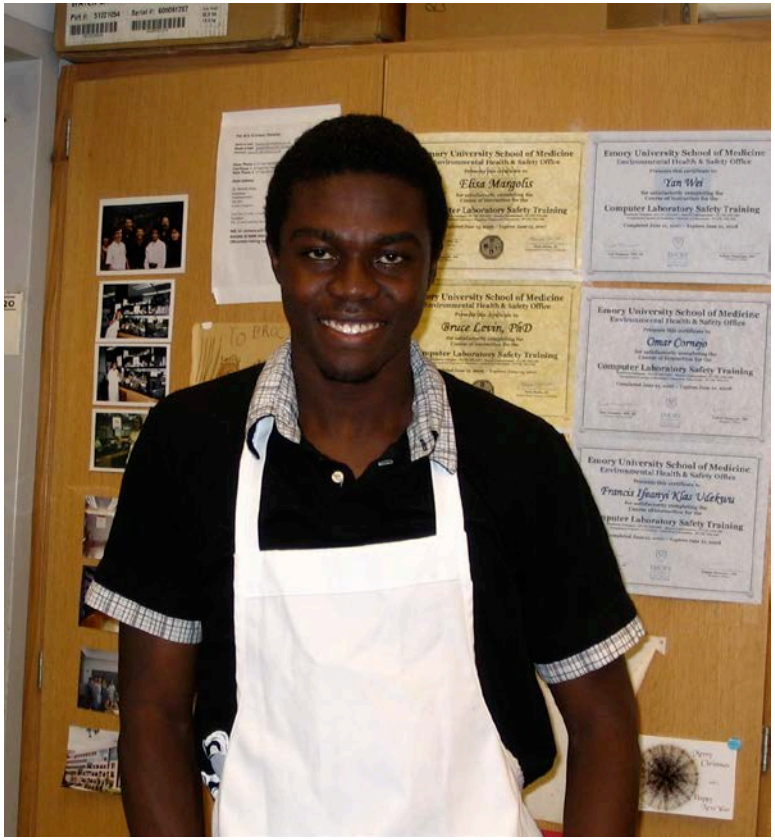
Antibiotic Treatment of Bacterial Infections: Pharmacodynamics Meets Population Dynamics Meets Immunology

Bruce R. Levin
Department of Biology
Emory University
Atlanta, GA
blevin@emory.edu
www.eclf.net

Symposium, Palmerston North, NZ October 23, 2012

Work with



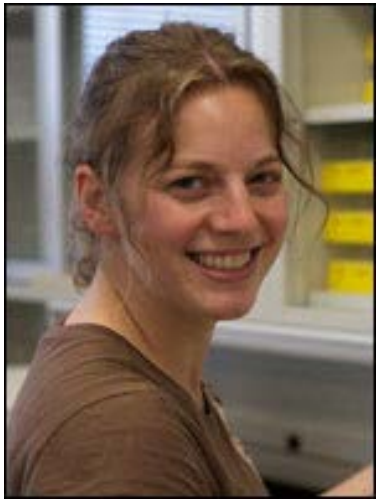
Pierre Ankomah

Rustom Antia

Immediate Motivation (inspiration?)

