EMA Workshop
Non-Clinical Models to Identify PK/PD Indices and PD Targets
In Vitro

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Institute for Therapeutic Innovation

University of Florida

In Vitro

- There are many *in vitro* systems for delineating the relationships between drug regimen intensity and *in vitro* outcomes of interest (bacterial cell kill and resistance suppression)
- I will limit myself to the system we employ in our laboratory – the hollow fiber infection model – first employed by Jurg Blaser and Steve Zinner and used extensively by Mike Dudley
- I have no experience with the other systems

In Vitro

- There are many advantages and disadvantages to both *in vitro* systems, as well as *in vivo* systems – I will concentrate on *in vitro* systems
- **Advantages:**
  1) any half-life can be simulated
  2) any bacterial burden can be examined
  3) any organism can be studied
  4) Resistance emergence is straightforward to find and study
  5) Other physiologic states can be induced and studied (e.g. Non-Replicative Persister Phenotype)

In Vitro

- **Advantages** (cont'd):
  6) The system can be employed at any stage of discovery/development
- Really? Even if I do not know the PK in man?
- Yes! Simply look at a small animal half-life for effect and then empirically dial in longer half-lives likely to be seen in man (e.g. 2, 4, 6, 8 hr half-lives) and ascertain the impact on the dynamic index
- We have done this before (AAC 2011;55:1747-1753 and AAC 2015;59: 3771-3777)

In Vitro

- What are the **disadvantages**?
  1) THERE IS NO IMMUNE SYSTEM!
  2) There is no physiology
  3) you cannot look at issues such as tissue penetration and effect on outcome
  4) cannot look directly at protein binding issues (we do employ free drug concentration-time curves, but there are other issues)

In Vitro

Cell Kill and Resistance Emergence

MAJOR ARTICLE

Bacterial-Population Responses to Drug-Selective Pressure: Examination of Garenoxacin's Effect on *Pseudomonas aeruginosa*

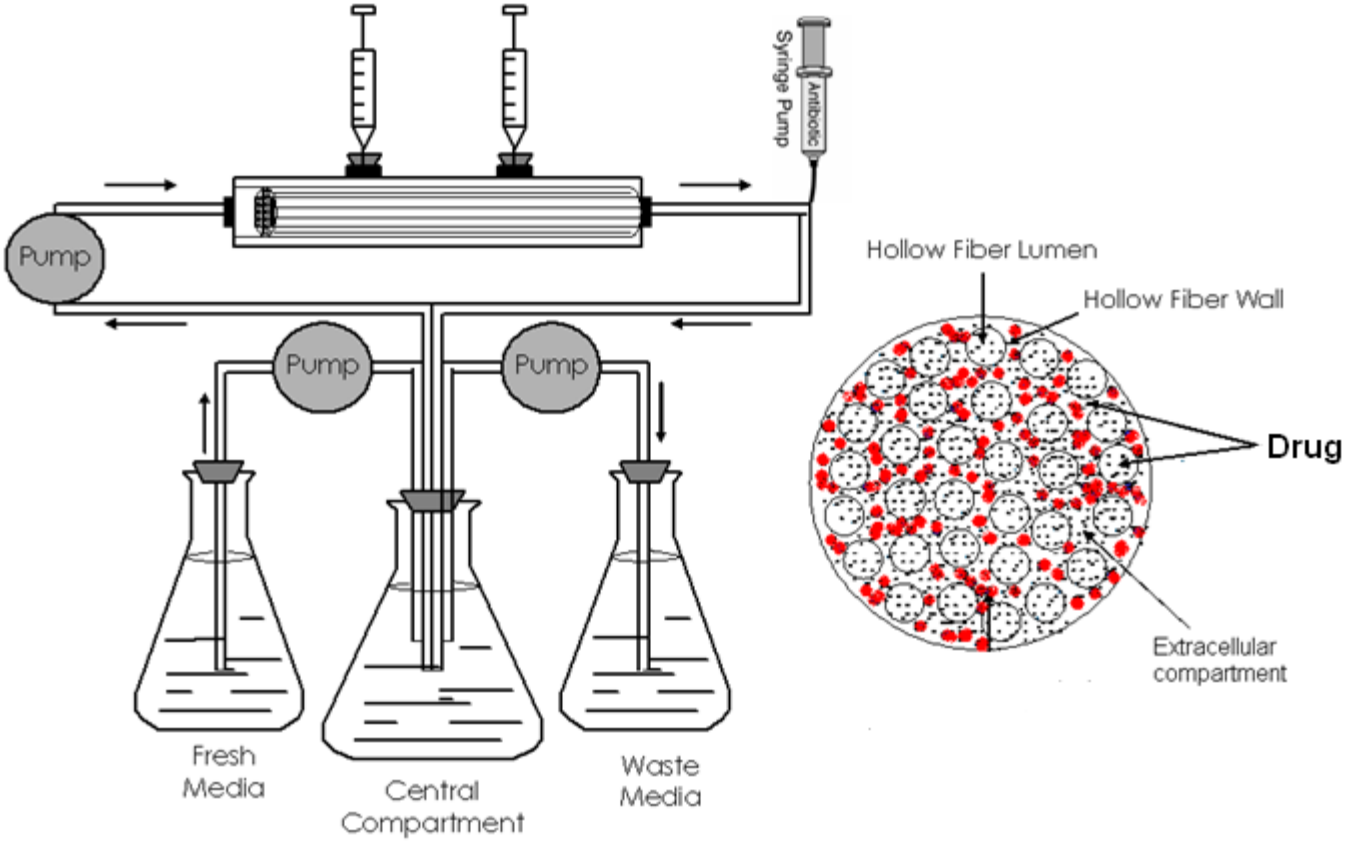
Vincent H. Tam,[1,a] Arnold Louie,[1] Mark R. Deziel,[1] Weiguo Liu,[1] Robert Leary,[2] and George L. Drusano[1]

[1]Emerging Infections and Host Defense Theme, Ordway Research Institute, Albany, New York; [2]San Diego Super Computer Center, University of California San Diego, La Jolla

The Journal of Infectious Diseases 2005; 192:420–8

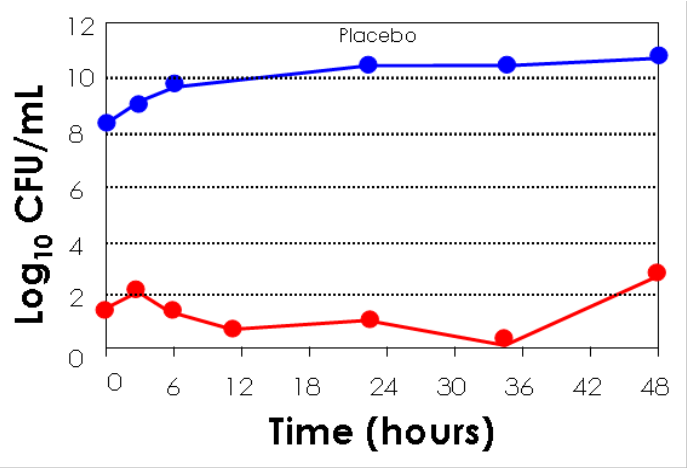
0022-1899/2005/19203-0009$15.00

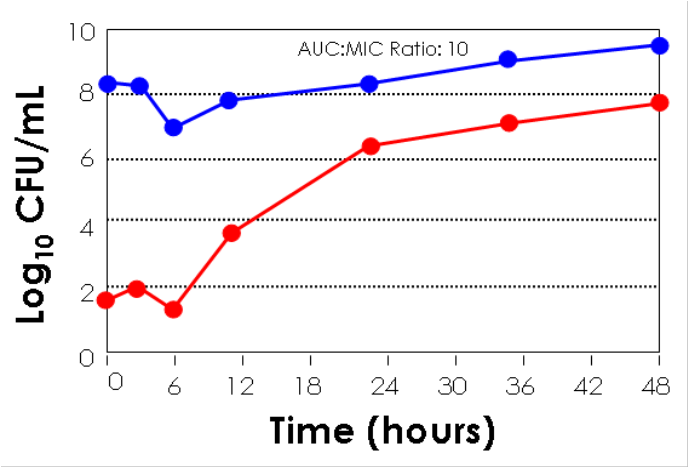
Resistance Suppression in *Pseudomonas aeruginosa*

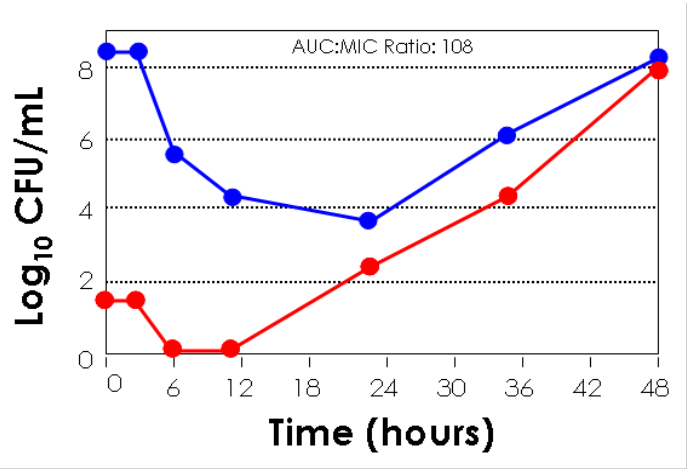


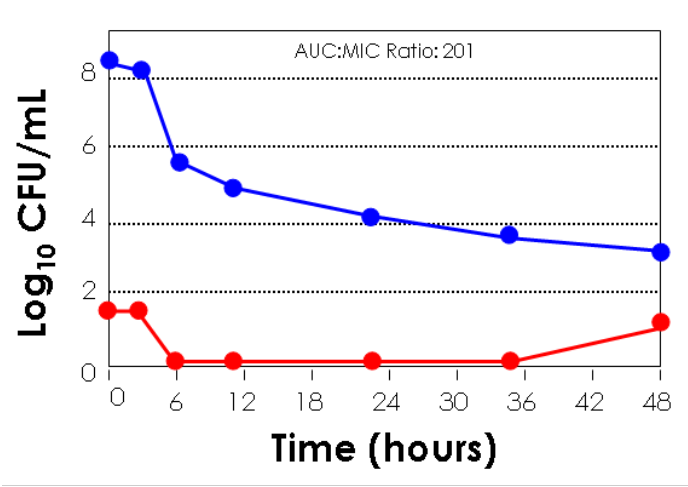
The use of the hollow fiber model for studying antimicrobial regimens was described by Blaser and Zinner and employed extensively by Dudley

