PK/PD Principles for Decreasing the Development of Drug Resistance in Antimicrobials: a Road Map

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Combating Resistance

- Requires a concerted effort:
- In the clinic:
  - Effective infection control programs.
  - Strong antibiotic stewardship programs.
- On the farm: Reduce antibiotic use in livestock.
- On the benchtop: Identify new targets for anti-infective therapies that lack cross-resistance with existing medications.
- In the Lab and Clinic: Optimize the PD of drugs to suppress resistance. (This usually requires higher dosages. Toxicity?)

PK-PD for Suppressing Resistance

1. Impact of bacterial burden on antibiotic dose/exposure intensity. Describe the “inverted U” as it relates to resistance amplification/suppression.
2. Therapy duration and its impact on drug exposures for suppressing resistance.
3. Pharmacodynamic indices for suppressing resistance.
4. Effect of granulocytes in controlling the infection: Possible avenue for identifying a target for maximizing treatment efficacy for VAP?

(I will not be discussing how to choose the best *in vivo* or *in vitro* infection models for resistant suppression)

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Bacterial burden in people with VAP

- Zaccard et al. conducted bilateral BALs to quantitate the bacterial burden in the lungs of 134 patients with suspected ventilator-associated pneumonia (VAP) due to *P. aeruginosa*, *Acinetobacter* spp., *Klebsiella* spp., and other GNRs.

Quantitative culture range	Percentage of patients
$\geq 3x10^5$ to $< 3x10^6$ CFU/mL	36.6%
$\geq 3x10^6$ to $< 3x10^7$ CFU/mL	37.3%
$\geq 3 x10^7$ CFU/mL	26.1%

- The mutation frequency for most antibiotics are between 10^{-5} to $8x10^{-7}$ CFU.
- The higher the bacterial burden, the higher the probability pre-existing isolates with reduced susceptibilities to the drug are present at the infection site prior to the start of therapy.

Zaccard et al. AAC 2011; 55: 1606.

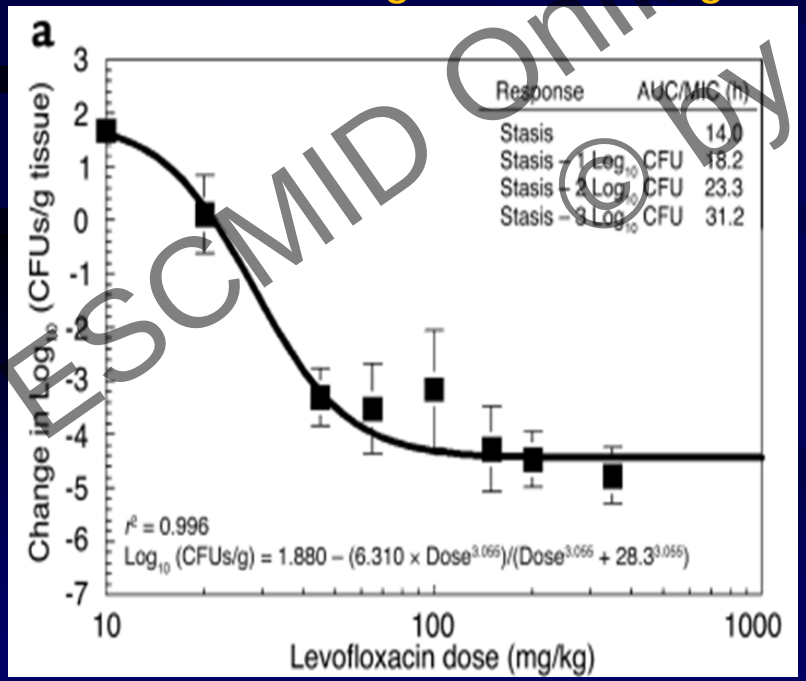
Impact of bacterial burden on antibiotic exposures needed to achieve microbiological endpoints in a immunocompetent murine thigh model of *P. aeruginosa*

Two bacterial inocula: 10^7 and 10^8 CFU/thigh MF to 3x MIC: 2 x 10^{-6} CFU.

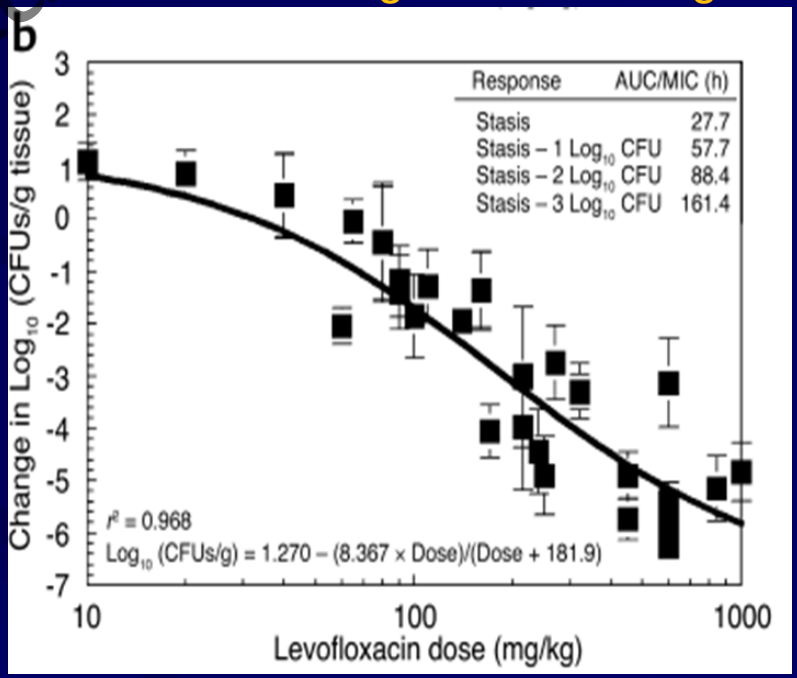
The two bacterial challenge inocula are higher than the inverse of the MF value:
10^7 CFU/thigh: ~ 5 resistant CFU/thigh. 10^8 CFU/thigh: ~ 50 resistant CFU/thigh

Dose-range study over 24 hours for single-dose levofloxacin: 0 to 1,000 mg/kg IP.