Andrew Read

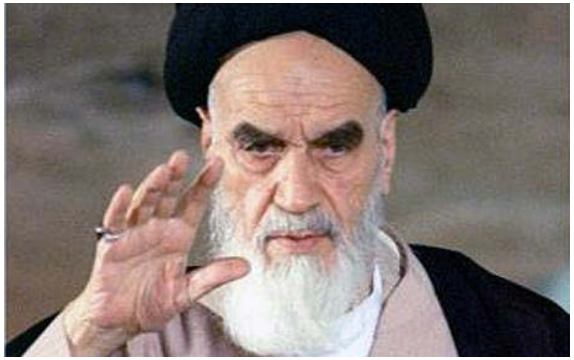
Troy Day

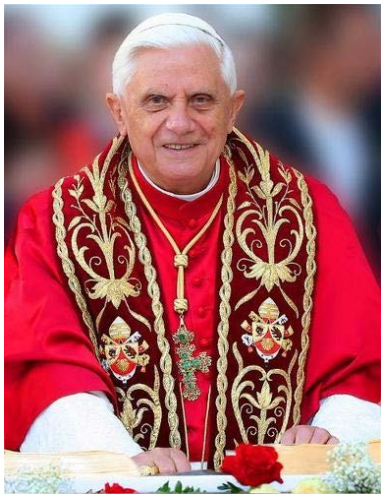


Silvie Huijben

The Evolution of drug resistance and the curious orthodoxy of aggressive chemotherapy PNAS June 28, 2011 vol. 108 no. Supplement 2 10871-10877

Defenders of Orthodoxy





OVERVIEW

- The problem/questions
- Treatment failure and the role of resistance
- The Rational Design Perspective: The PK/PD Mafia and resistance as a continuum
- Population Biologists on the case
- A mathematical model of antibiotic treatment I.
  - Pharmacodynamics meets population dynamics
  - Treatment in the absence of an immune response
- A mathematical model of antibiotic treatment II.
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  - Treatment of self-limiting infections
  - Treatment of potentially lethal infections
- Summary and Conclusions

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THE PROBLEM/GOALS

The Problem (Question)

How do we design optimal antibiotic treatment protocols?

- Choice of drugs
- Dose
- Frequency
- Term of administration

The (pretentious?) Goals

To Minimize:

- Likelihood of mortality
- Term and magnitude of morbidity
- Likelihood of relapse
- Side-effects of treatment (including collateral resistance)
- Likelihood of acquired resistance

