

Mathematical and Computer Simulation Models of Antibiotic Treatment with Non-Inherited Resistance to Antibiotics: Phenotypic Switching and SOS-Induction.

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The purpose of this website is to illustrate with simple mathematical models how non-inherited resistance to antibiotics of the sorts considered in the articles by Balaban et al. (1) (phenotypic switch) and Miller et al. (2) (SOS-induction) mechanisms can account the kinetics of antibiotic killing and **may** affect the course of treatment. Our models are heuristic and general rather than quantitatively precise analogues of specific situations. Although the parameters we use for the numerical analysis of their properties (computer simulations) are in the range of those estimated *in vitro* estimates for *E. coli* and the antibiotics to which it is susceptible (3, 4) these models do not capture the complexity of interaction between antibiotics and bacteria *in vitro* much less that of treatment of patients with intact constitutive and immune defenses and the variation among them (5).

The approach we use to explore the effects of non-inherited resistance on the efficacy of treatment is similar to that we used in (3, 4). We simulate treatment by combining a model of the **pharmacokinetics** (the changes in the concentration of the antibiotic) in treated hosts with one of the **pharmacodynamics** of the antibiotic and the infecting bacteria (i. e. the relationship between the concentration of the drug and rate of bacterial growth or death). In the present case we extend the simulations used in (3, 4) to include two subpopulations that are generated from each other via phenotypic switching or an antibiotic-mediated (SOS-) induction mechanism. One subpopulation of density T bacteria per ml grows more slowly and is more tolerant to the killing effects of the antibiotic than the other more rapidly growing and more antibiotic sensitive subpopulation of density S. The Berkeley MadonnaTM programs used in this website can be downloaded from www.eclf.net.

Pharmacodynamics - In the absence of the antibiotic, the sensitive S and tolerant T subpopulations grow exponentially at rates $\psi^{(S)}_{MAX}$ and $\psi^{(T)}_{MAX}$ per hour, respectively. When the antibiotic is present their rates of growth are reduced and when the concentration of the antibiotic exceeds the minimum inhibitory concentration MIC_S or MIC_T of the S and T subpopulations these growth rates are negative. For the relationship between the antibiotic concentration, a mg/kg and the reduction in growth rate of the bacteria $\mu_S(a)$ and $\mu_T(a)$, we assume a Hill function for both the S and T subpopulations (3, 6)

$$\mu_S(a) = (\psi^{(S)}_{MAX} - \psi^{(S)}_{MIN})(a/MIC_S)^{k_S} / [(a/MIC_S)^{k_S} - \psi^{(S)}_{MIN} / \psi^{(S)}_{MAX}]$$

$$\mu_T(a) = (\psi^{(T)}_{MAX} - \psi^{(T)}_{MIN})(a/MIC_T)^{k_T} / [(a/MIC_T)^{k_T} - \psi^{(T)}_{MIN} / \psi^{(T)}_{MAX}]$$

where $\psi_{\text{MIN}}^{(S)}$ and $\psi_{\text{MIN}}^{(T)}$ are the maximum rates of killing (negative growth rates) and κ_S and κ_T are the Hill coefficients of the S and T subpopulations, respectively.

Hill function parameters – In our simulations, we use the same Hill function parameters for both the phenotypic switch and SOS-induction models. For the S population we assume the maximum growth rates are $\psi_{\text{MAX}}^{(S)}=1$ and minimum growth rate are $\psi_{\text{MIN}}^{(S)}=-10$. We assume the T population, replicates at a lower rate than S, $\psi_{\text{MAX}}^{(T)}=0.1$ and are killed by the antibiotic at lower rate as well, $\psi_{\text{MIN}}^{(T)}=-0.20$. For convenience in our simulations we assume $\text{MIC}_S = \text{MIC}_T=1$ and $\kappa_S = \kappa_T=1$. The relationship between the antibiotic concentration and the rate of growth for the S and T populations over the range of antibiotic concentrations used in the simulations are presented in Figure 1.