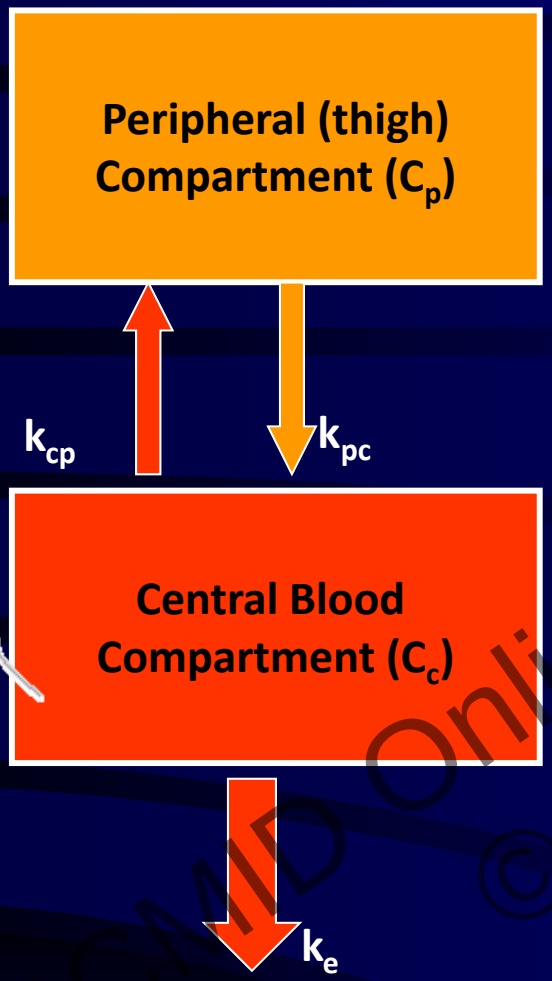
Initial Challenge 10^7 CFU/thigh

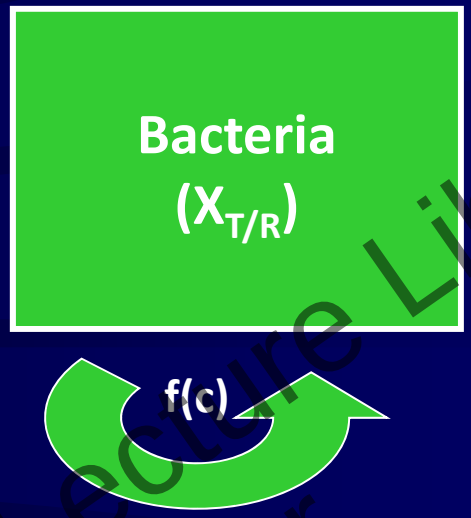


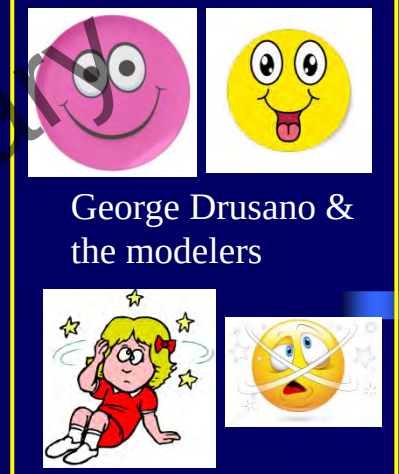
Initial Challenge 10^8 CFU/thigh



Jumbe et al. J Clin Invest 2003;112:275







[1] $\frac{dC_a}{dt} = -k_a C_a$

[2] $\frac{dC_c}{dt} = k_a C_a + k_{pc} Cp - k_{cp} C_c - k_e C_c$

[3] $\frac{dC_p}{dt} = k_{cp} C_c - k_{pc} C_p$

$$\frac{dX_S}{dt} = K_{GS} \times X_S \times L - f_{KS}(C_c^{H\xi}) \times X_S \quad [4]$$

$$\frac{dX_R}{dt} = K_{GR} \times X_R \times L - f_{KR}(C_c^{H\xi}) \times X_R \quad [5]$$

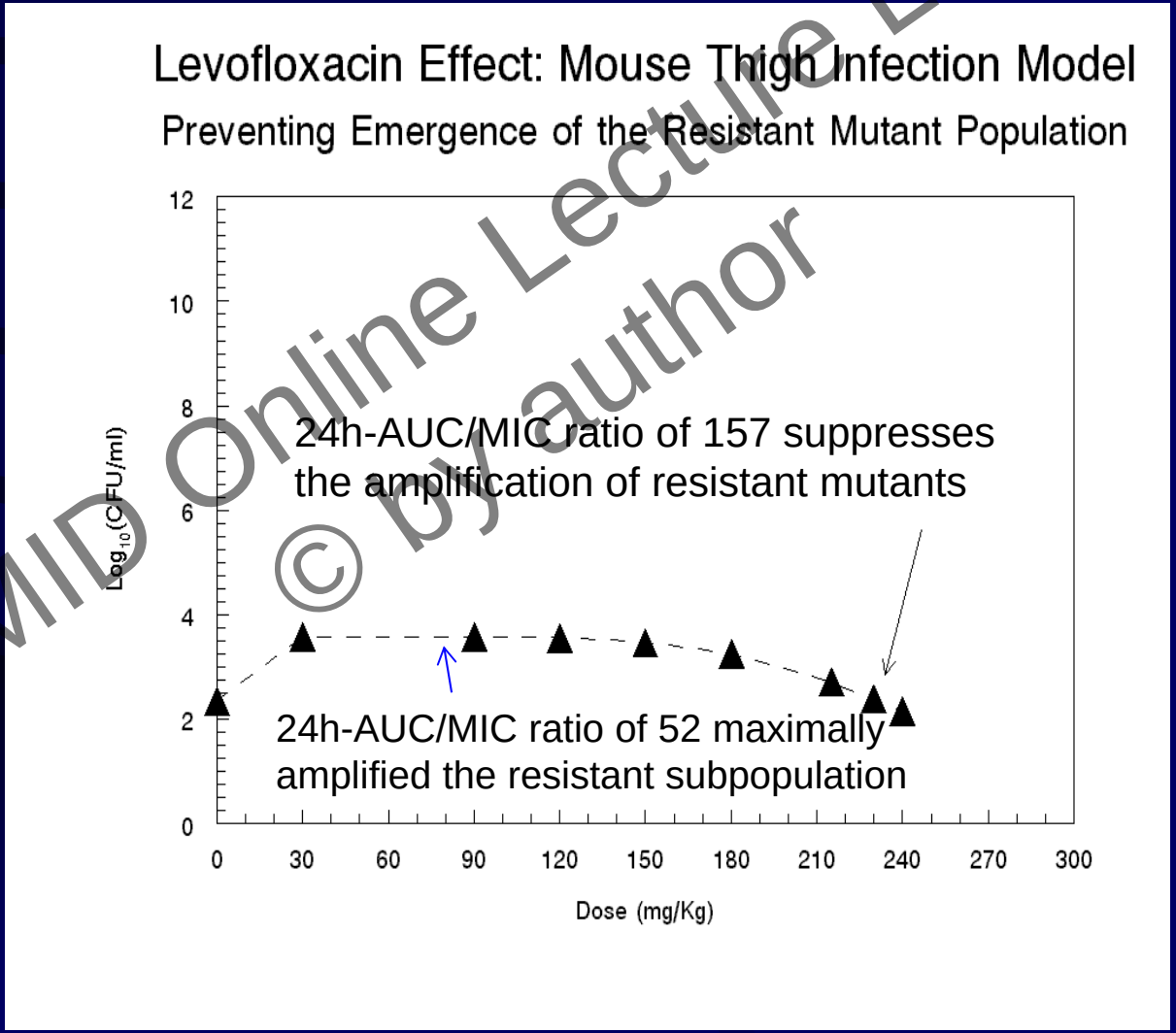
$$L = (1 - (X_R + X_S)/POPMAX) \quad [6]$$

$$f_{\psi\xi}(C_c^{H\xi}) = \frac{K_{max\,\xi} \bullet C_c^{H\xi}}{C^{H\xi}_{50\xi} + C_c^{H\xi}}, \quad \psi = K \text{ and } \xi = S,R \quad [7]$$

$$Y_1 = X_T = X_S + X_R \quad [8]$$

$$Y_2 = X_R \quad [9]$$

The mathematical modeling outputs: Levofloxacin doses that amplify or suppress the growth of the levofloxacin-resistant *P. aeruginosa* subpopulations in mice infected with 10^8 CFU/thigh