Resistance Suppression in *Pseudomonas aeruginosa*

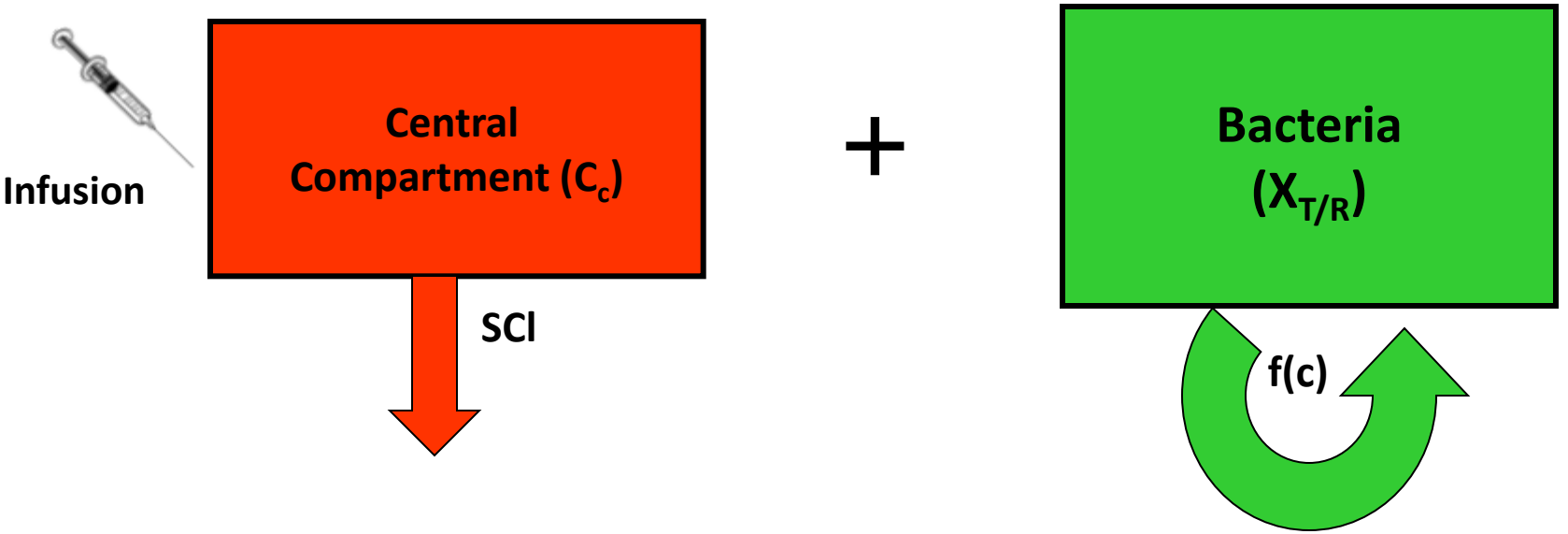








Tam V et al. Bacterial-population responses to drug selective pressure: Examination of garenoxacin's effect on *Pseudomonas aeruginosa*. J Infect Dis 2005;192:420-428



[1] $\frac{dC_c}{dt} = \text{Infusion} - (SCl/V) \times C_c$

$$\frac{dX_S}{dt} = K_{GS} \times X_S \times L - f_{KS}(C_c^{H\xi}) \times X_S \quad [2]$$

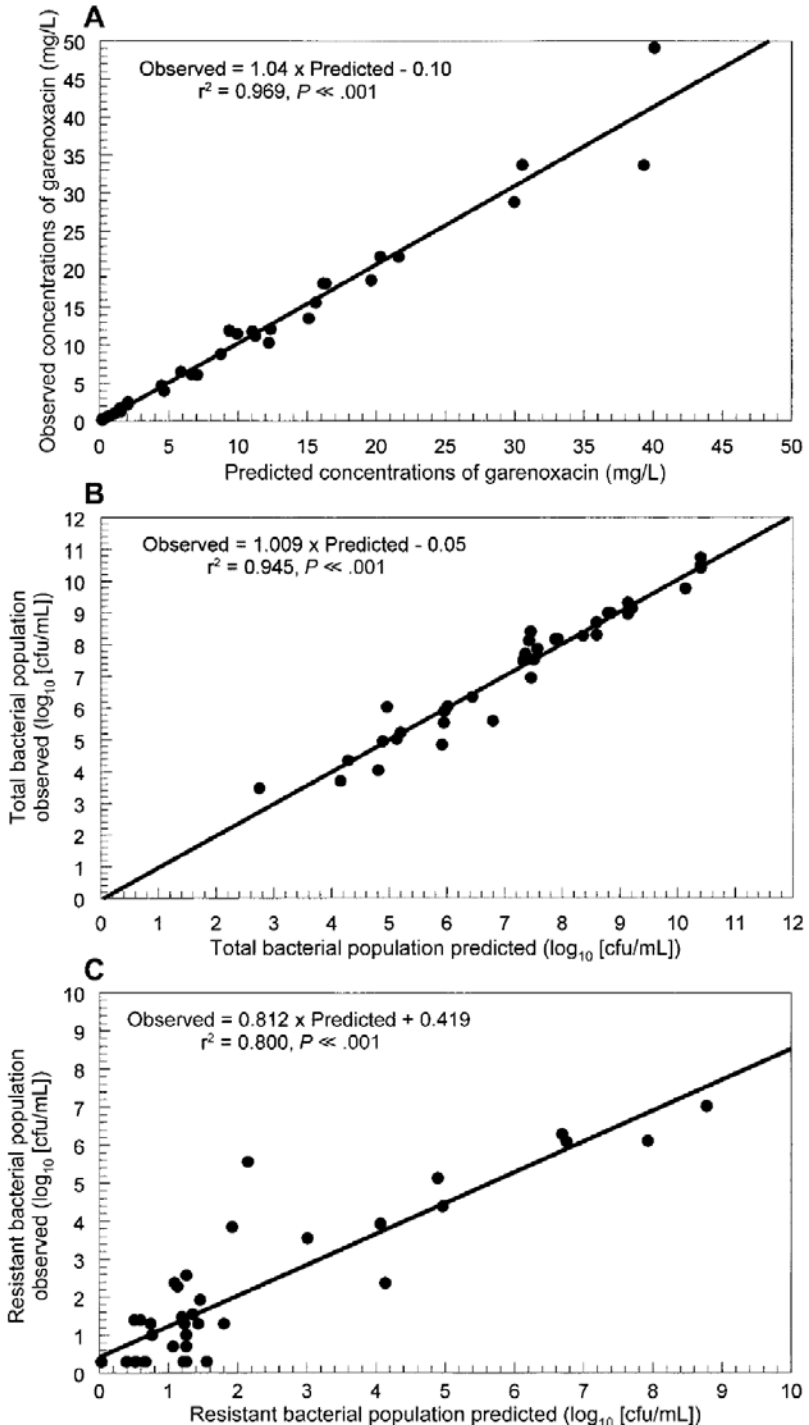
$$\frac{dX_R}{dt} = K_{GR} \times X_R \times L - f_{KR}(C_c^{H\xi}) \times X_R \quad [3]$$

$$L = (1-(X_R + X_S)/POPMAX) \quad [4]$$

$$f_{\psi\xi}(C_c^{H\xi}) = \frac{K_{max\ \xi} \bullet C_c^{H\xi}}{C^{H\xi}_{50\ \xi} + C_c^{H\xi}}, \ \psi = K \text{ and } \xi = S,R \quad [5]$$

$$Y_1 = X_T = X_S + X_R,\ IC(1) = 1.01 \times 10^8 \quad [6]$$

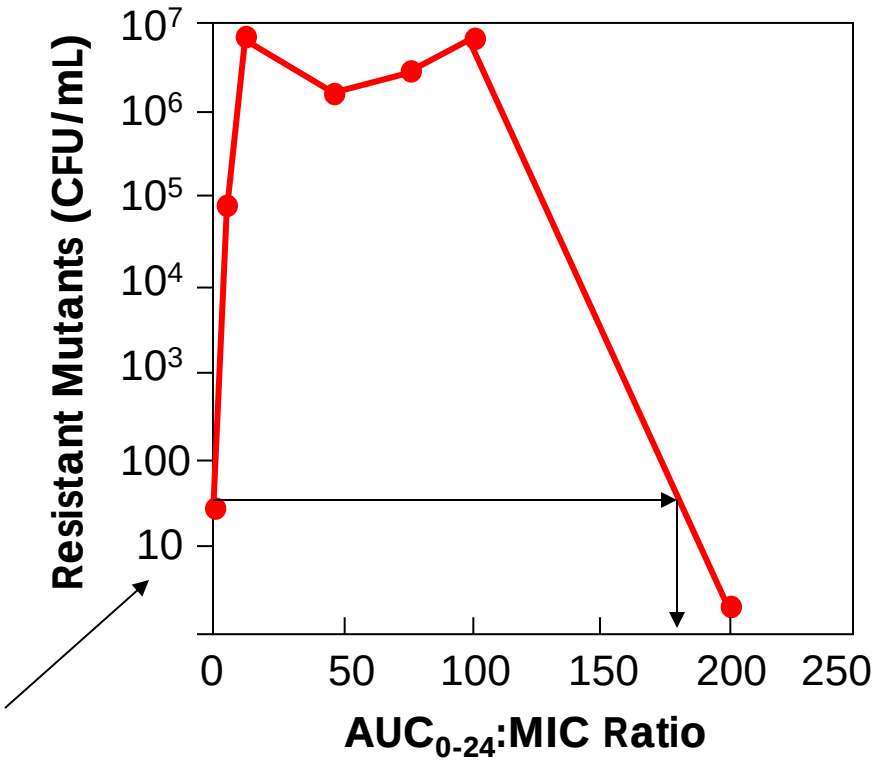
$$Y_2 = X_R,\ IC(2) = 58 \quad [7]$$


A
Observed = 1.04 x Predicted - 0.10
r² = 0.969, P ≪ .001
Observed concentrations of garenoxacin (mg/L)
Predicted concentrations of garenoxacin (mg/L)
B
Observed = 1.009 x Predicted - 0.05
r² = 0.945, P ≪ .001
Total bacterial population observed (log10 [cfu/mL])
Total bacterial population predicted (log10 [cfu/mL])
C
Observed = 0.812 x Predicted + 0.419
r² = 0.800, P ≪ .001
Resistant bacterial population observed (log10 [cfu/mL])
Resistant bacterial population predicted (log10 [cfu/mL])

