

Title: Mechanism-based Models for Growth and Killing of *Pseudomonas aeruginosa* by Tobramycin to Quantify and Predict the Inoculum Effect

Authors: Jürgen B. Bulitta* (1), Neang S. Ly (1), Brian T. Tsuji (1), William J. Jusko (1), Alan Forrest (1)

Institution: (1) School of Pharmacy and Pharmaceutical Sciences, SUNY Buffalo, NY, USA.

Objectives: Published pharmacodynamic (PD) models for bacterial growth and killing by antibiotics cannot describe the time course of killing at various initial inocula (bacterial concentrations at time zero, CFU₀). Such models would allow one to account for differences in bacterial inocula and potentially to limit emergence of resistance during optimization of dosage regimens. The aims of this study were: 1) To develop a mechanism-based PD model that describes bacterial killing and growth of *P. aeruginosa* at various CFU₀. 2) To compare external validation with standard diagnostic plots as tools for model qualification.

Methods: Time kill experiments were performed in duplicate using nine tobramycin concentrations from 0 to 64 mg L⁻¹ against *Pseudomonas aeruginosa* (strain PAO1, MIC = 1 mg L⁻¹) initially at inocula of 10⁶ and 10⁹ CFU mL⁻¹ (CFU: colony forming units). An inoculum of 10^{7.5} CFU mL⁻¹ was studied for prospective model validation. Samples (n=13) were serially collected over 48h. Empirical models that estimate one parameter set at each inoculum were compared to a mechanism-based model that describes data at all inocula simultaneously by one parameter set. All data were co-modeled for both cases. Log-transformed bacterial counts were fitted in NONMEM[®] VI by the FOCE method with an additive error model.

Results: At the 10⁹ CFU mL⁻¹ inoculum the bacterial killing rate constant was approximately 5-times smaller compared to the 10⁶ CFU mL⁻¹ inoculum at the same drug concentrations. Literature models required different parameter estimates at each inoculum to adequately describe the data. A new model was developed that included three bacterial subpopulations with different susceptibility. The observed concentration dependent lag-time of bacterial killing could be described by a time-delayed bacterial killing starting at lower concentrations than the immediate bacterial killing. The time-delayed effect was modeled by a series of transit compartments. An inoculum effect compartment whose input is determined by the number of viable bacteria could successfully describe the slower bacterial killing at high inocula. The individual fitted vs. observed bacterial counts for the Meagher & Forrest 2004 model and for the new inoculum effect model were precise and unbiased for both models (Fig. 1). The new model had three more parameters and a 187 points better objective function compared to the literature model. The dual mechanisms of drug action of the new model could better capture the concentration dependent lag-time of bacterial killing at both inocula (Fig. 2). After estimating the parameters of the new model from data at the 10⁶ and 10⁹ CFU mL⁻¹ inoculum, CFU vs. time profiles were simulated for other starting values. The 10^{7.5} CFU mL⁻¹ inoculum was chosen for prospective validation, as these profiles had a different shape than the profiles at the 10⁶ and 10⁹ CFU mL⁻¹ inocula. The model predicted that a 6 h lag-time of bacterial killing with a nadir of bacterial counts at about 16 h would occur for a concentration of 0.5 mg L⁻¹ (Fig. 3). The experimental data showed this unusual time course of bacterial killing for a concentration of 1 mg L⁻¹. Model parameters were subsequently re-estimated based on the whole dataset.

Conclusions: 1) The new proposed model successfully described data at all three initial inocula with one parameter set. 2) Incorporating a dual mechanism of action significantly improved the ability of the model to describe the shape of the bacterial counts vs. time profiles across all three inocula. 3) Prospective validation was a valuable tool for model qualification. Mechanism-based models for anti-infectives represent an important tool to design optimal dosage regimens that maximize effectiveness, minimize emergence of resistance, and account for the time course of disease progression.