Jumbe et al. J Clin Invest 2003;112:275

Prospective Validation Experiment –

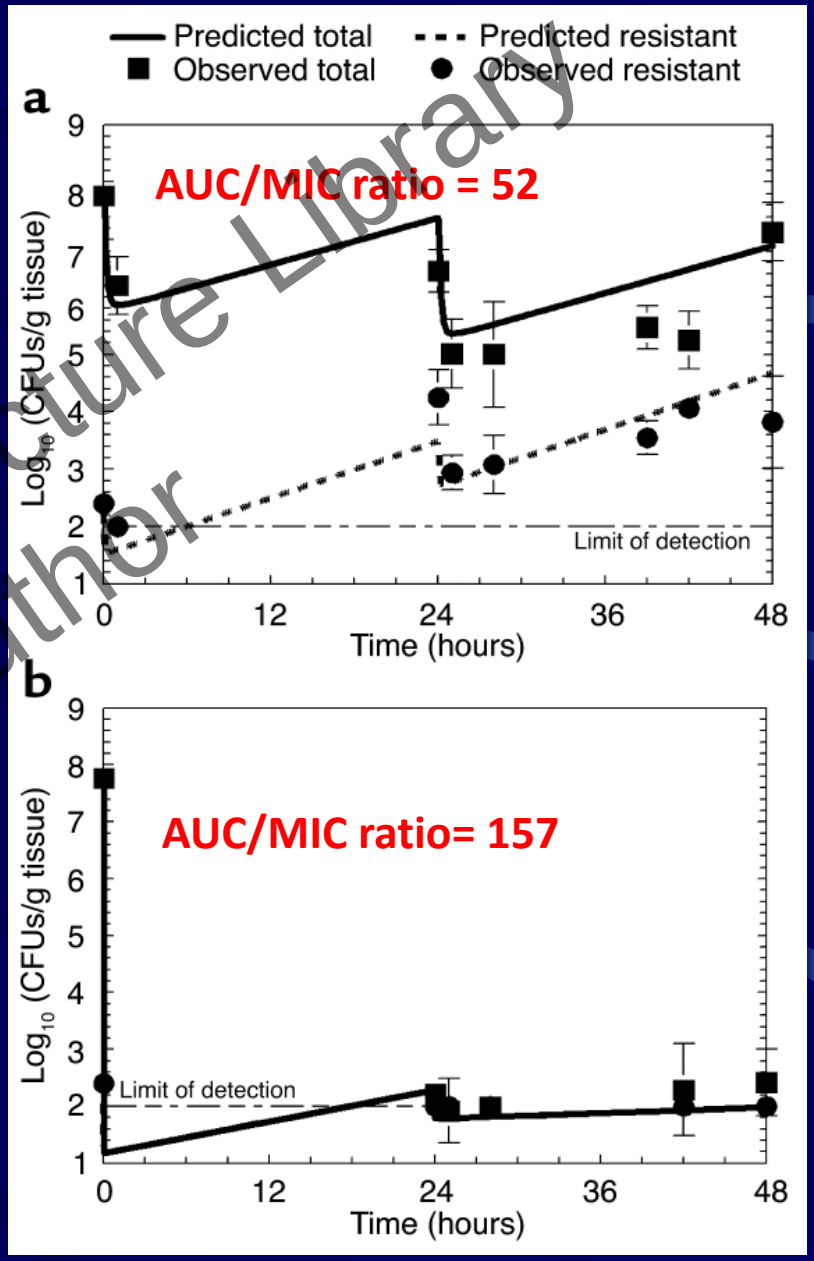
Immune normal mice were inoculated with 10^8 CFU/thigh inoculum and were treated with these two levofloxacin exposures that had not been explicitly examined before.

Levofloxacin was dosed once-daily.

We also extended the study duration from 24h to 48h to further test the predictions.

The lines in the figures are NOT best-fit lines.

They are prospective prediction lines about which the data have been scattered.



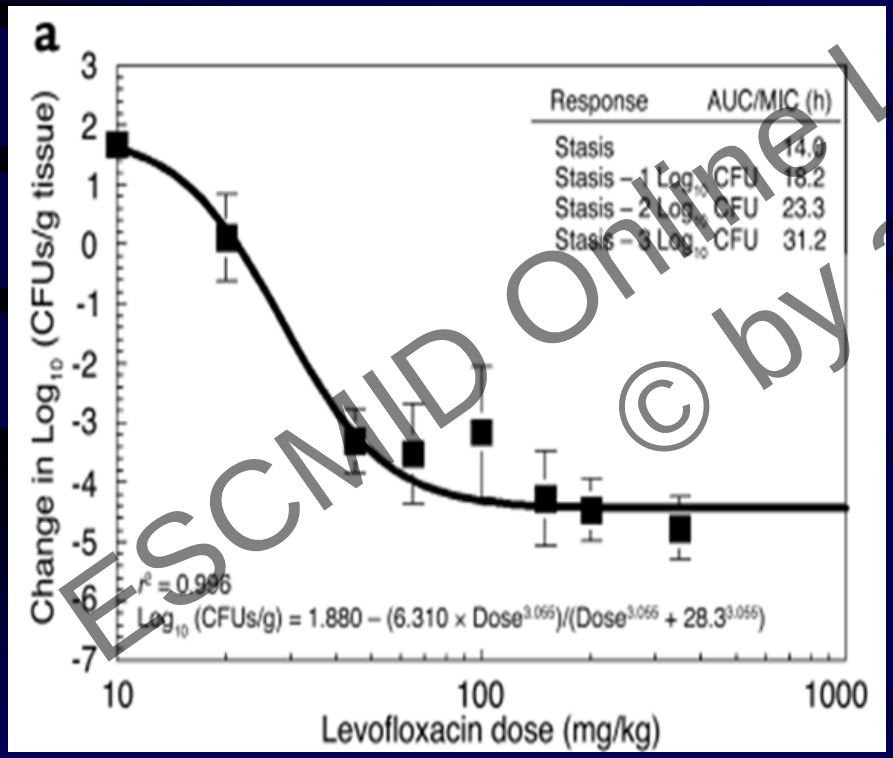
Jumbe et al. J. Clin. Invest. 2003;112:275 and
Drusano et al. Nature Reviews Microbiol. 2004;2:289

Implications for drug development for resistance prevention

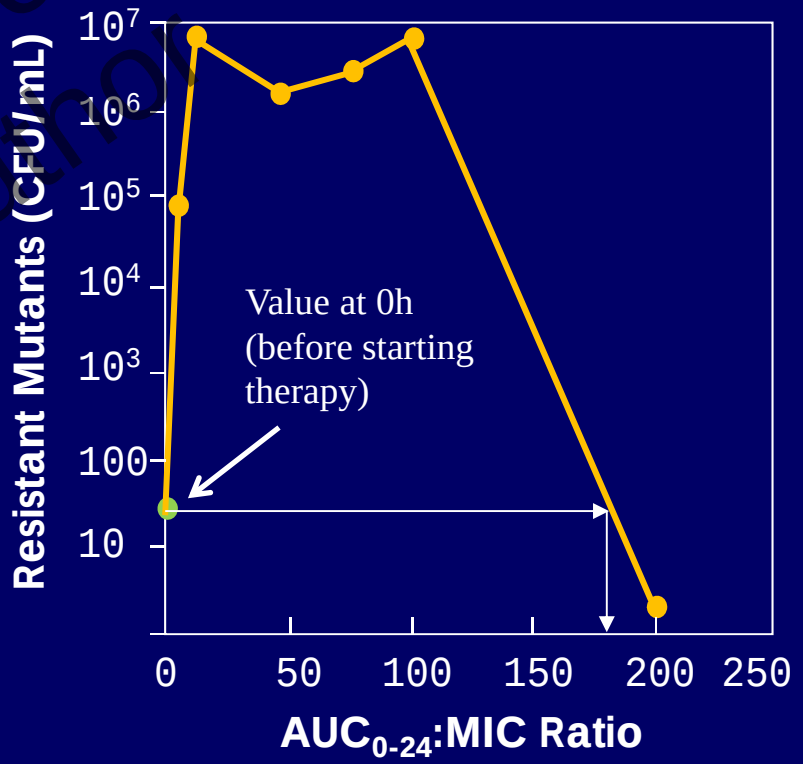
- Bacterial burden does matter when evaluating the efficacy of a compound for resistance suppression.
- Mathematical modeling of the dose-range data can be used to identify the drug exposures that are predicted to amplify or prevent the emergence of the drug-resistant microbial subpopulations.
- Monte Carlo simulations predicted that only 61.2% of people given levofloxacin 750 mg po QD would achieve the AUC/MIC target of 157 that would counterselect for resistance.
  - Higher dosages of levofloxacin would be needed to prevent resistance in a larger proportion of patients.