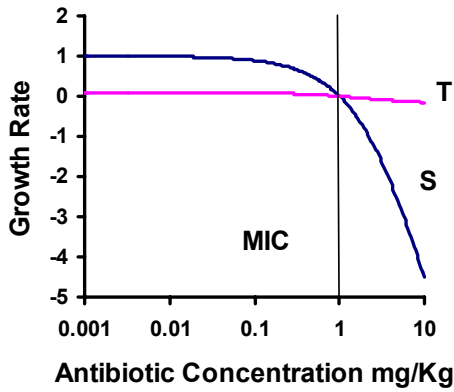


Figure 1 - The relationship between the antibiotic concentration and the rate of growth or death (negative growth) of the S and T populations. Hill function parameters: $\text{MIC}_S = \text{MIC}_T=1$, $\kappa_S = \kappa_T= 1$, $\psi_{\text{MAX}}^{(S)}=1$, $\psi_{\text{MIN}}^{(S)} = -10$, $\psi_{\text{MAX}}^{(T)}=0.1$ and $\psi_{\text{MIN}}^{(T)} = -0.20$.

Phenotypic Switch Model - In this model the variables S and T are what Balaban et al. (1) call normal and persister cells, n and p , respectively. Since we are restricting this consideration to a population that in the absence of antibiotics would be growing exponentially, we only consider those persisters that are generated in the course of cell division, Balaban et al's type II, rather than those produced at stationary phase, their type I. During the course of growth S generate T at a rate x and T produce S at a rate y per cell per hour. With these definitions and assumptions, the rates of change in the concentration of the antibiotic and densities of the S and T populations are given by,

$$dS/dt = S \psi_{\text{MAX}}^{(S)} - xS + yT - \mu_S(a)$$

$$dT/dt = T \psi_{\text{MAX}}^{(T)} - yT + xS - \mu_T(a)$$

SOS-induction Model - We assume that induction of S and the production of T occur at rates that are directly proportional to the concentration of the antibiotic. For this we use hyperbolic function used for enzyme kinetics as well as bacterial growth (7), where the

per-capita rate at which S produce T is given by $v a/(a+k)$ where v is the maximum rate at which T are produced by induction of S and k the concentration of the antibiotic where the rate of induction is half its maximum. We also assume that T bacteria become un-induced (repressed) and enter the S state at a rate that is a declining function of the antibiotic, $u (1-a/(a+k))$. With these definitions and assumptions then rates of change in the viable cell densities of S and T are given by,

$$dS/dt = S \psi^{(S)}_{MAX} - S v a/(a+k) + T u (1- a/(a+k)) - \mu_S(a)$$

$$dT/dt = T \psi^{(T)}_{MAX} + S v a/(a+k) - T u (1- a/(a+k)) - \mu_T(a)$$

Simulated Time-Kill Curves - In Figure 2 we illustrate the dynamics of the change in total density of viable bacteria exposed to a constant concentration of an antibiotic in the absence of non-inherited resistance and with the phenotypic switch (Figure 2a) and SOS-induction models for non-inherited resistance (Figure 2b).