Topic area: Mechanistic models / systems biology

Figure 1: Individual fitted vs. observed bacterial counts for a literature model (Meagher AK, Forrest A, et al. AAC 2004, 48:2061-8) with different parameter sets for each inoculum (left) and for the new model that can describe data at various inocula with one parameter set (right)

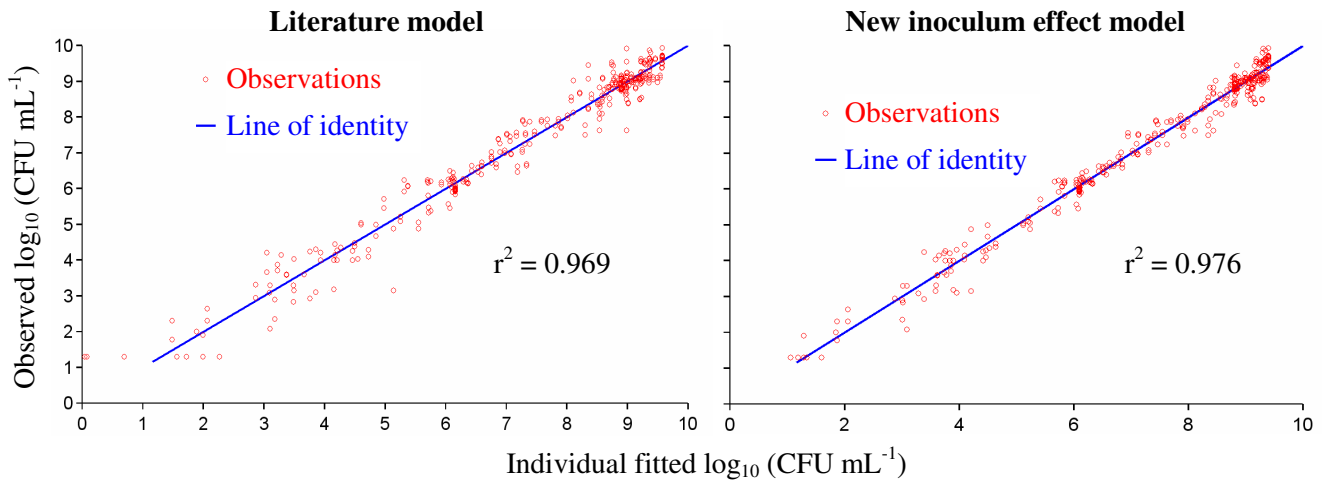


Figure 2: Individual curve fits (one replicate) for the literature model and the new proposed model

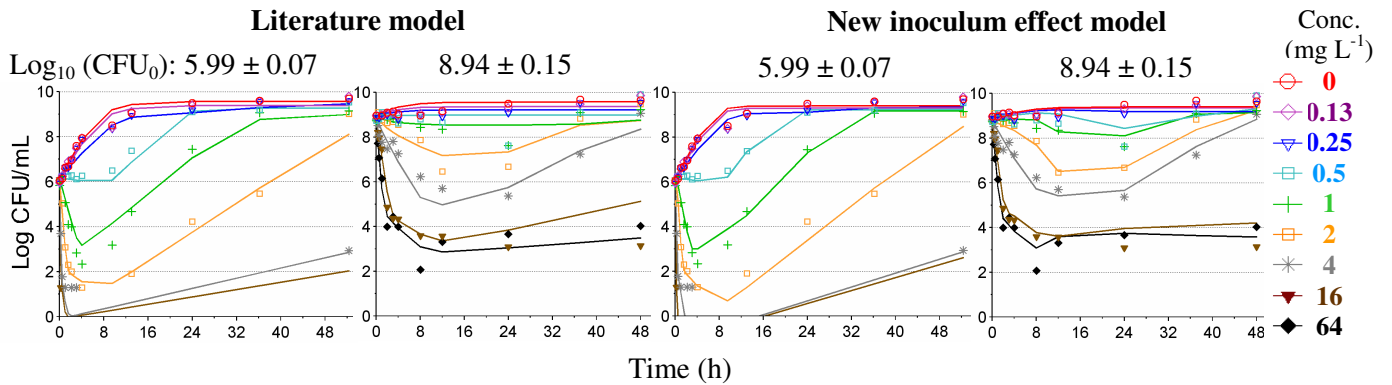


Figure 3: Prospective validation – simulated bacterial counts for an unstudied inoculum ($10^{7.5}$ CFU mL⁻¹) for a model estimated from data at 10^6 & 10^9 CFU mL⁻¹ compared to experimental data at $10^{7.5}$ CFU mL⁻¹