The exposure-response effect of antibiotics on the susceptible and resistant microbial populations differ.

Killing of the susceptible population is characterized by a monotonic function



Killing of the less-susceptible population is described by an "Inverted U."



Jumbe et al. J Clin Invest 2003; 112: 275

Tam et al. AAC 2007; 51: 744.

PD indices for killing and resistance amplification

			PD Index-intensities killing for:		
Antibiotic	Pathogen	PDI-killing	0 to 1 log kill	resistance selection	References
Garenoxacin	*S. aureus*	AUC/MIC	10 - 35	10 – 35	Tam et al. AAC 2007
Moxifloxacin	*Y. pestis*	AUC/MIC	31 – 63.5	31 – 127	Louie et al. AAC 2011
Garenoxacin	*K. pneumoniae*	AUC/MIC	10 – 35	10 – 35	Tam et al. AAC 2007
Linezolid	*B. anthracis*	AUC/MIC	45 – 56	45 – 56	Louie et al. AAC 2008
Vancomycin	Staph spp.	AUC/MIC	100 – 200	100 – 400	Ramos-Martin JAC 2016
Daptomycin	*S. aureus*	AUC/MIC	14 – 71	0.5 – 40	Bowker et al. JAC 2009
Telavancin	Enterococcus	AUC/MIC	6 – 60	1 – 50	MacGowan AAC 2011
Ceftaroline	*S. aureus*	T>MIC(%)	13 – 40	15 – 30	MacGowan AAC 2013
Doripenem	*P. aeruginosa*	T>MIC(%)	14 – 42	12 – 37	Bowker et al. AAC 2012
	A. baumannii	T>MIC(%)	9 – 32	12	Bowker et al. AAC 2012
Ceftolozane/tazo	*E. coli*	T>threshold(%)	87.5 (tazo)	75 – 98 (tazo)	VanScoy et al. AAC 2013
Rifampin	*M. tuberculosis*	AUC/MIC	224 – 448	224 – 700	Gumbo et al. AAC 2007

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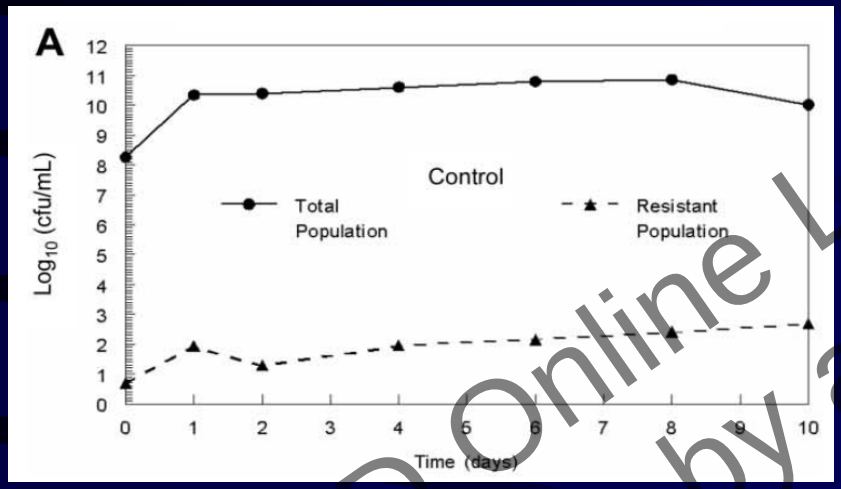
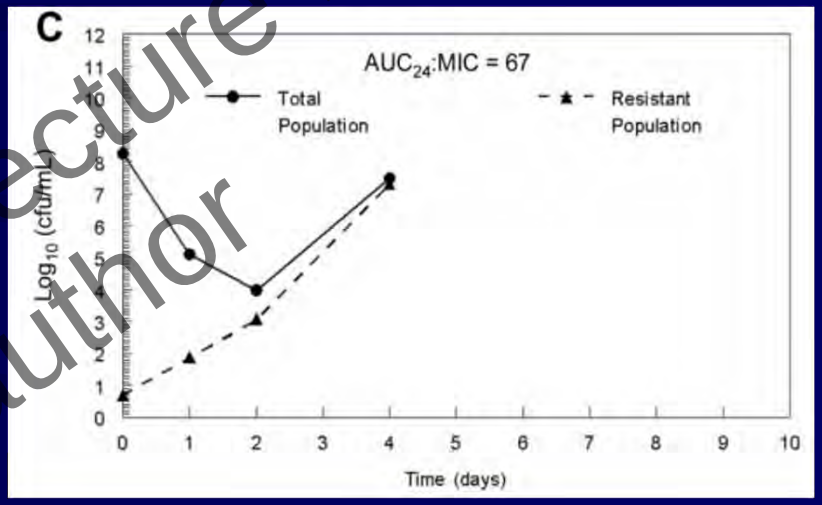
Suppressing Resistance – Therapy Duration

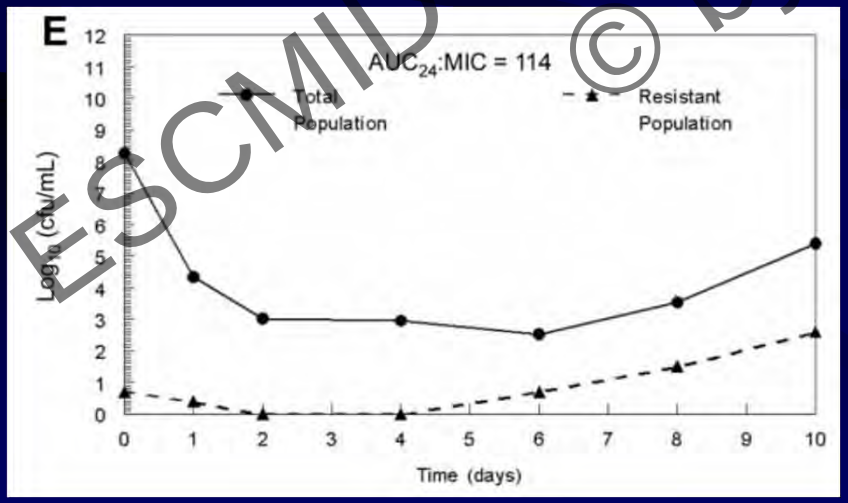
- Therapy duration is one of the great understudied areas of Infectious Diseases.
- There are only a few infectious diseases for which duration of therapy has been established based on firm scientific data – Strep throat, endocarditis, UTI, and some STDs.
- But what is known about the impact of duration of antibiotic therapy on the amplification or suppression of the antibiotic-resistant subpopulations?

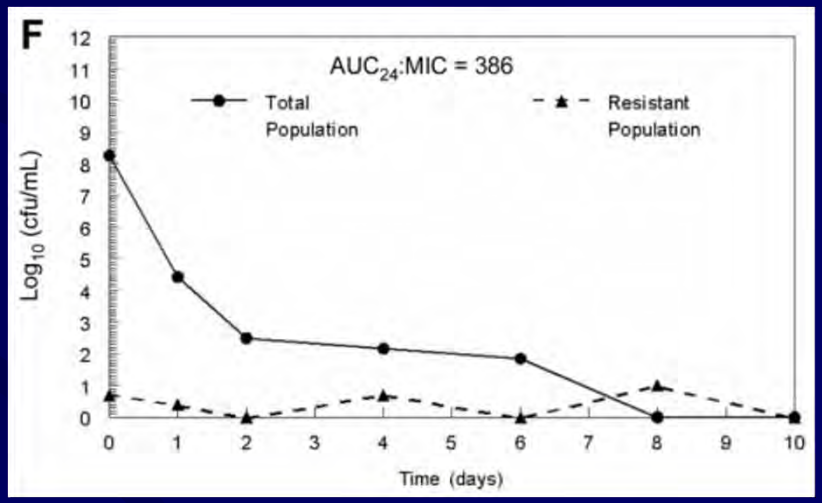
Suppressing Resistance – Therapy Duration

We conducted a 10 day dose-range study with the fluoroquinolone garenoxacin vs *S. aureus* in a HFIM.