Resistance Suppression in *Pseudomonas aeruginosa*

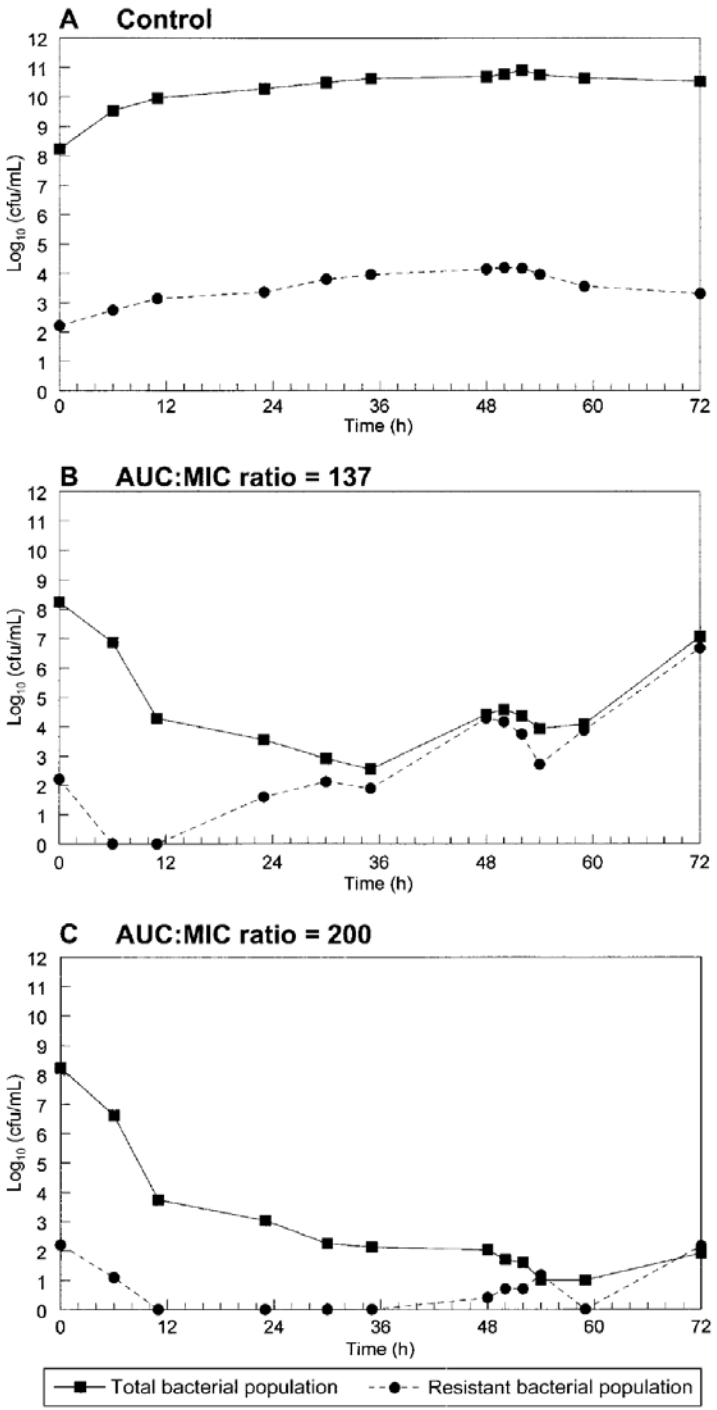
P. aeruginosa - Prevention of Amplification of Resistant Subpopulation

- The amplification of the resistant sub-population is a function of the AUC/MIC ratio
- The response curve is an inverted “U”.
- The AUC/MIC ratio for resistant organism stasis is circa 185/1



Resistant organisms at baseline

All other data points represent resistant organism counts at 48 hours of therapy



Prospective Validation Experiment

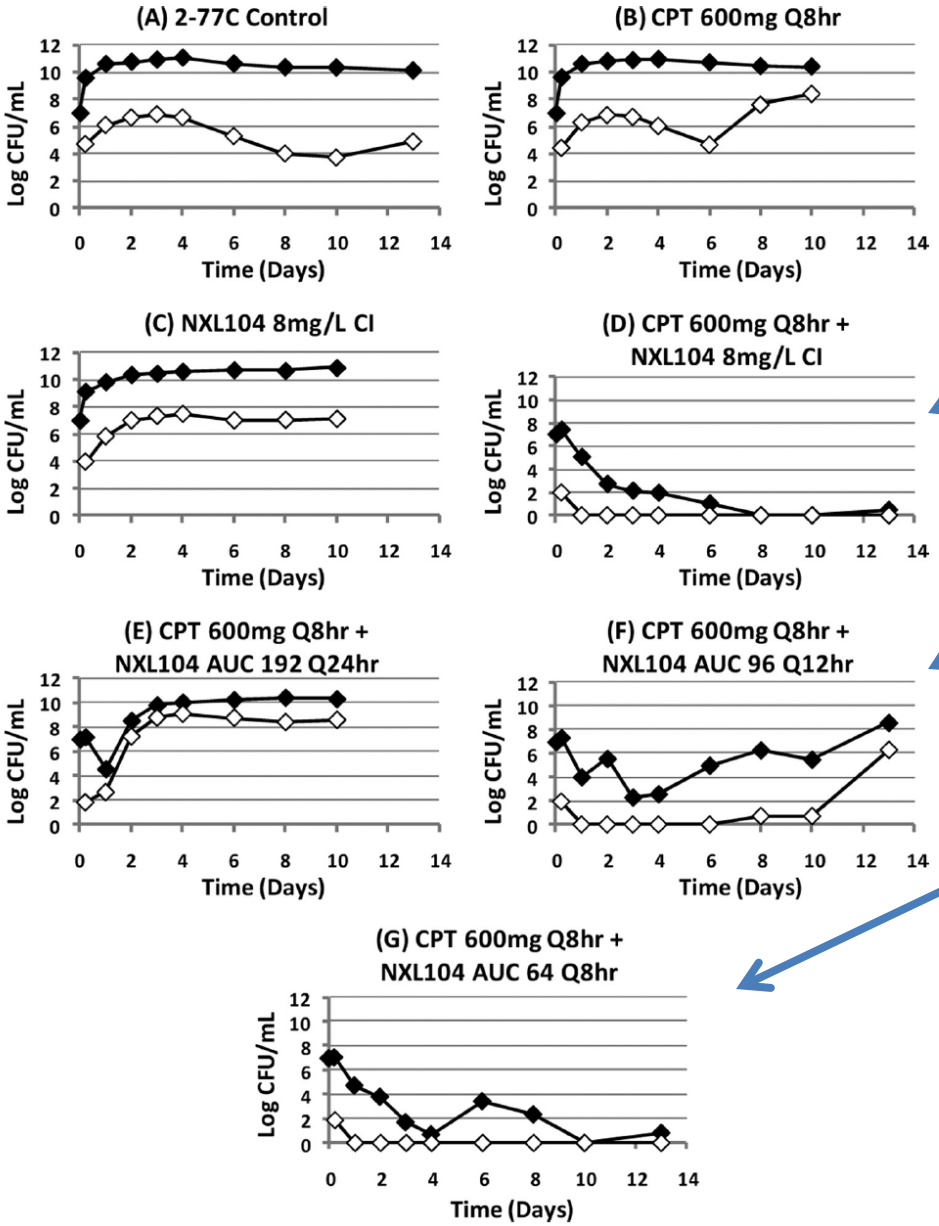
AAC
Journals.ASM.org

Pharmacodynamics of β-Lactamase Inhibition by NXL104 in Combination with Ceftaroline: Examining Organisms with Multiple Types of β-Lactamases

Arnold Louie,[a]* Mariana Castanheira,[b] Weiguo Liu,[a]* Caroline Grasso,[a] Ronald N. Jones,[b] Gregory Williams,[c] Ian Critchley,[c] Dirk Thye,[c] David Brown,[a]* Brian VanScoy,[a]* Robert Kulawy,[a]* and G. L. Drusano[a]*

Ordway Research Institute, Emerging Infections Pharmacodynamics Laboratory, Albany, New York, USA[a]; JMI Laboratories, North Liberty, Iowa, USA[b]; and Cerexa, Inc., Oakland, California, a wholly owned subsidiary of Forest Laboratories, Inc., New York, New York, USA[c]

In Vitro – Time to Resistance



Continuous infusion of Avibactam (AUC = 8 x 24 = 192 - then called NXL104) worked and suppressed resistance for the duration of the experiment (D);

AUC=192 Q 24 h (E) failed, as did AUC = 96 Q12 h (F)

AUC = 64 Q 8 h (G) succeeded for the whole experiment, implying that for this agent Time > Threshold (or Cmin) drives β-lactamase inhibition

Note in (F) that resistance did not emerge until after day 10 – you must study long enough

In Vitro

Impact of Therapy Duration

MAJOR ARTICLE

Impact of Drug-Exposure Intensity and Duration of Therapy on the Emergence of *Staphylococcus aureus* Resistance to a Quinolone Antimicrobial

V. H. Tam,[1,a] A. Louie,[1] T. R. Fritsche,[2] M. Deziel,[1,b] W. Liu,[1] D. L. Brown,[1] L. Deshpande,[2] R. Leary,[3,a] R. N. Jones,[2] and G. L. Drusano[1]

[1]Emerging Infections and Host Defense Laboratory, Ordway Research Institute, Albany, New York; [2]JMI Laboratories, North Liberty, Iowa; [3]University of California, San Diego, Supercomputer Center

