-H}}{x}\ln\frac{(e^x-1)^H + (e^x xy)^H}{(e^x-1)^H + (xy)^H} \qquad (6)$$

where $x \triangleq T/\tau$, $y \triangleq \frac{C_{avg}}{C_{50}} \triangleq \left(1/T\right)\int_0^T C(\eta)/C_{50}\,d\eta$, and $z \triangleq \text{MIC}/C_{50}$. Limits of C- or t-dependent activity in terms of two indices, H & MIC/C_{50} are shown in Figure 4.

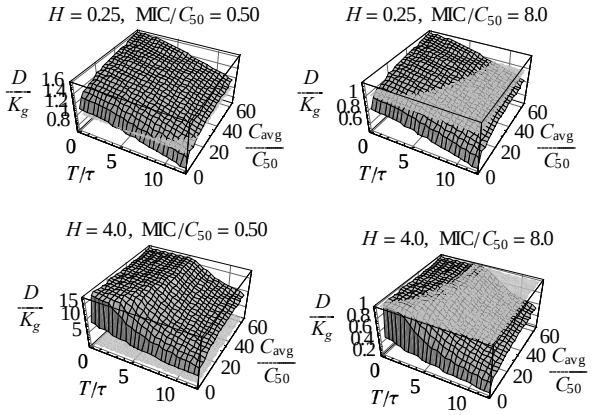

Figure 4. Modes of antibiotic bactericidal activity vs. the pharmacodynamic indices H (Hill exponent, Eq. (1)) and MIC/C_{50}.

Discussion and future directions

In this publication we have presented a very brief overview of some new basic modeling tools we have developed to quantify the bactericidal effect of antibiotics. Preliminary validation on in vitro experimental data was successful. We want to emphasize that even though the suggested tools are fairly general, what we have presented here is only elements of a basic framework. We hope that

this framework both will be used as a basic analytical tool by experimentalists and will be further enhanced by theoreticians. Our long-term goal is to improve clinical practice and the development process for antibiotics. As a next step, the following issues are worth exploring:

- Sensitivity to experimental errors.
- Limits of resistant subpopulation detectability.
- Characterization of critical measurements.
- Effect of spontaneous emergence of resistant mutants.
- Extension to combination therapy (simultaneous use of multiple antibiotics).
- Use in the design of feedback-based strategies (Bayard et al., 1994; Garraffo, 1994; Iliadis and Barbolosi, 1994; Jelliffe et al., 1994; Park et al., 1998; Jelliffe, 2000) for optimal antibiotic dosing regimens.
- Assess how densely the classes delineated in Figure 4 are populated.

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References

Ariens, E. J., and A. M. Simonis (1964). *J. Pharm. Pharmacol.*, **16**: 137–157.

Bayard, D. S., M. H. Milman et al. (1994). *Intl. J. Bio-Medical Computing*, **36**: 103-115.

Centers for Disease Control and Prevention (2000). http://www.cdc.gov/drugresistance/actionplan/html/index.htm.

Centers for Disease Control and Prevention (2004). http://www.cdc.gov/drugresistance/.

Chadwick, D. J., and J. Goode, Eds. (1997). *Antibiotic resistance: Origins, Evolution, Selection and Spread*, John Wiley & Sons.

Chemical Heritage Foundation (2002). http://www.chemheritage.org/EducationalServices/pharm/antibiot/readings/resist.htm.

Clark, A. J. (1933). *Mode of action of drugs on cells.* London, Edward Arnold.

Coates, A., Y. Hu et al. (2002). *Nature Reviews Drug Discovery*, **1**: 895 -910.

Cohen, M. L. (1992). *Science*, **257**(5073): 1050-1055.

Corvaisier, S., P. H. Maire et al. (1998). *Antimicrobial Agents And Chemotherapy*, **42**(7): 1731-1737.

Craig, W. A. (1998). *Clinical Infectious Diseases*, **26**: 1-12.

Cutler, N. R., J. J. Sramek et al., Eds. (1994). *Pharmacodynamics and drug development: perspectives in clinical pharmacology*, Wiley.

Drlica, K. A. (2001). *ASM News*, **67**(1): 27-33.

Firsov, A. A., I. Y. Lubenko et al. (2001). *Antimicrobial Agents And Chemotherapy*, **45**(3): 927-931.

Firsov, A. A., S. N. Vostrov et al. (1997). *Antimicrobial Agents And Chemotherapy*, **41**(6): 1281-1287.