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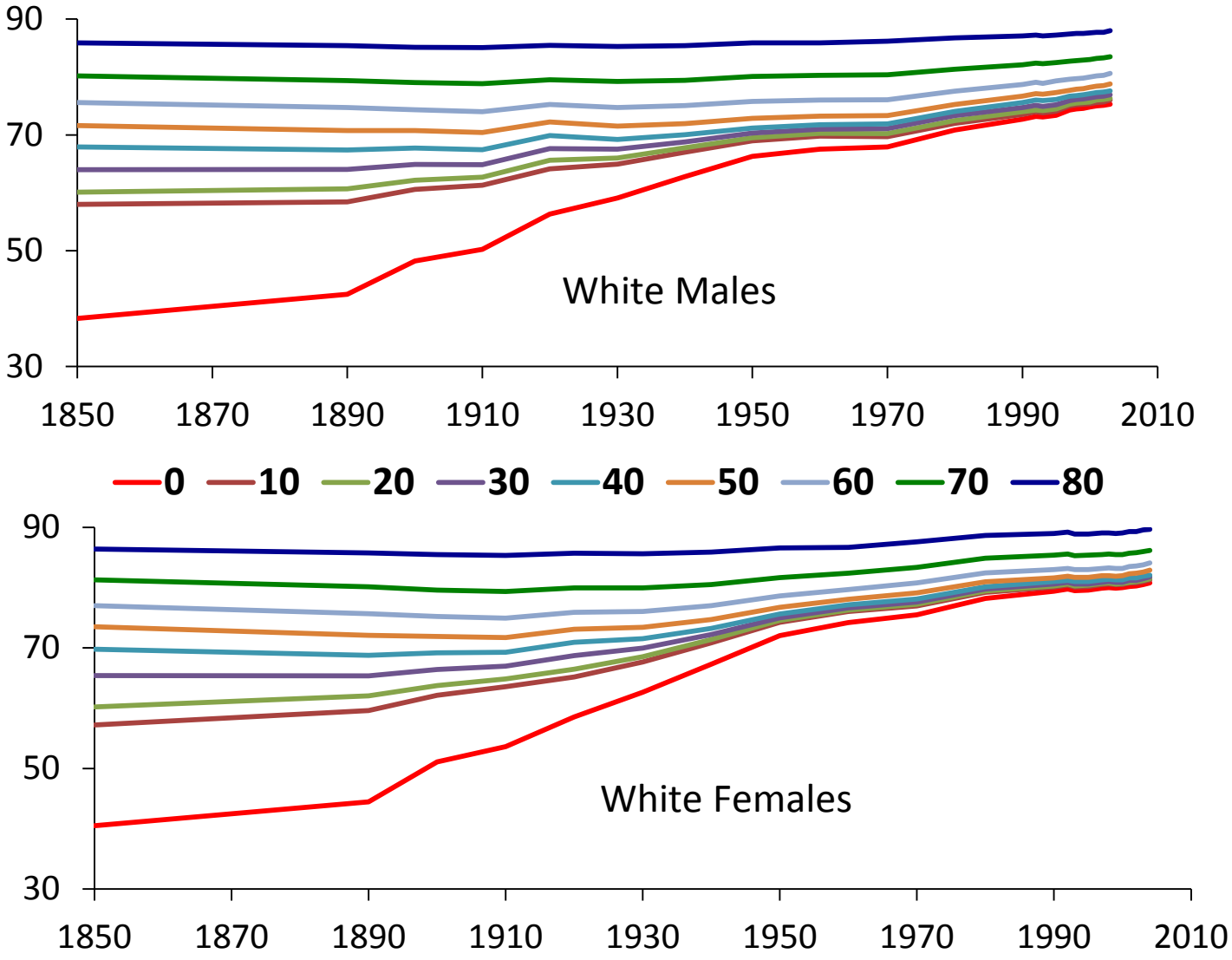
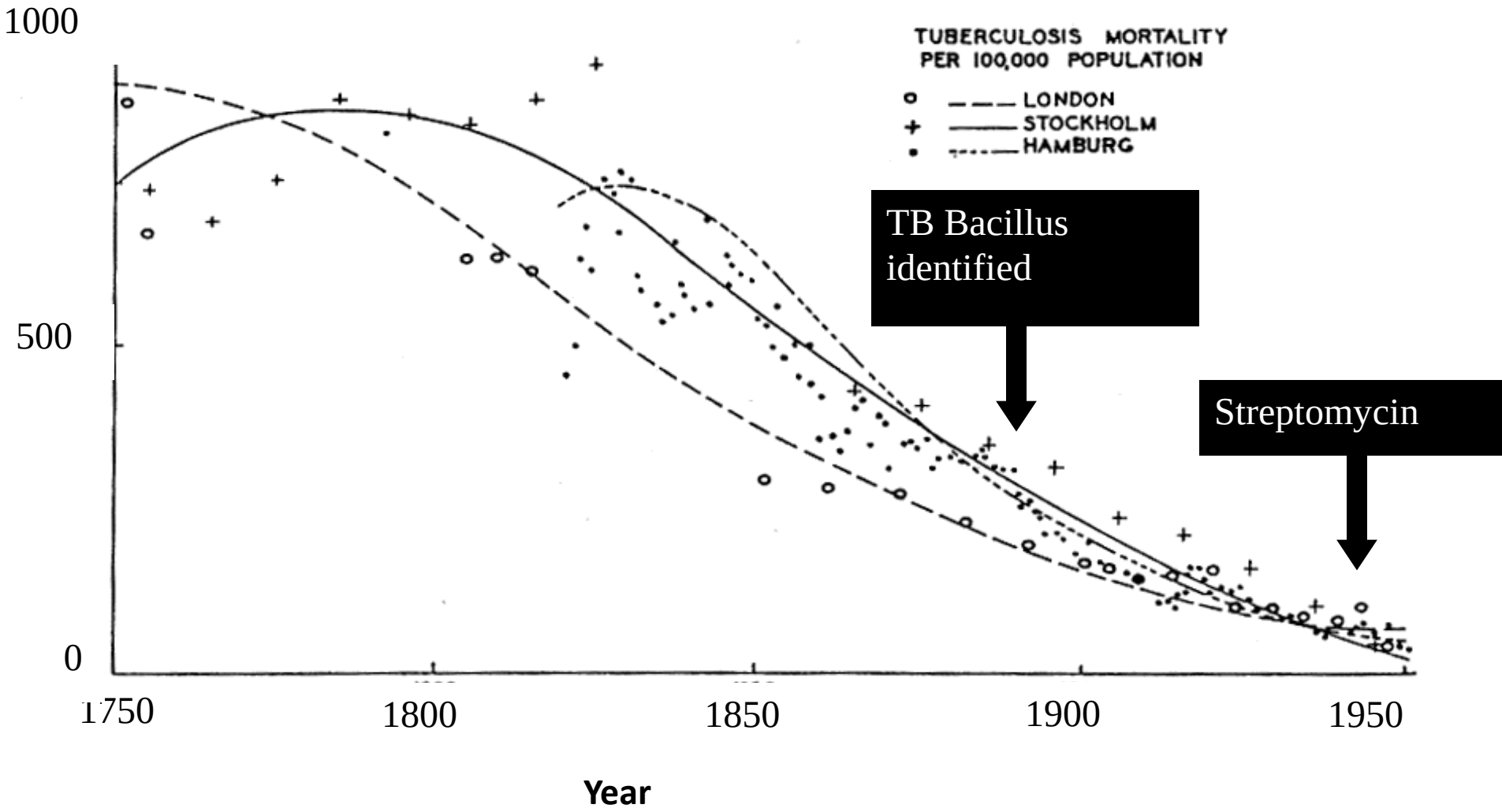
A necessary perspective on the role of antibiotics