The Journal of Infectious Diseases 2007; 195:1818–27

0022-1899/2007/19512-0013$15.00

DOI: 10.1086/518003

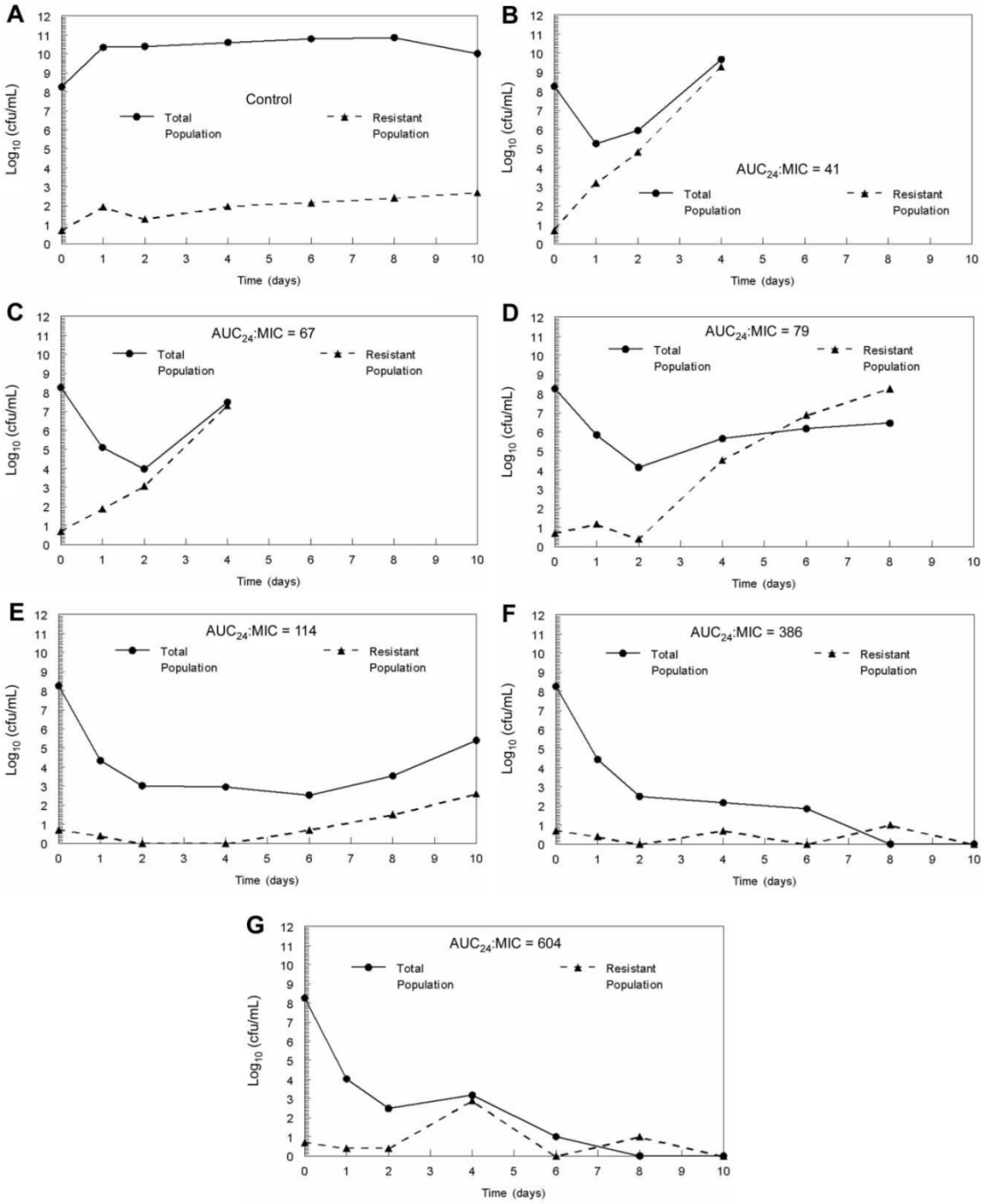

A
Control
Total Population
Resistant Population
Log10 (cfu/mL)
Time (days)
B
AUC24:MIC = 41
Total Population
Resistant Population
Log10 (cfu/mL)
Time (days)
C
AUC24:MIC = 67
Total Population
Resistant Population
Log10 (cfu/mL)
Time (days)
D
AUC24:MIC = 79
Total Population
Resistant Population
Log10 (cfu/mL)
Time (days)
E
AUC24:MIC = 114
Total Population
Resistant Population
Log10 (cfu/mL)
Time (days)
F
AUC24:MIC = 386
Total Population
Resistant Population
Log10 (cfu/mL)
Time (days)
G
AUC24:MIC = 604
Total Population
Resistant Population
Log10 (cfu/mL)
Time (days)

Table 1. Population mean parameter estimates for the pharmacodynamic model using only the first 2 days of therapy.

Parameter	Mean ± SD
Clearance, L/h	6.19 ± 1.59
Volume of central compartment, L	87.4 ± 13.5
$K_{gmax\text{-}S}$	1.14 ± 2.02
$K_{gmax\text{-}R}$	0.107 ± 0.0958
$K_{kmax\text{-}S}$	22.9 ± 11.3
$EC_{50\text{-}S}$, mg/L	12.1 ± 6.80
H_S	0.951 ± 0.312
$K_{kmax\text{-}R}$	22.7 ± 7.14
$EC_{50\text{-}R}$, mg/L	21.8 ± 16.4
H_R	3.04 ± 1.74
POP_{max}, cfu/mL	$4.53 \pm 4.30 \times 10^{10}$
Initial total population, cfu/mL	$9.94 \pm 6.44 \times 10^{7}$
Initial resistant subpopulation, cfu/mL	4.94 ± 0.791