Garraffo, R. (1994). *Intl. J. of Biomedical Computing*, **36**: 43-57.

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

Grevel, J. (1987). *Pharm. Res.*, **4**: 86-91.

Hill, A. V. (1910). *J. Physiol.*, **40**: iv-vii.

Holford, N. H. G., and L. B. Sheiner (1981). *Clin. Pharmacokinet.*, **6**: 429-453.

Iliadis, A., and D. Barbolosi (1994). *Intl. J. Bio-Medical Computing*, **36**: 87-93.

Infectious Diseases Society of America (2004). http://www.idsociety.org/Template.cfm?Section=Home&CONTENTID=7455&TEMPLATE=/ContentManagement/ContentDisplay.cfm.

Jelliffe, R. (2000). *Therapeutic Drug Monitoring*, **22**: 325-329.

Jelliffe, R. W., P. Maire et al. (1994). *Intl. J. Bio-Medical Computing*, **36**: 1-23.

Jusko, W. (1971). *J Pharm Sci*, **60**: 892-895.

Landman, D., J. M. Quale et al. (2002). *Arch Intern Med*, **162**(13): 1515-20.

Laxminarayan, R. (2001). http://www.rff.org/Documents/RFF-Resources-143-antibiotic.pdf.

Levy, S. B. (1998). *Scientific American*: 53.

Mann, J. (1999). *The Elusive Magic Bullet.* Oxford University Press.

Miesel, L., J. Greene et al. (2003). *Nature Reviews Genetics*, **4**: 442 -456.

Morens, D. M., G. K. Folkers et al. (2004). *Nature*, **430**(08 July): 242 - 249.

Mueller, M., A. de la Peña et al. (2004). *Antimicrobial Agents and Chemotherapy*, **48**(2): 369-377.

Nathan, C. (2004). http://www.nature.com/news/2004/041018/pf/431899a_pf.html#B9.

Neu, H. C. (1992). *Science*, **257**: 1064-1073.

Nikolaou, M., and V. H. Tam (2005). *Journal of Mathematical Biology*, **submitted**.

Nikolaou, M., and V. H. Tam (2005). *Journal of Antimicrobial Chemotherapy*, **submitted**.

Park, K., D. Verotta et al. (1998). *Journal of Pharmacokinetics and Biopharmaceutics*, **26**(4): 471-492.

Projan, S. J. (2003). *Curr. Opin. Microbiol.*, **6**(5): 427-430.

Radetsky, P. (1998). *Discover*(November): 76-85.

Robertson, T. B. (1923). *The chemical basis of growth and senescence.* Philadelphia, Lippincott.

Schentag, J. J., D. J. Swanson et al. (1985). *J. Antimicrob. Chemother.*, **15 (Suppl. A)**: 47-57.

Schwinghammer, T. L., and P. D. Kroboth (1988). *J. Clin. Pharmacol.*, **28**: 388-394.

Service, R. (2004). *Science*, **303**(5665): 1796-1799.

Shah, P. M., W. Junghanns et al. (1976). *Dtsch. Med. Wochenschr.*, **101**: 325-328.

Struelens, M. (1998). *BMJ*, **317**: 652-654.

Tally, F. P., and M. F. DeBruin (2000). *J. Antimicrob. Chemother.*, **46**(4): 523-526.

Tedesco, K. L., and M. J. Rybak (2004). *Pharmacotherapy*, **24**(1): 41-57.

U.S. Food and Drug Administration. http://www.fda.gov/fdac/features/2002/402_bugs.html.

Varaldo, P. E. (2002). *Journal of Antimicrobial Chemotherapy*, **50**: 1-4.

Vogelman, G., and W. A. Graig (1986). *Journal of Pediatrics*(108): 835-840.

Wagner, J. (1968). *J. Theoret. Biol.*, **20**: 173-201.

Walsh, C. (2003). *Nature Reviews Microbiology*, **1**: 65 -70.

Weisstein, E. W. (2004). http://mathworld.wolfram.com/Cumulant.html.

Wenzel, R. P. (2004). *N. Engl. J. Med.*, **351**(6): 523-526.

World Health Organization (2001). http://www.who.int/emc/amr.html.

Yacobi, A., J. P. Skelly et al., Eds. (1993). *Integration of pharmacokinetics, pharmacodynamics, and toxicokinetics in rational drug development*. New York, Plenum Press.