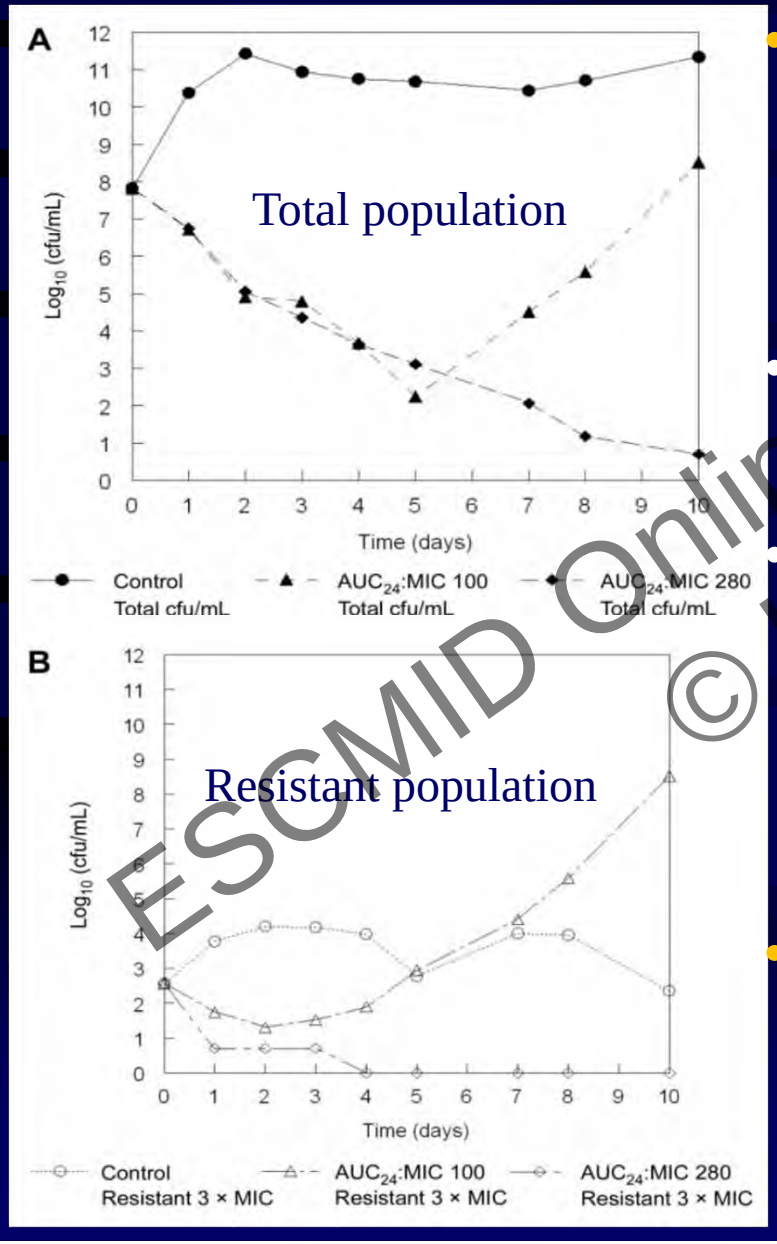


Tam et al. J. Infect. Dis. 2007; 195: 1818.

Suppressing Resistance – Therapy Duration

- We modeled the dose-ranging data to ascertain the drug exposure that would suppress resistance amplification after 2 days of garenoxacin therapy and after 10 days of therapy.
- We also modeled the time-to-failure for the less intense exposure.
- Model predictions:
    1. A daily administered AUC/MIC ratio of 100 would suppress resistance for at least 2 days.
    2. A daily administered AUC/MIC ratio of 280 would suppress resistance for the duration of the 10-day study.
    3. Time to failure due to resistance amplification for the daily-dosed AUC/MIC ratio of 100 would be 5 days.
- A prospective validation study was conducted to validate these predictions.

Suppressing Resistance – Validation Study



- No-treatment control provided no selective pressure. The resistant population increased in parallel to the total population.
- The AUC/MIC ratio of 100 given once daily for 10 days failed after day 5.
- The AUC/MIC ratio of 280 eliminated the less-susceptible subpopulation by day 4 and resulted in a progressive reduction in the total population for the entire 10-day treatment period.
- With longer treatment duration, the drug exposure needed to suppress the resistant subpopulation increased 28-fold.

Tam et al. J Infect Dis. 2007;195:1818.

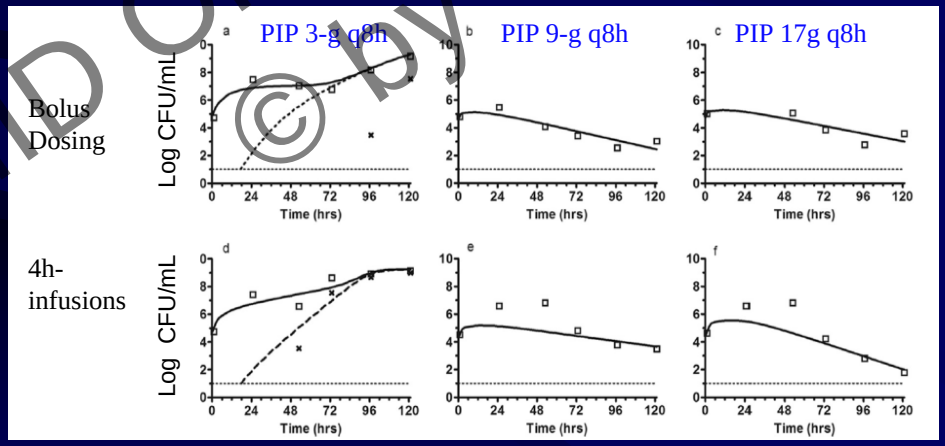
Common mode of administration of drugs and their impact on resistance amplification

- Extended infusion of beta-lactam drugs is now in vogue for killing the drug-susceptible infecting bacterium.
- Felton et al. evaluated 0.5 h bolus vs 4 h extended infusions of 3 to 17g piperacillin (with tazobactam) given q8h on resistance amplification in *P. aeruginosa* in a 5 day *in vitro* HFIM experiment.
  - High inoculum ($8x10^8$ CFU/mL): all arms failed with resistance.
  - Low inoculum ($4x10^5$ CFU/mL):
    - 0.5 h bolus dosing every 8 h: trough/MIC ratio for piperacillin of 3.4 was needed to suppress resistance.
    - 4 h extended infusions every 8 h: trough/MIC ratio of 10.4 was required.

Felton et al. AAC 2013; 57: 5811.

Common mode of administration of drugs and their impact on resistance amplification

- Felton et al. evaluated 0.5 h bolus vs 4 h extended infusions of 3, 9, & 17 g of piperacillin (with tazobactam) given every 8 h on resistance amplification in *P. aeruginosa* in a 5 day *in vitro* HFIM study.
  - High and low starting inocula: $4x10^5$ and $8x10^8$ CFU/mL (VAP)
- Results:
  - High inoculum: all regimens failed due to resistance amplification.
  - Low inoculum: In order to suppress resistance, modeling of the results:



- 0.5 h bolus dosing every 8 h: trough/MIC ratio of 3.4 was needed
- 4 h extended infusions every 8 h: trough/MIC ratio of 10.4 was required.