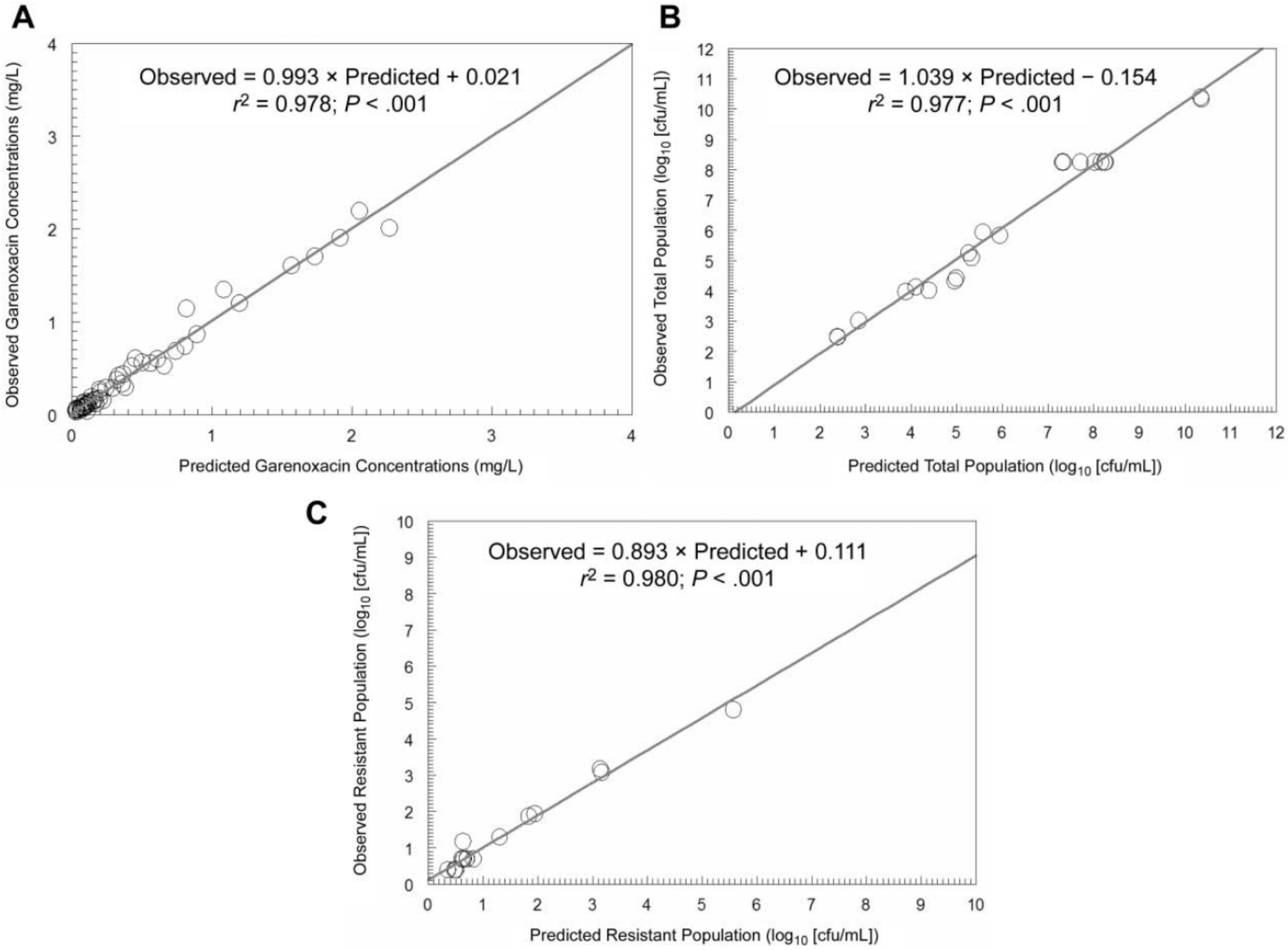
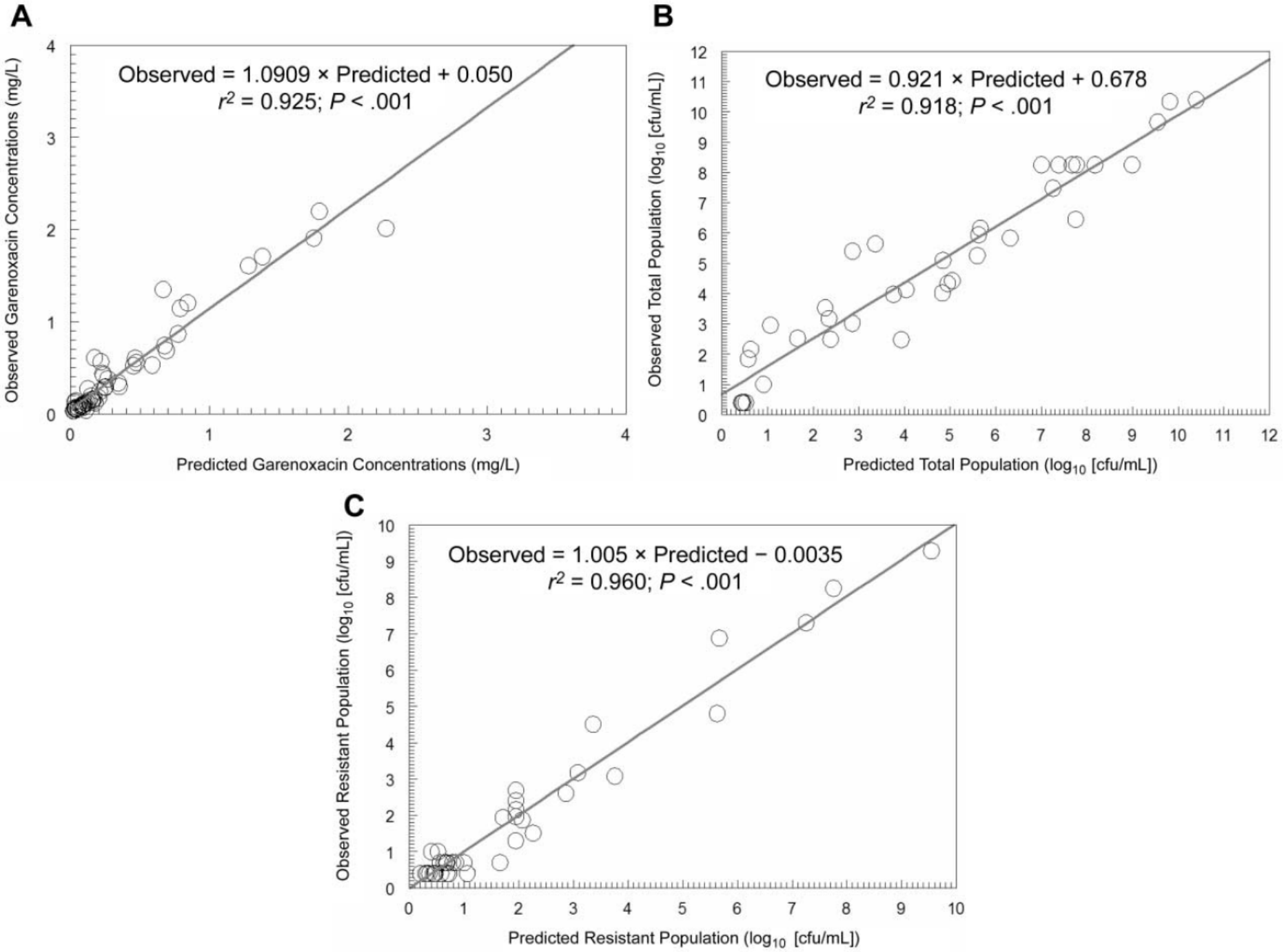
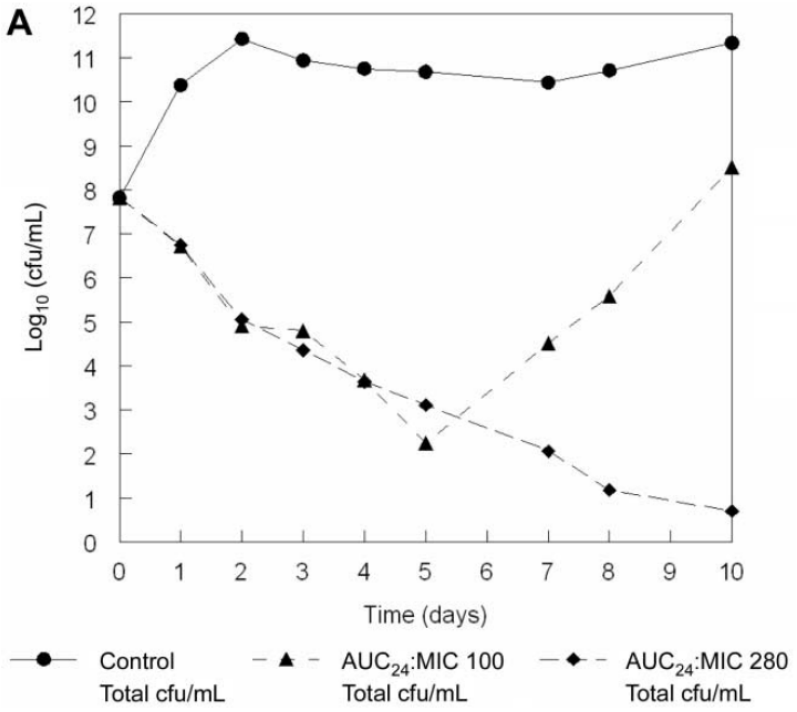
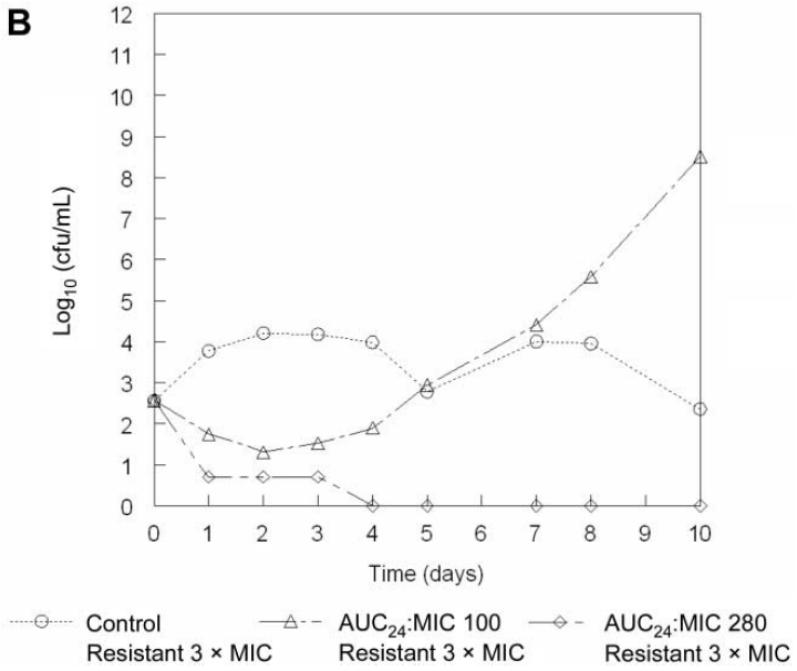

A
Observed = 0.993 × Predicted + 0.021
r^2 = 0.978; P < .001
Observed Garenoxacin Concentrations (mg/L)
Predicted Garenoxacin Concentrations (mg/L)
B
Observed = 1.039 × Predicted − 0.154
r^2 = 0.977; P < .001
Observed Total Population ($\log_{10}$ [cfu/mL])
Predicted Total Population ($\log_{10}$ [cfu/mL])
C
Observed = 0.893 × Predicted + 0.111
r^2 = 0.980; P < .001
Observed Resistant Population ($\log_{10}$ [cfu/mL])
Predicted Resistant Population ($\log_{10}$ [cfu/mL])

Table 2. Population mean parameter estimates for the pharmacodynamic model using all 10 days of therapy.

Parameter	Mean ± SD
Clearance, L/h	6.84 ± 1.20
Volume of central compartment, L	88.6 ± 18.5
$K_{gmax\text{-}S}$	0.107 ± 0.105
$K_{gmax\text{-}R}$	0.179 ± 0.0975
$K_{kmax\text{-}S}$	8.22 ± 3.93
$EC_{50\text{-}S}$, mg/L	14.5 ± 5.05
H_S	0.837 ± 0.364
$K_{kmax\text{-}R}$	46.3 ± 10.3
$EC_{50\text{-}R}$, mg/L	8.04 ± 4.66
H_R	1.81 ± 0.325
POP_{max}, cfu/mL	$1.08 \pm 1.16 \times 10^{10}$
Initial total population, cfu/mL	$2.02 \pm 3.19 \times 10^{8}$
Initial resistant subpopulation, cfu/mL	5.88 ± 2.07


A
Observed = 1.0909 × Predicted + 0.050
r^2 = 0.925; P < .001
Observed Garenoxacin Concentrations (mg/L)
Predicted Garenoxacin Concentrations (mg/L)
B
Observed = 0.921 × Predicted + 0.678
r^2 = 0.918; P < .001
Observed Total Population ($\log_{10}$ [cfu/mL])
Predicted Total Population ($\log_{10}$ [cfu/mL])
C
Observed = 1.005 × Predicted − 0.0035
r^2 = 0.960; P < .001
Observed Resistant Population ($\log_{10}$ [cfu/mL])
Predicted Resistant Population ($\log_{10}$ [cfu/mL])





Prospective Validation Experiment

MAJOR ARTICLE

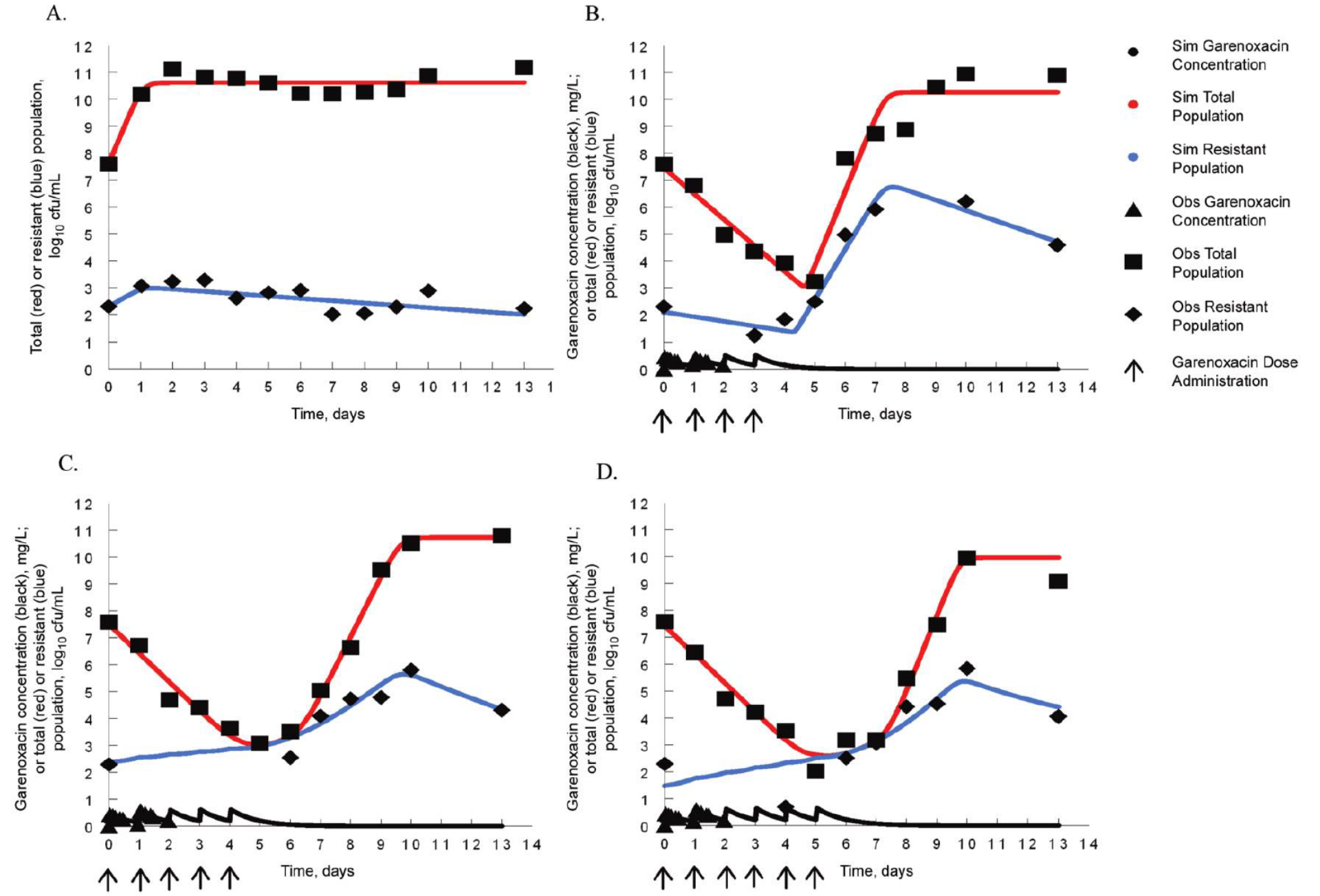
Impact of Short-Course Quinolone Therapy on Susceptible and Resistant Populations of *Staphylococcus aureus*