Changes in Expected Life Span at Different Ages in the United States



National Vital Statistics Reports, vol 54., no. 19, June 28, 2006. Web: www.dhhs.gov .

Tuberculosis mortality per 100,000 per year



Death Rates for Common Infectious Diseases in the United States per 100,000 Population

	1900	1935	1970
Influenza and Pneumonia	202.2	103.9	30.9
Tuberculosis	194.4	55.1	2.6
Gastroenteritis	142.7	14.1	1.3
Diphtheria	40.3	3.1	0.0
Typhoid fever	31.3	2.7	0.0
Measles	13.3	3.1	0.0
Dysentery	12.0	1.9	0.0
Whooping Cough	12.0	3.7	0.0
Scarlet fever (including Strep. throat)	9.6	2.1	0.0
Meningococcal infections	6.8	2.1	0.3

H.F. Dowling, 1977, *Fighting Infection*, Harvard Press

Also see, McKeowen (1976) "The Role of Medicine: Dream, Mirage or Nemesis?" Princeton Univ. Press.

Antibiotic Resistance is a great career opportunity as well as a major and increasing problem.

Resistance is not the only reason antibiotic treatment fails and for some infections not the major reasons.

TREATMENT FAILURE: IT'S NOT JUST ABOUT RESISTANCE

Mortality rates of patients with bacteremic pneumococcal pneumonia

Treatment	% mortality
Symptomatic[1]	80
Specific Serum[1]	45
Penicillin[1] (1940s)	17

[1]M. Finland. Clinical Pharmacology and Therapeutics 13:469-511, 1972.