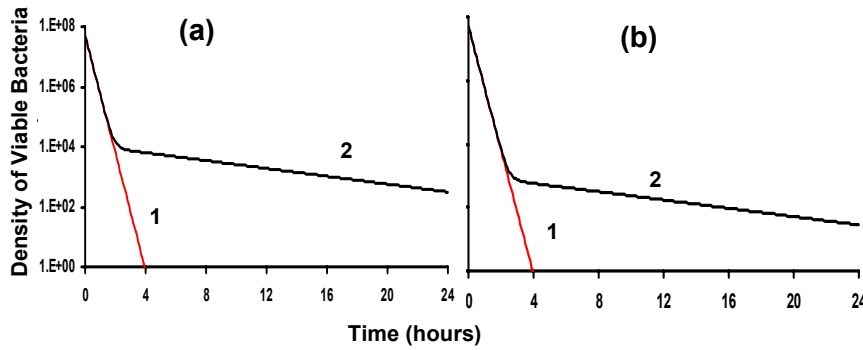


Figure 2. Simulated time kill experiments: Change in the total density of viable bacteria exposed to 10 mg/kg of the antibiotic; Hill Function Parameters $\psi^{(S)}_{MAX}=1$, $\psi^{(S)}_{MIN} = -10$, $\psi^{(T)}_{MAX} =0.1$, $\psi^{(T)}_{MIN} = -0.20$, $MIC_S=MIC_T 1.0$, $\kappa_S = \kappa_T=1.0$. (a) Phenotypic switch; 1- no non-inherited resistance $x=y=0$, and 2- non-inherited resistance $x=0.001$, $y=0.00001$, (b) SOS induction, 1- no non-inherited resistance $v=u=0$, 2- non-inherited resistance, $v=0.0001$, $u=0.01$, $k=1$. In these runs $S(0) =5 \times 10^7$ and $T(0) =0$.

Becoming sensitive again - There is a common reason why in both models when the antibiotic is removed and the bacteria grow, their populations become dominated by S cells, the persisters and the induced tolerant cells replicate at much lower rates than the susceptible $\psi^{(T)}_{MAX} \ll \psi^{(S)}_{MAX}$. Also contributing to the high rate of replacement of T by S are; (i) in the phenotypic switch model a much higher rate of transition from S to T, than from T to S, $y \gg x$ and (ii) in the SOS-induction model is that the absence of the antibiotic T cells are not produced. In this model we also assume the maximum rate of de-induction (repression) is greater than rate of induction $u > v$. To illustrate the reversion to sensitivity, we use the above models but assume that population growth is limited as logistic function so that the rate of change in the density of bacteria is multiplied by

$(1-N/K_{max})$, where N is the total population size and K_{max} the maximum density of bacteria that can be maintained (Figure 3).

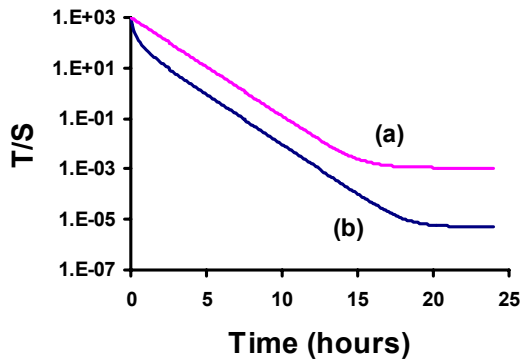


Figure 3- Change in the ratio of T and S , in the absence of antibiotics. The pharmacodynamic parameters are the same as in Figure 2. $K_{max}=10^9$, $T(0) = 10^3$, $S(0)=1$. (a) Phenotypic switching, (b) SOS-induction.

Treatment simulations - The pharmacokinetics model used here is the same as that in (3, 4). At any given time, the effective concentration of a bactericidal antibiotic at the site of the infection is a (mg/kg). As a result of clearance by kidneys and other processes a declines. For simplicity, we assume this decline is exponential with constant rate d per hour. Thus between treatments the rate of change in the concentration of the antibiotic is given by,

$$da/dt = -da$$

To mimic treatment, in the computer simulation of these pharmacokinetics every C hours, D mg/kg of the antibiotic are added and instantly becomes accessible see Figure 4.

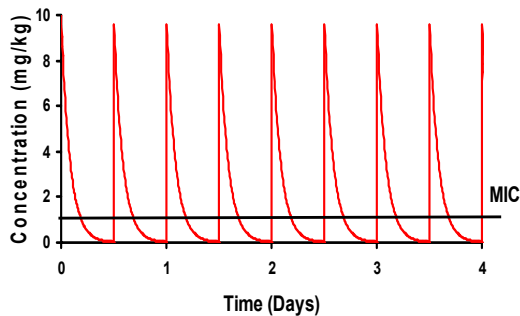


Figure 4 - Pharmacokinetic simulation- Change in the concentration of the antibiotics. Parameters $D=10$ (mg/kg), $C=12$ hours, $d=0.50$ per hour, $MIC=1$.