Felton et al. AAC 2013; 57: 5811.

- Overall, the data suggests that to minimize resistance amplification:
  - antibiotics should be given at higher dosages for a shorter duration of time.
  - Piperacillin-tazobactam, should be given as bolus doses and not as extended infusions.

Clinical Correlation

- Prospective, randomized, double-blinded clinical trial comparing 8 vs 15 days of antibiotic therapy for VAP.
  - VAP dx'ed by quantitative cultures of BAL specimens
  - Findings:
    - No differences in overall mortality (18.8% vs 17.2%).
    - More antibiotic free days with 8 days of therapy ($p < 0.001$).
    - For VAP caused by non-fermenting GNRs, including *P. aeruginosa*, more recurrence was observed with shorter treatment (8 vs 15 days of therapy: 40.6% vs 25.4%, difference 15%, 90% CI, 3.9% to 26.6%).
    - But 8 days of treatment had less recurrences due to multidrug-resistant bacteria than 15 days of treatment (42.1% vs 62.3%, respectively, $p = 0.04$).

Chastre et al. JAMA 2003;209:2588.

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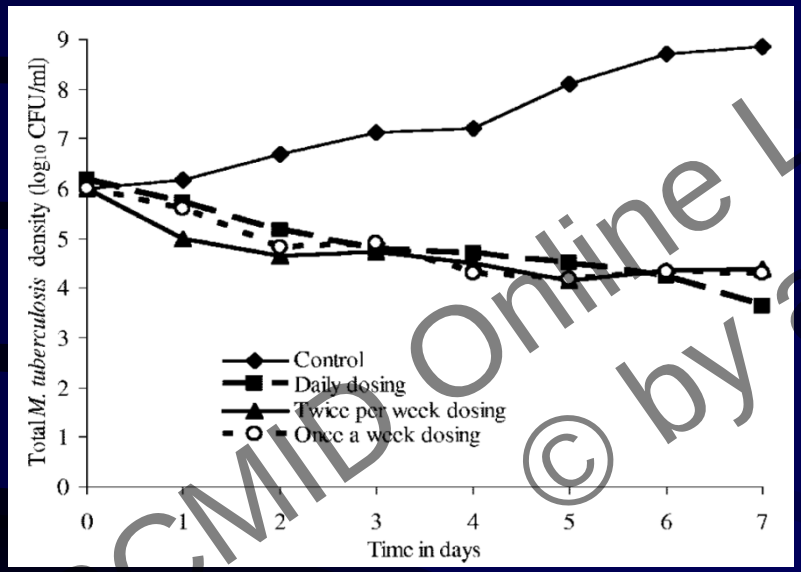
Suppressing Resistance-Pharmacodynamic Drivers

- For many antibiotics, the PD indices for killing the parent isolate and for suppressing resistance differ.
  - Minimizing resistance amplification requires antibiotics to be given using a different schedule of administration compared with regimens aimed that maximizing the killing of the parent isolate.
  - However, the higher dosages needed to suppress resistance often
- Examples:
  1. Rifampin for *Mycobacterium tuberculosis*
  2. Vancomycin for *S. aureus*

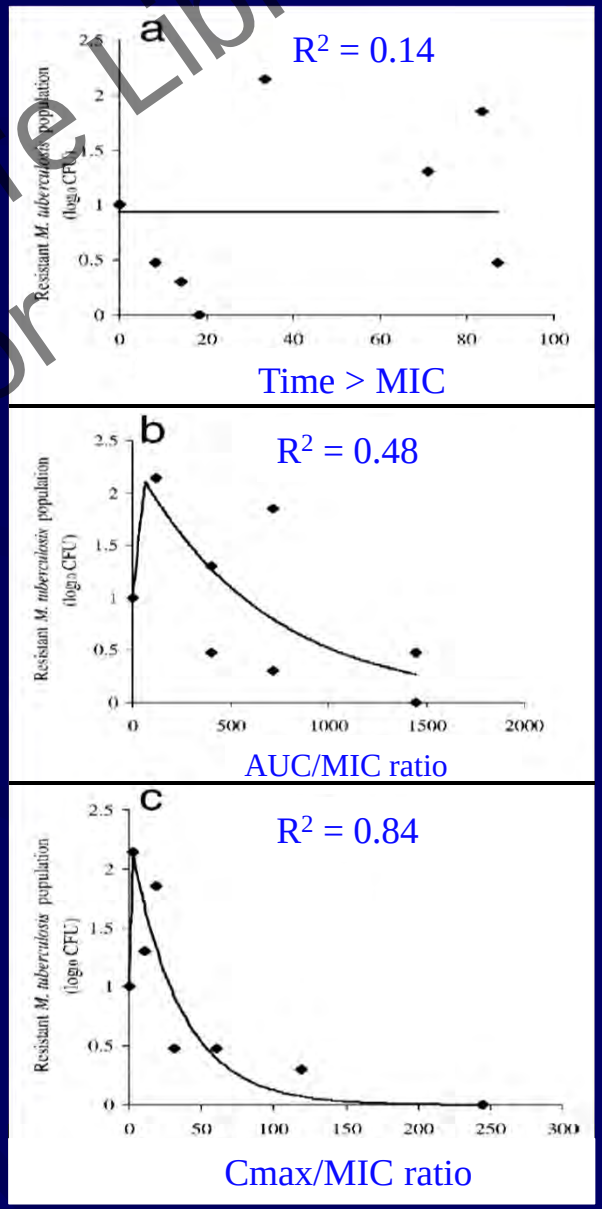
Rifampin suppressing resistance-pharmacodynamic drivers for *M. tuberculosis*