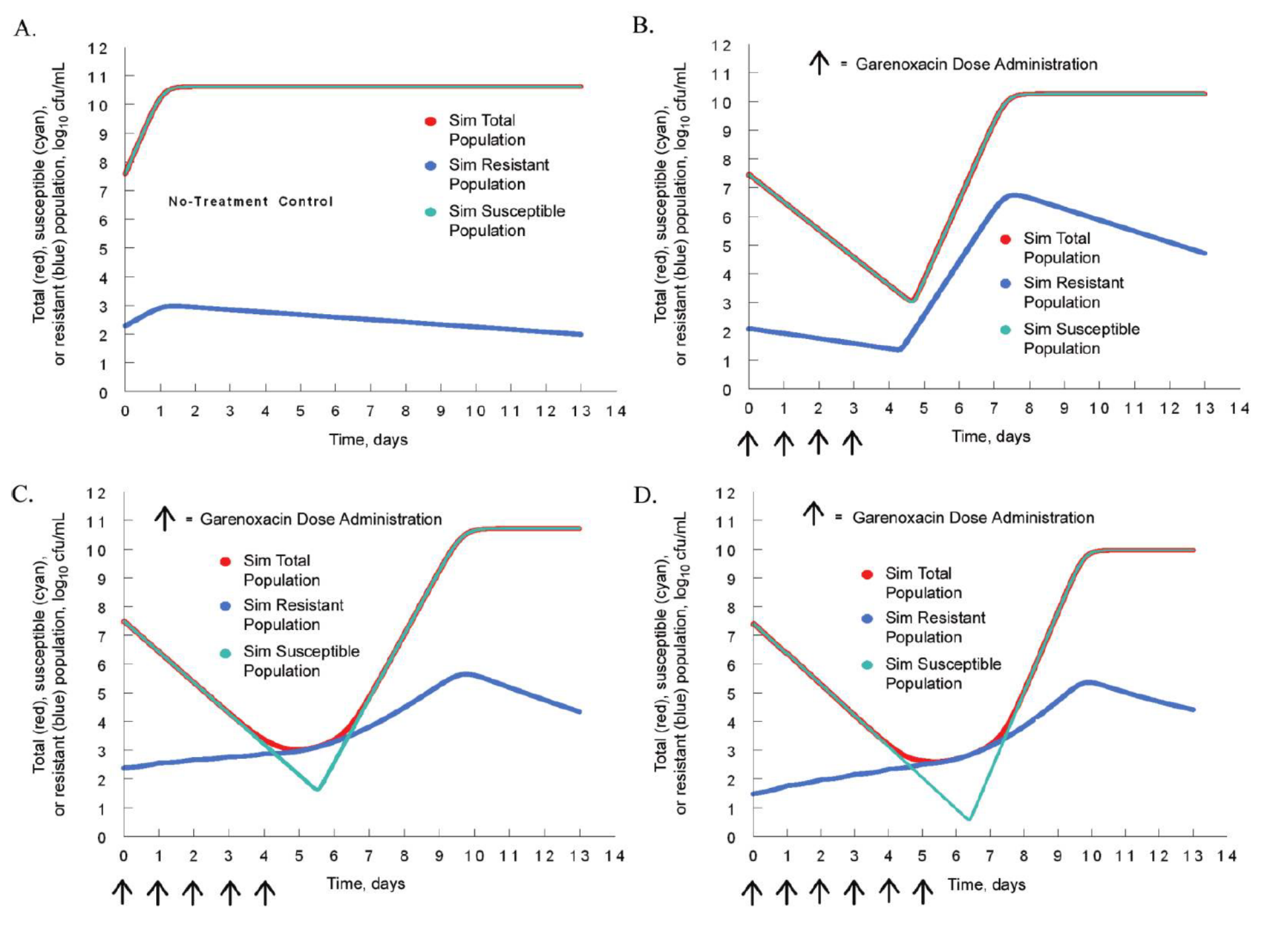
G. L. Drusano,[1] W. Liu,[1] D. L. Brown,[1] L. B. Rice,[2] and A. Louie[1]

[1]Emerging Infections and Host Defense Laboratory, Ordway Research Institute, Albany, New York; [2]Louis Stokes Cleveland Veterans Affairs Medical Center, Cleveland, Ohio

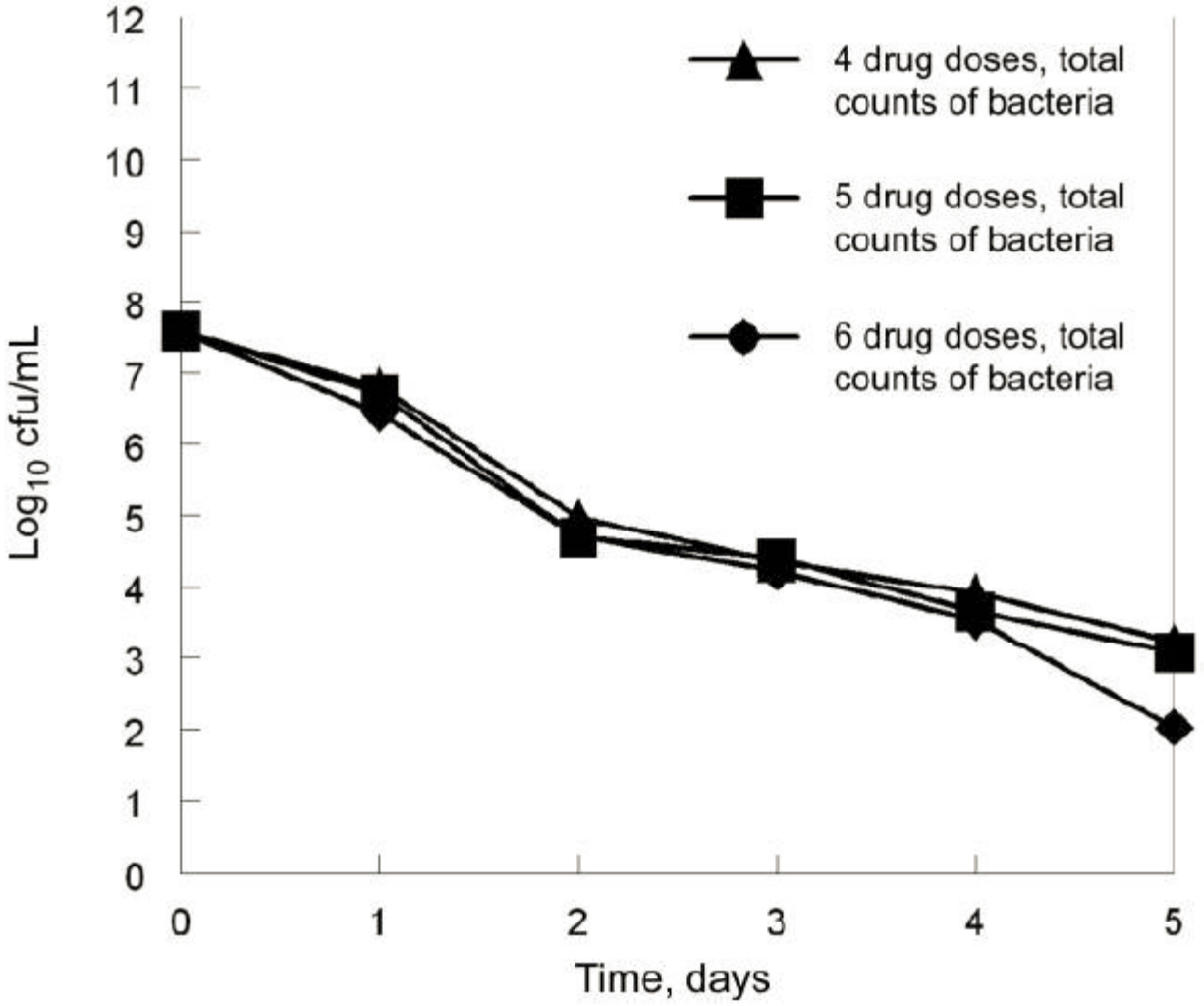
The Journal of Infectious Diseases 2009; 199:219–26

0022-1899/2009/19902-0010$15.00
DOI: 10.1086/595739


A.
B.
C.
D.
Total (red) or resistant (blue) population, log10 cfu/mL
Garenoxacin concentration (black), mg/L; or total (red) or resistant (blue) population, log10 cfu/mL
Time, days
Sim Garenoxacin Concentration
Sim Total Population
Sim Resistant Population
Obs Garenoxacin Concentration
Obs Total Population
Obs Resistant Population
Garenoxacin Dose Administration


A.
B.
C.
D.
Total (red), susceptible (cyan), or resistant (blue) population, log10 cfu/mL
Time, days
No-Treatment Control
Sim Total Population
Sim Resistant Population
Sim Susceptible Population
= Garenoxacin Dose Administration