In Figure 5 we follow the changes density of bacteria in a simulated treatment of an infection with an antibiotic and bacteria with the same pharmacodynamic parameters as in Figures, 1 and 2 and the pharmacokinetics depicted in Figure 4 and a dose of the

antibiotic $D=10\text{ mg/kg}$ administered every 12 or 8 hours. Without the phenotypic switch generating the 12 hour treatment regime is effective in clearing the infection within three days (Figure 5a). While there is an initial reduction

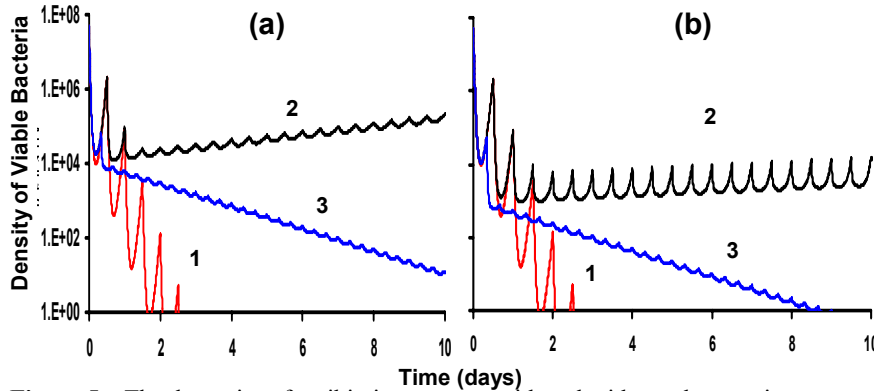


Figure 5 – The dynamics of antibiotic treatment with and without phenotypic switching; changes in the density of viable bacteria. The pharmacodynamics parameters in this simulation are identical to those in Figure 2. The pharmacokinetics are those depicted in Figure 3, $D=10\text{mg/kg}$ but with $C=8$ as well as 12 hours. (a) Phenotypic switching – 1- no switching ($x=y=0$), 2- $x=0.001$, switching $y=0.00001$, $C=12$, 3- switching $x=0.001$, $y=0.00001$, $C=8$, (b) SOS Induction- 1- no induction, $v=u=0$, 2- induction, $v=0.0001$, $u=0.01$, $k=1$, $C=12$, 3- induction, $v=0.0001$, $u=0.01$, $k=1$, $C=8$. In these runs, $S(0)=5 \times 10^7$ and $T(0)=0$.

in the density bacteria with phenotypic switching treatment every 12 hours is not sufficient to clear the infection. By increasing the frequency of dosing to every 8 hours, treatment can once again lead to the clearance of the infection, but the term is considerably longer than in the absence of non-inherited resistance. Similar results obtain with the SOS-induction model (Figure 5b).

Implications, other models and other parameter values: We interpret the above to suggest that non-inherited resistance to antibiotics of the sorts considered in the Balaban et al. and Miller et al articles **may** have a considerable effect on the microbiological course and outcome of antibiotic treatment. Quite independently of these two investigations we came to the same conclusion using a different model for the generation of non-inherited resistance (4). We conjecture that at least qualitatively the same conclusion would be drawn with other models of antibiotic treatment in which cells with non-inherited resistance are generated and parameter values in realistic ranges. As noted, the Berkeley Madonna™ programs used for the above simulations are available from www.ecdf.net. The reader is most welcome to explore the properties of these models with parameters in ranges they consider realistic.

Theoretical Humility - We intentionally chose the verb “may” for our interpretation of the clinical relevance of non-inherited resistance for humility. We believe theory should guide experimental and other empirical studies rather than serve as a substitute for empirical evidence. The experimental results reported by Balaban et al (1), Miller et al. (2), our own studies (4) as well as many much older investigations point to the existence of non-inherited resistance to antibiotics. We believe that these *in vitro* observations along with the above theoretical results call for *in vivo* experimental studies with laboratory animals that explore the contribution of non-inherited resistance to the efficacy of antibiotic treatment.

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8. This endeavor was supported by grants from the US National Institutes of Health GM33782 and AI40662 and the British Wellcome Trust (IPRAVE project).