TREATMENT FAILURE: IT'S NOT JUST ABOUT RESISTANCE

Mortality rates of patients with bacteremic pneumococcal pneumonia

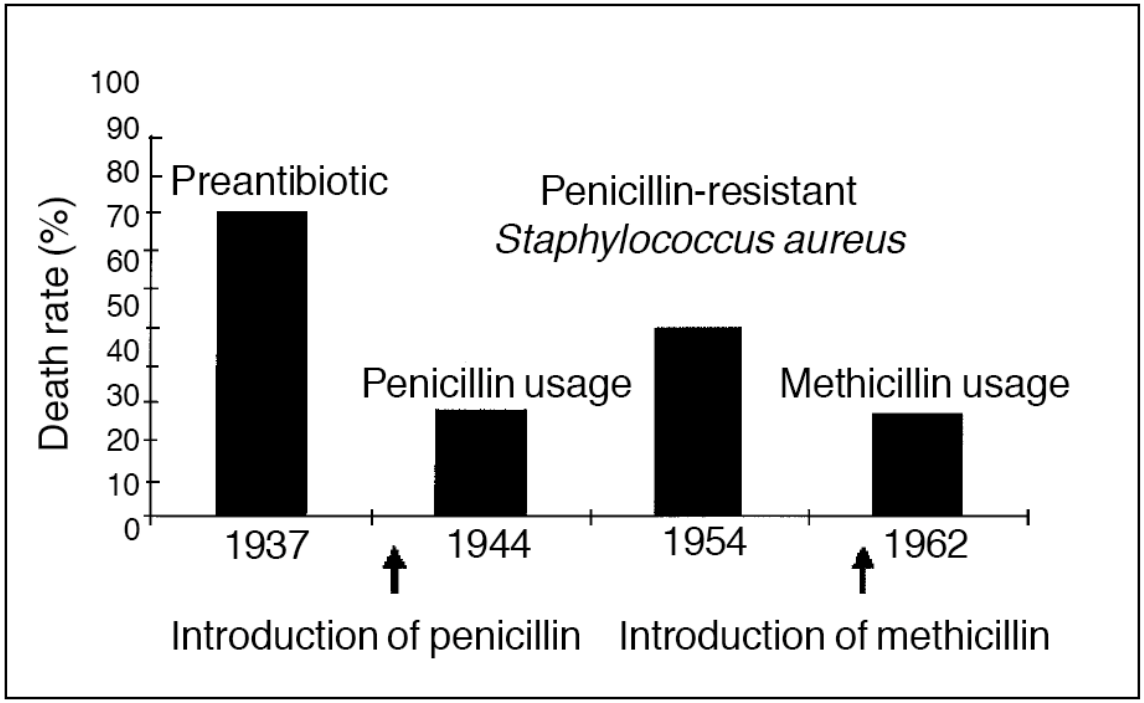
Treatment	% mortality
Symptomatic[1]	80
Specific Serum[1]	45
Penicillin[1] (1940s)	17
1995-1997[2]	12*
1998-2001[3]	17*

*Patients with resistant pneumococcus did not have a higher death rate

[1]M. Finland. Clinical Pharmacology and Therapeutics 13:469-511, 1972.
[2]Feikin, D.R., et. al. Am J Public Health 90(2): 223-9, 2000.
[3]Yu, V. L. et. al. Clin. Infect. Dis. 37(2):230-7, 2003.