In Vitro – Very Reproducible



In Vitro

Looking at Agents in Combination

Mono-Rx
Pseudomonas aeruginosa

Antimicrobial Agents and Chemotherapy

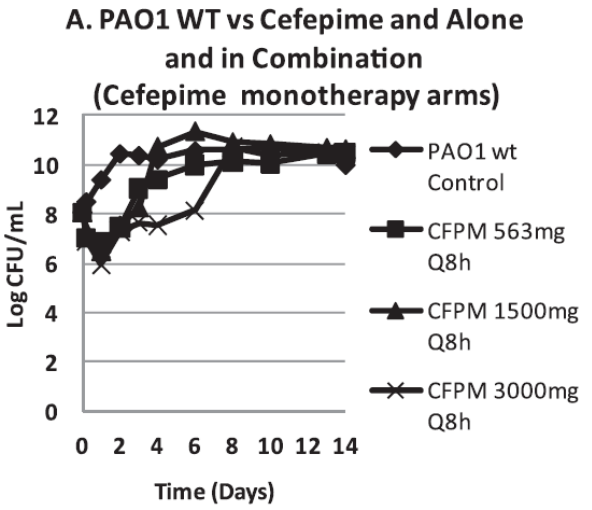
Resistance Emergence Mechanism and Mechanism of Resistance Suppression by Tobramycin for Cefepime for Pseudomonas aeruginosa

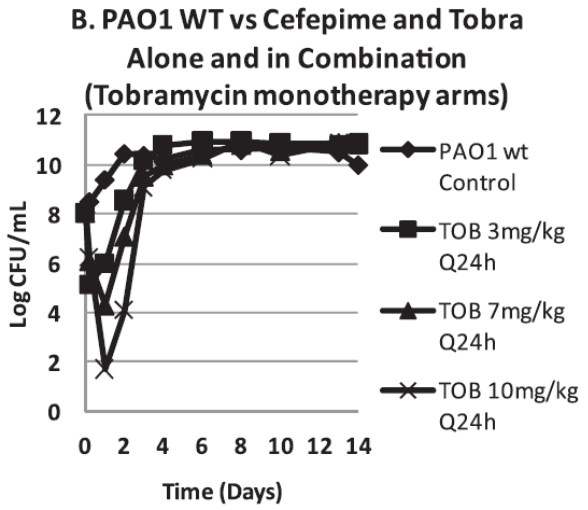
G. L. Drusano, Robert A. Bonomo, Nadzeya Bahniuk, Juergen B. Bulitta, Brian VanScoy, Holland DeFiglio, Steven Fikes, David Brown, Sarah M. Drawz, Robert Kulawy and Arnold Louie

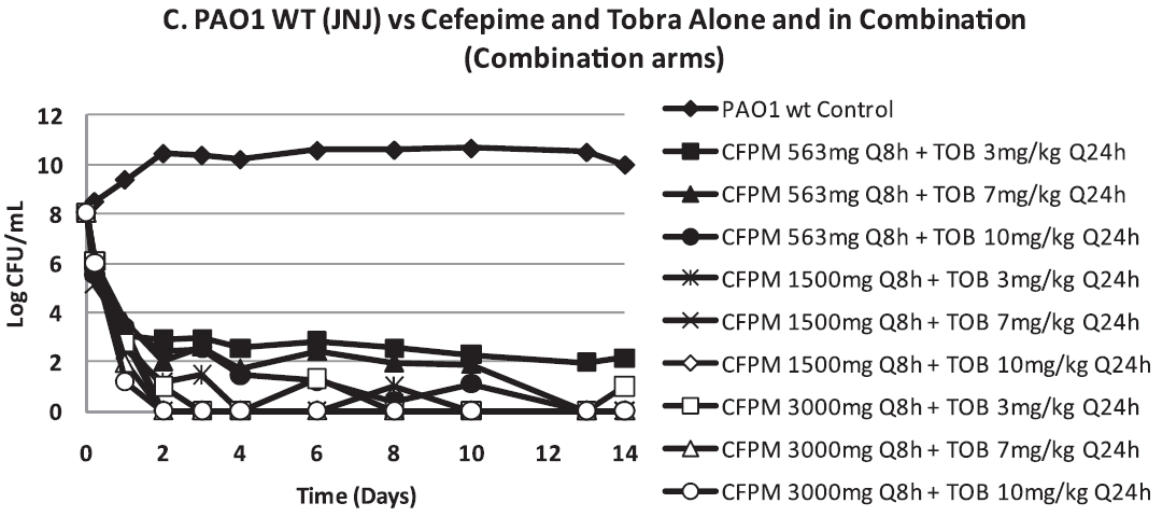
Antimicrob. Agents Chemother. **2012, 56(1):231. DOI:**

In Vitro

So, what's going on? Why the failure of mono-Rx and why the success of combo-Rx?