Cell kill is AUC/MIC-driven

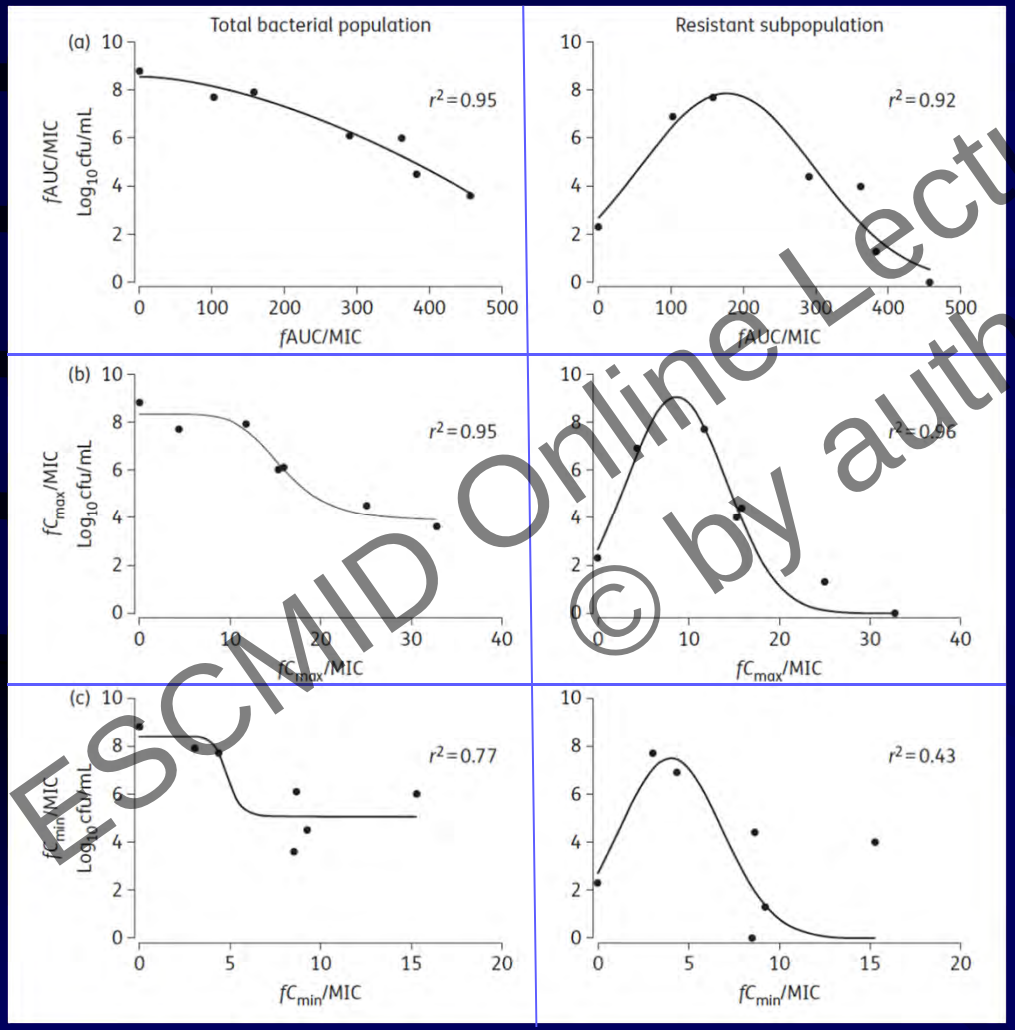


Resistance suppression is Cmax/MIC-driven (but the exposure is <u>not</u> achievable in humans)

Gumbo et al. AAC 2007;51:3781.



Vancomycin PD for killing and resistance prevention (10-day HFIM)



Ramos-Martin et al. JAC 2016; 72: 992

PD index for killing the susceptible-bacteria: AUC/MIC or Cmax/MIC

PD index for suppressing resistant subpopulations: Cmax/MIC ratio.

Resistance amplification and suppression follows the “inverted U” paradigm.

PD indices for killing and resistance suppression

Antibiotic	Pathogen	PDI-killing	PDI-killing for: 0 to 1 log kill	PDI-killing for: resistance selection	PDI: resistance prevention
Garenoxacin	*S. aureus*	AUC/MIC		10 – 35	Cmax/MIC
Moxifloxacin	*Y. pestis*	AUC/MIC	31 – 63.5	31 – 200	Cmax/MIC ≥ 14.4
Moxifloxacin	*B. anthracis*	AUC/MIC	100 – 200	100 – 200	T>MIC
Garenoxacin	*K. pneumoniae*	AUC/MIC		10 – 35	Cmax/MIC
Linezolid	*B. anthracis*	AUC/MIC	45 – 56	45 – 56	Cmax/MIC ≥ 9.8
Vancomycin	Staph spp.	AUC/MIC	100 – 200	100 – 400	Cmax/MIC
Daptomycin	*S. aureus*	AUC/MIC	14 – 71	0.5 – 40	??
Telavancin	Enterococcus	AUC/MIC	6 – 60	1 – 50	??
Doripenem	*P. aeruginosa*	T>MIC	14 – 42	12 – 37	Tr/MIC
Meropenem	*P. aeruginosa*	T>MIC			Tr/MIC ≥ 6.2
Pip/tazo	*P. aeruginosa*	T>threshold			Tr/MIC (≥ 3.4, bolus)
Ceftolozane/tazo	*E. coli*	T>threshold	87.5 (tazo)	75 – 98 (tazo)	T>threshold or Tr/MIC
Rifampin	*M. tuberculosis*	AUC/MIC	224 – 448	224 – 700	Cmax/MIC (≥ 175)

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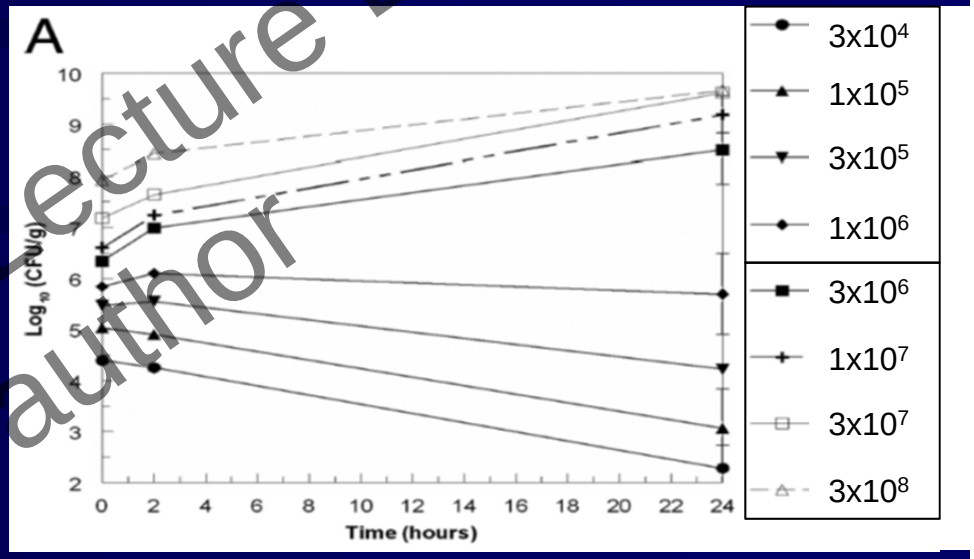
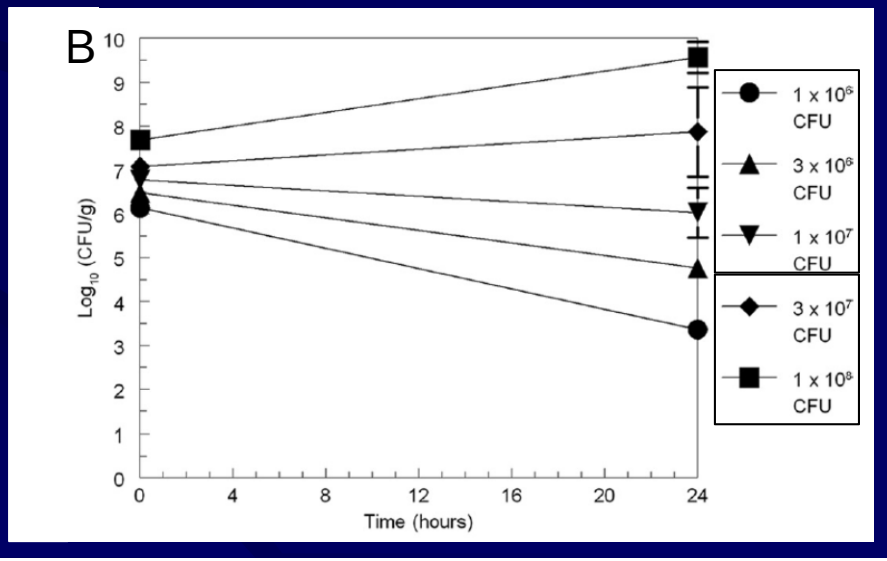
What about the role of the innate immune system?

- Inoculum range study for *P. aeruginosa* in immune normal mice.
  - A. Thigh infection model
  - B. Pneumonia model
- A Michaelis-Menten model was fit to the data:
  - A. For the thigh model, the K_m or the bacterial burden that half saturates neutrophil function, was 4.3 x 10^6 CFU/g.
  - B. For the pneumonia model, the K_m was 2.15 x 10^6 CFU/g.