TREATMENT FAILURE: IT'S NOT JUST ABOUT RESISTANCE

Death rate of staphylococcal bacteremia over time

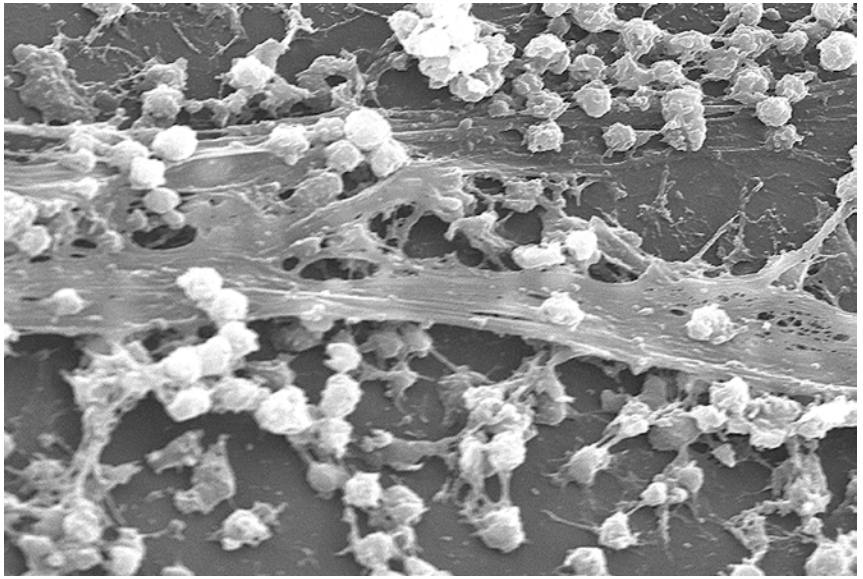


Even in the absence of resistance, a substantial fraction of treated patients die

Rubin et al. (1999) Emerg. Infect. Dis. 5:9-17

TREATMENT FAILURE: IT'S NOT JUST ABOUT RESISTANCE

- **Host-mediated Factors**
  - Age
  - Underlying Disease
  - Improper Immune Response
- **Non-inherited Resistance**
  - Persistence
  - Latency
  - Biofilms
  - Abscesses
  - Empyema



S. aureus biofilm

Levin, B.R., and Rozen, D.E. (2006) *Nat. Rev. Micro*. 4: 556-562

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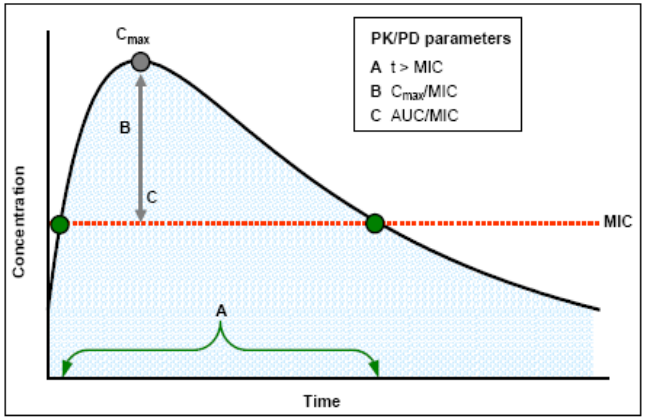
Rational Design of Antibiotic Treatment
PK/PD (PK/MIC) Indices

In vivo Pharmacodynamics (PK)

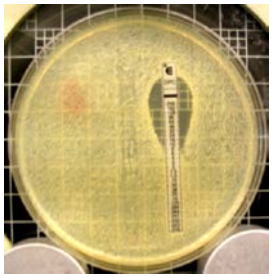
Figure 1. Illustration of the main PK/PD parameters that correlate with efficacy against extracellular infections.



A Time (t) during which the concentration remains above the minimum inhibitory concentration (MIC) of the antibiotic against the pathogen, B ratio between the peak plasma concentration (C_{max}) of the antibiotic reached in the serum and the MIC, C ratio between the 24-h area under the concentration-time curve (AUC) and the MIC.

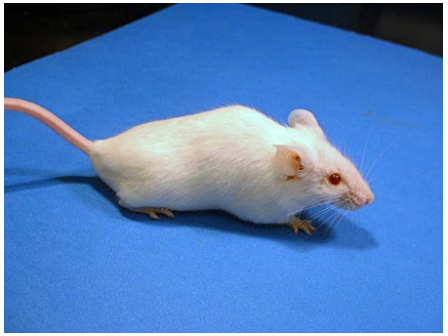
* Van Bambeke, et al (2006). *Curr Opin Drug Discov Devel* ***9, 218-30.***

In vitro pharmacodynamics (PD)



MIC estimation

Treatment experiments



"The Gold Standard"

Rational Design of Antibiotic Treatment

PK/PD Indices (PK/MIC)

In vivo Pharmacodynamics (PK)