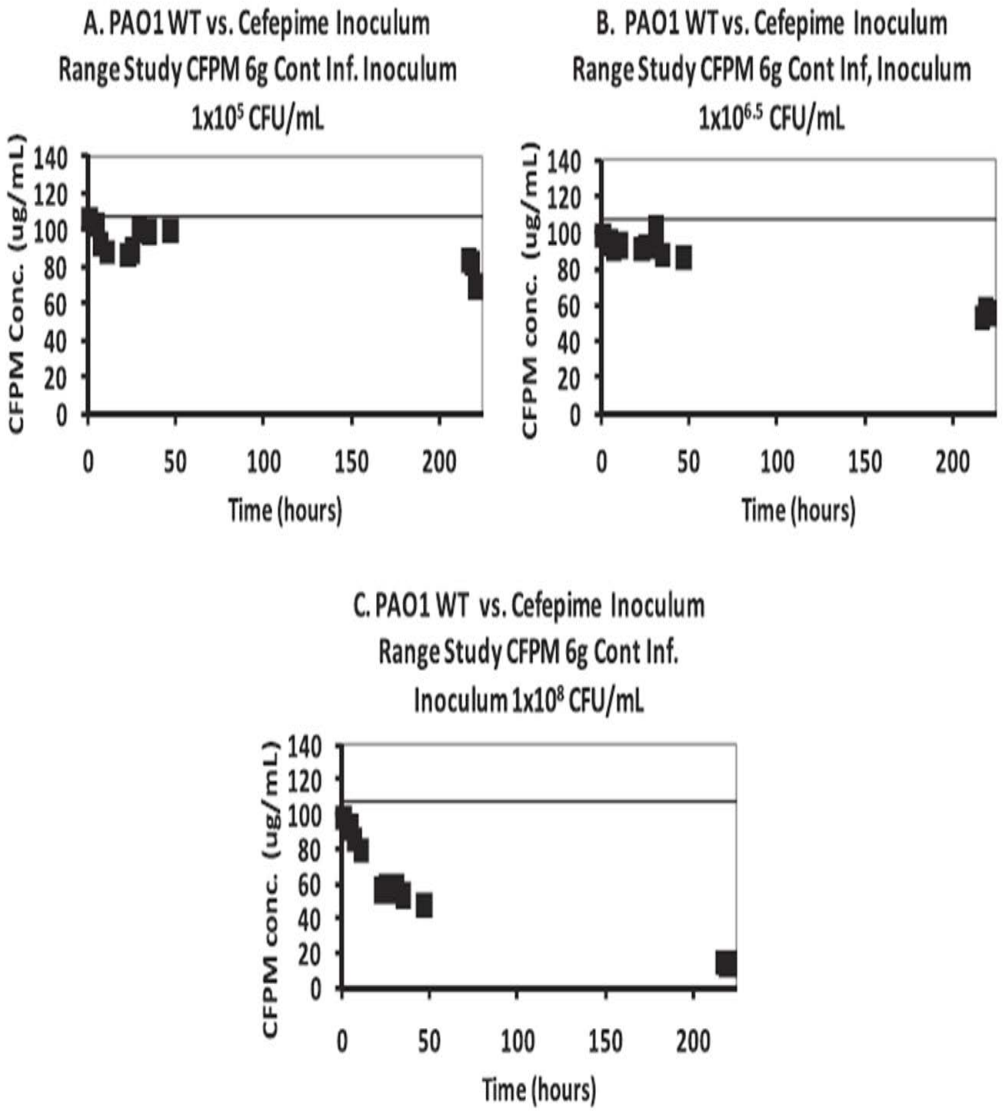




AAC 2012; 56:231-242

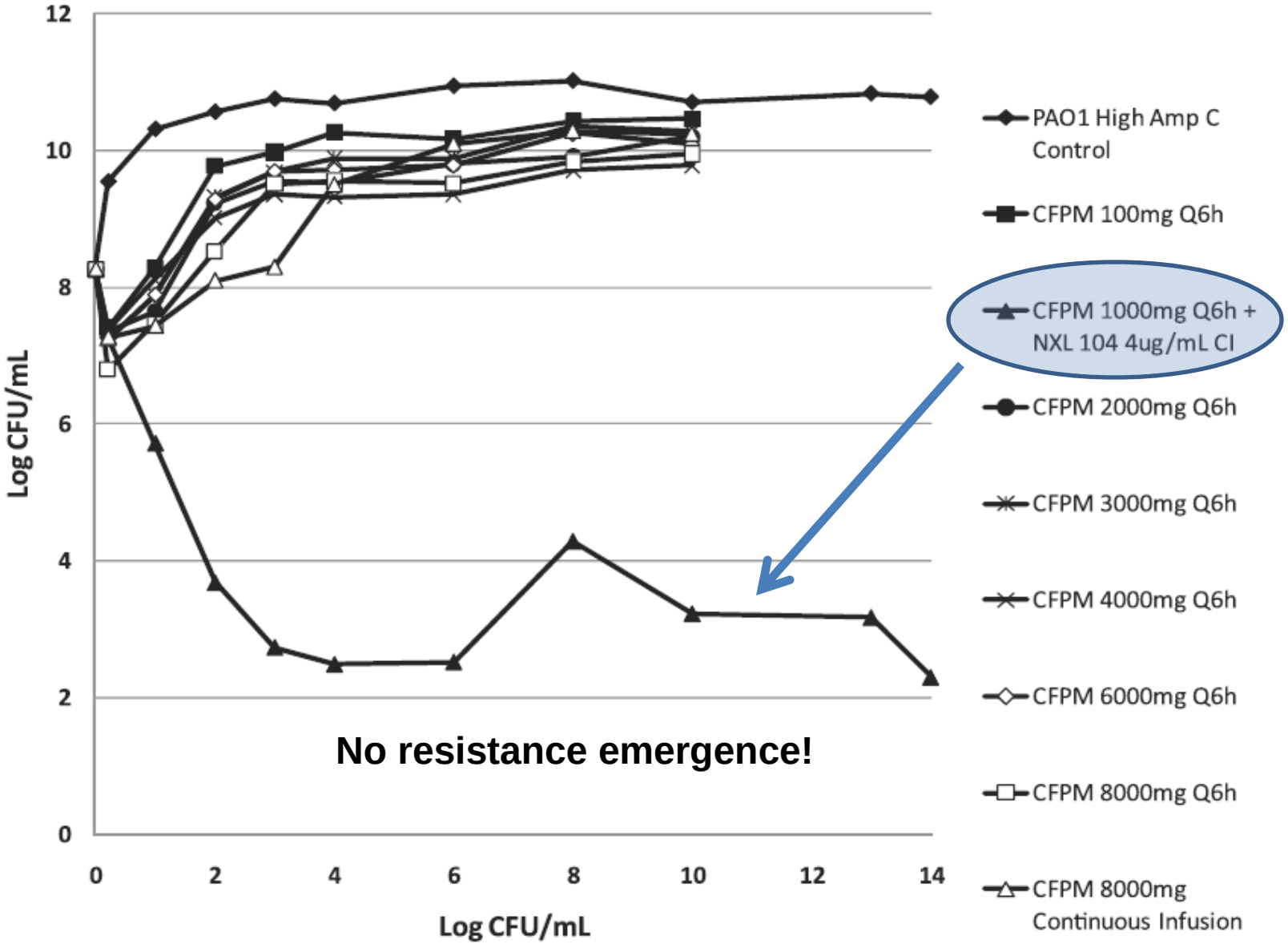
In Vitro

- So, what is going on?
- We looked at the stability of cefepime over time at different baseline inocula
- Inoculum and time-dependent hydrolysis was seen
- Hypothesis: β-lactamase mediated problem



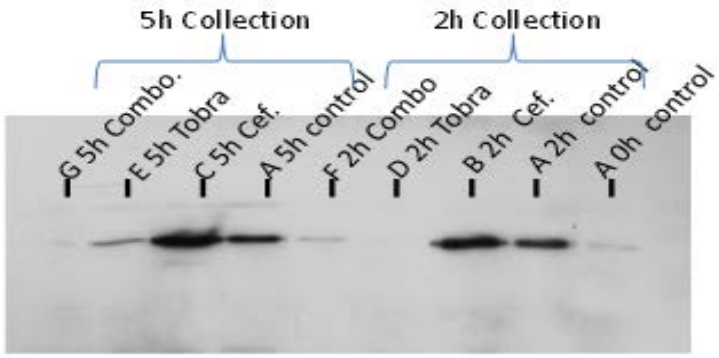
Antimicrob Agents Chemother 2012;56:231-242

In Vitro


12
10
8
6
4
2
0
Log CFU/mL
0
2
4
6
8
10
12
14
Log CFU/mL
No resistance emergence!
PAO1 High Amp C Control
CFPM 100mg Q6h
CFPM 1000mg Q6h + NXL 104 4ug/mL CI
CFPM 2000mg Q6h
CFPM 3000mg Q6h
CFPM 4000mg Q6h
CFPM 6000mg Q6h
CFPM 8000mg Q6h
CFPM 8000mg Continuous Infusion

In Vitro
Success of Combination Therapy

- As a protein synthesis inhibitor, we hypothesize that the aminoglycoside shuts down the expression of the ampC β-lactamase



AAC 2012; 56:231-242

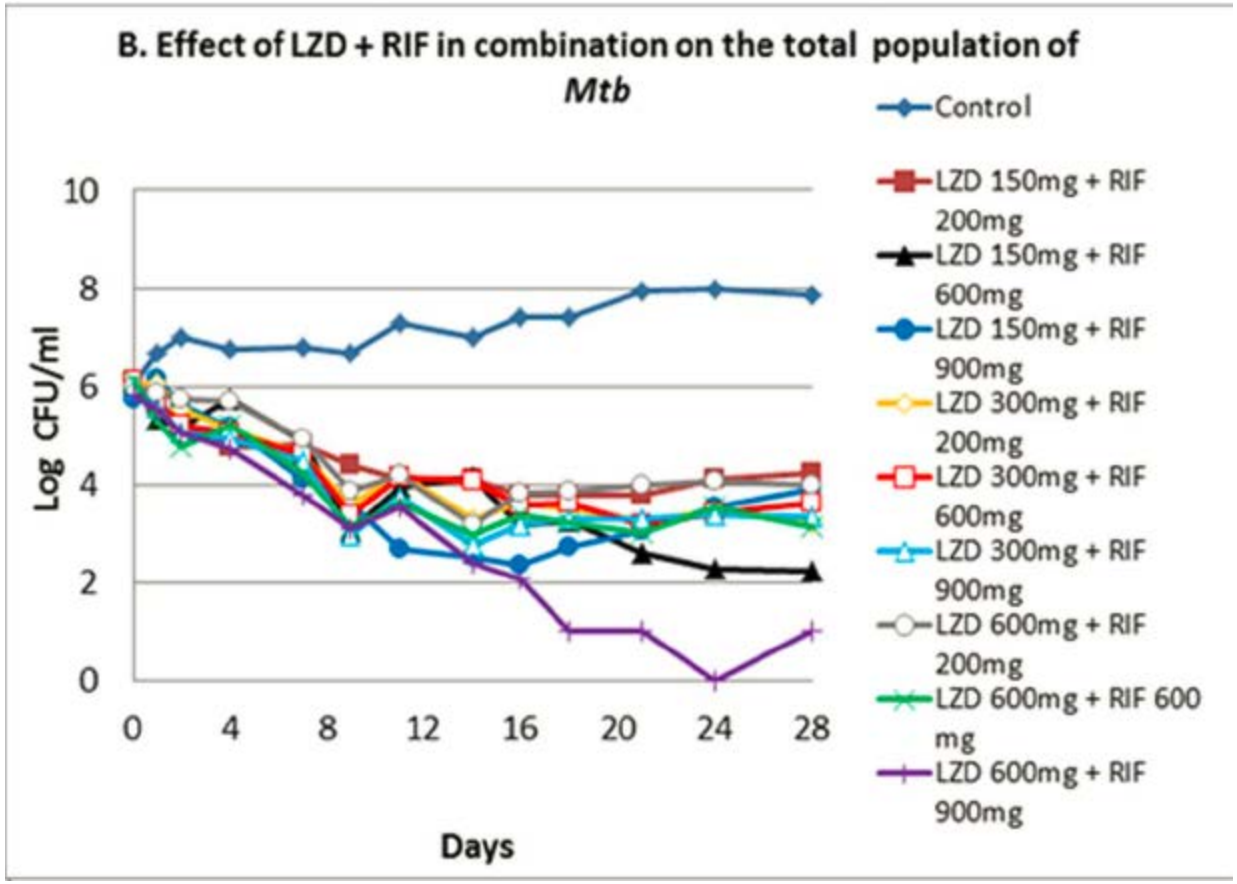
OPEN ACCESS Freely available online

PLOS ONE

Analysis of Combination Drug Therapy to Develop Regimens with Shortened Duration of Treatment for Tuberculosis

George L. Drusano[1]*, Michael Neely[2], Michael Van Guilder[2], Alan Schumitzky[2], David Brown[1], Steven Fikes[1], Charles Peloquin[3], Arnold Louie[1]

In Vitro



We have gone as long as 6 months; 1-2 months is standard for us in MTB studies

In Vitro - Conclusions

- This *in vitro* system is flexible, powerful and reproducible
- It allows study of differences in PK, organisms, bacterial burden and resistance emergence
- It allows linkage of measures of regimen intensity to effect (cell kill and resistance suppression)
- It allows experiments to be carried out for clinically-relevant durations
- All the data are straightforwardly able to be modeled fully parametrically to increase insight and allow design of validation experiments

In Vitro - Conclusions

- WHAT IS MISSING IS MODELING ALL THE OUTPUTS AND USING THE DATA TO PEFORM A PROSPECTIVE VALIDATION STUDY – THIS WILL IMPROVE CONFIDENCE!

Thank You for Your Attention!

$$dX_1/dt = R(1) - (SCL/V_c) \times X_1; \quad (1)$$

$$dN_S/dt = K_{g\text{-}S} \times E \times N_S - K_{\text{kill-S}} \times M \times N_S$$

$$\rightarrow \quad - K_{\text{kill-nat-S}} \times N_S; \quad (2)$$

$$dN_R/dt = K_{g\text{-}R} \times E \times N_R - K_{\text{kill-R}} \times M \times N_R$$

$$\rightarrow \quad - K_{\text{kill-nat-R}} \times N_R. \quad (3)$$

$$E = (1 - (N_S + N_R)/\text{POP}_{\text{max}}) . \quad (4)$$

$$(X_1/V_c)^H/((X_1/V_c)^H + C_{50\text{-}k}^H) . \quad (5)$$

Table 1. Population-mean parameter estimates for pharmacodynamic model.

Parameter	Estimate (SD)
Clearance, L/h	57.1 (6.45)
Volume of central compartment, L	45.9 (3.67)
$K_{gmax\text{-}S}$, $\log_{10}$ (cfu/mL)/h	6.88 (0.722)
$K_{gmax\text{-}R}$, $\log_{10}$ (cfu/mL)/h	3.75 (1.08)
$K_{kmax\text{-}S}$, $\log_{10}$ (cfu/mL)/h	10.0 (2.86)
$C_{50k\text{-}S}$, mg/L	0.0849 (0.0480)
$H_{k\text{-}S}$	26.7 (7.83)
$Kk_{max\text{-}R}$, $\log_{10}$ (cfu/mL)/h	5.29 (0.871)
$C_{50k\text{-}R}$, mg/L	0.417 (0.199)
$H_{k\text{-}R}$	7.84 (8.13)
$K_{nat\text{-}S}$, $\log_{10}$ (cfu/mL)/h	0.768 (0.532)
$K_{nat\text{-}R}$, $\log_{10}$ (cfu/mL)/h	0.950 (0.342)
POP_{max}, cfu/mL	3.59×10^{10} (2.21×10^{10})
Initial total population, cfu/mL	3.00×10^{7} (5.50×10^{6})
Initial resistant population, cfu/mL	146 (80)