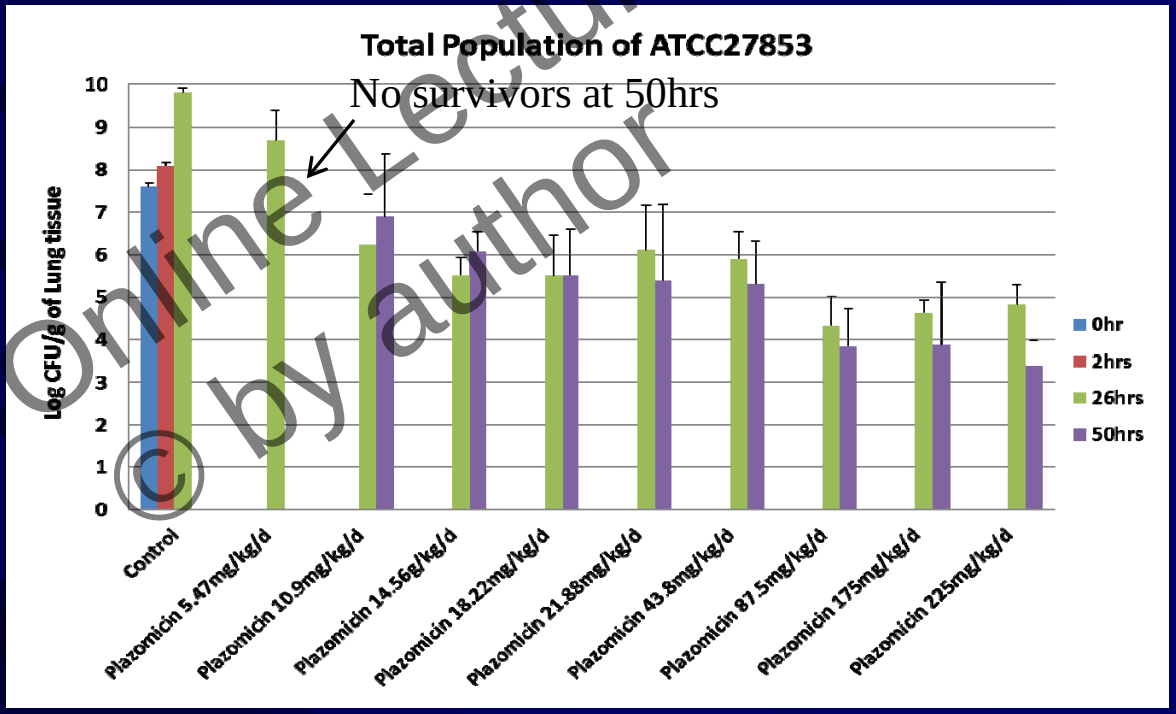




Drusano et al. AAC 2010; 54: 4368.
Drusano et al. AAC 2011; 55: 2693.

WBC and Plazomicin vs *P. aeruginosa*

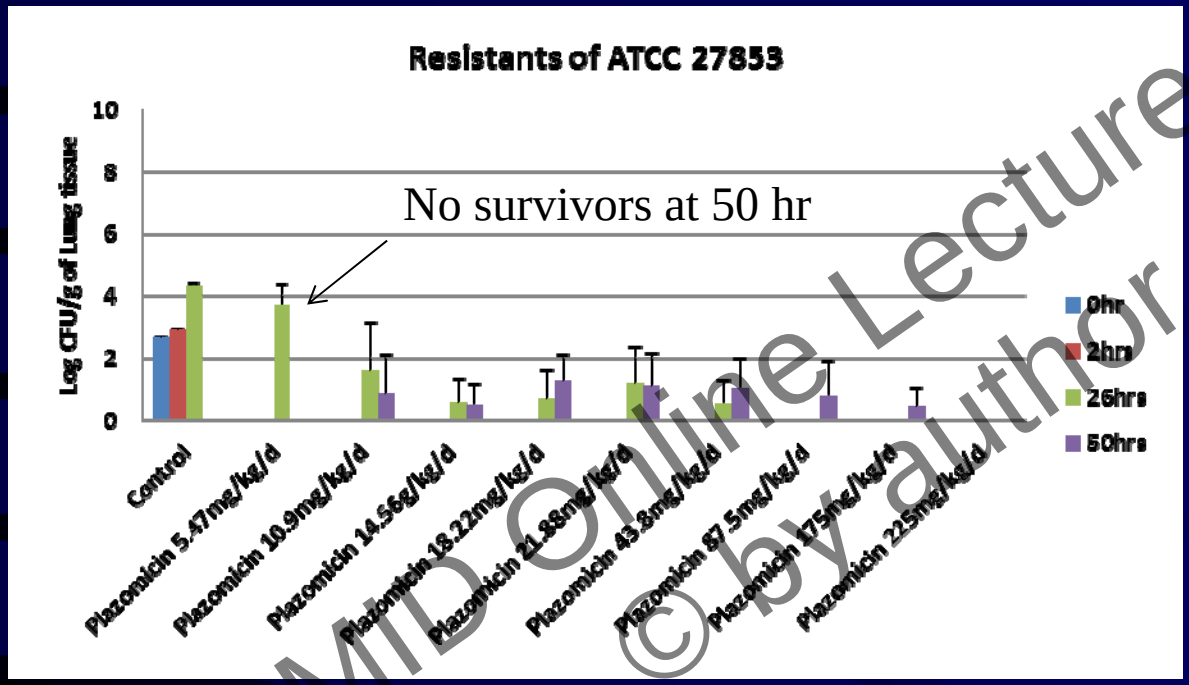
With the humanized plazomicin regimens, no drug would be detected in serum by hour 26.



Higher humanized plazomicin dosages given from 2 - 26 hr, reduced the *P. aeruginosa* burden to < 10^5 CFU/g. This restored granulocyte function, allowing the neutrophils to kill an additional 1 – 1.5 log CFU/g of bacteria (without antibiotic) between the 26 and 50 hr time points.

Drusano et al. J. Infect. Dis. 2014; 210: 1319.

WBC and Plazomicin vs *P. aeruginosa*



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$\geq 3x10^6$ to $< 3x10^7$ CFU/mL	37.3%
$\geq 3 x10^7$ CFU/mL	26.1%

Plazomicin dosages that reduce the bacterial burden to $\leq 10^5$ CFU/g in concert with WBCs prevented the amplification of the less-susceptible *P. aeruginosa* isolates.

Together with the Qcx data by Zaccard, this suggests a $\geq$ 2 - 3 log CFU/g reduction is needed to achieve this endpoint over ? Days.

Zaccard et al. AAC 2011; 55: 1606.

Drusano et al. J. Infect. Dis. 2014; 210: 1319.

An approach to Combating Resistance

- Thus, for single drug regimens in order to combat resistance we need to:
  - "Hit the bacteria hard and hit them fast"
  - Use higher dosages for a shorter duration to rapidly reduce the total (susceptible and less-susceptible) population to below 10^5 CFU/g of tissue, which is predicted to restore granulocyte function. The restored function of the granulocytes can eliminate the residual bacteria without additional antibiotics.
  - Drug tolerability may be a limiting factor for some drugs.
- Although not discussed today, combination antibiotic regimens is very effective for combating resistance.
  - For example: beta-lactam plus aminoglycoside.

Conclusions

- Drug exposures for the killing of the Abx-susceptible microbial population is a monotonic function.
- The killing of the less-susceptible population is characterized by an “inverted U.”
  - Doses of drugs that yield a stasis effect and 1-log reductions of the parent strain also amplify the less-susceptible subpopulations.
- The intensity of drug exposures needed to suppress resistance may increase with the duration of therapy.
- The PD indices for the killing of the parent strain and for resistance prevention are not the same.
- The immune system has a role in controlling infections provided the bacterial concentrations are < 10^5 CFU/mL.
- To prevent resistance: higher dosages for shorter durations .

Acknowledgements

George Drusano, M.D.

The laboratory crew