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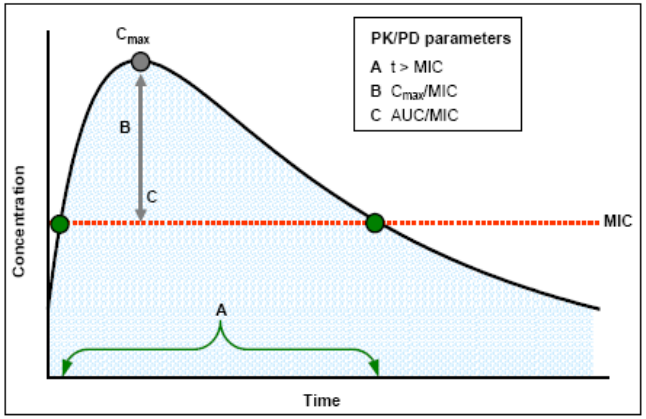

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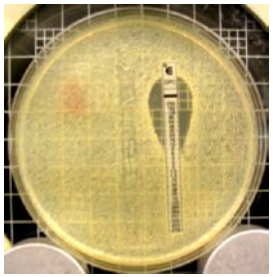
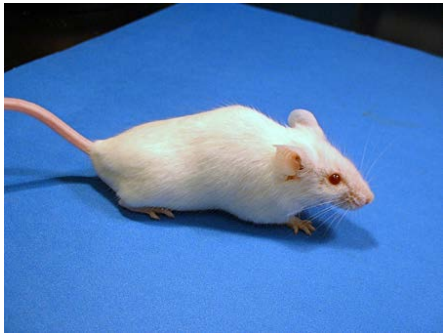


MIC estimation

Made under optimum conditions for the action of the drug: low densities of planktonic bacteria growing exponentially in medium where the antibiotic is most effective.

Treatment experiments



"The Gold Standard"

Does not account for much of what we called non-inherited resistance or other realities of bacterial infections.

RESISTANCE AS A CONTINUUM

	Streptococcus pneumoniae		*Staphylococcus aureus*	
Antibiotic	MIC-Sensitive (<µg/mL)	MIC-Resistant (> µg/mL)	MIC-Sensitive (< µg/mL)	MIC-Resistant (> µg/mL)
Levofloxacin	2	2	2	2
Vancomycin	2	2	2	2
Azithromycin	0.25	0.5	1	2
Tetracycline	1	2	1	2
Linezolid	2	4	4	4
Rifampicin	0.06	0.5	0.06	5

EUCAST
EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

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The *Ec*LF Antibiotic Treatment and Resistance Collective


Roland Regoes


Camilla Wiuff


Klas Udekwu


Nick Parrish


Amoolya Singh


Kim Garner


Renata Zappala


Pierre Ankomah


Pål Johnsen


Danny Rozen


Ellie Margolis


Amy Kirby


Marc Lipsitch


Nina Walker


Fernando Baquero


Paul Johnson


Bruce Levin

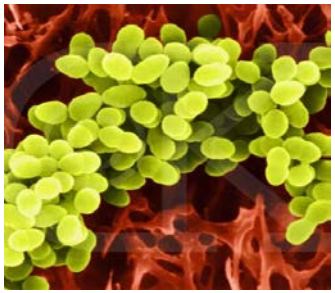
APPROACH: IMPROVING TREATMENT PROTOCOLS

Theory
Mathematical and computer simulation models

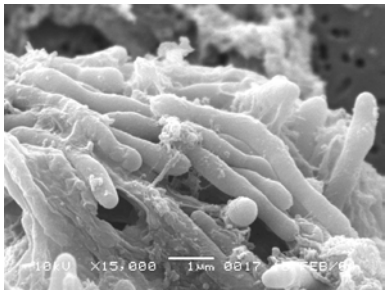
Experiments
Flasks, chemostats, bacteria and antibiotics

"All models are wrong, some are useful"

George Box



Staphylococcus aureus



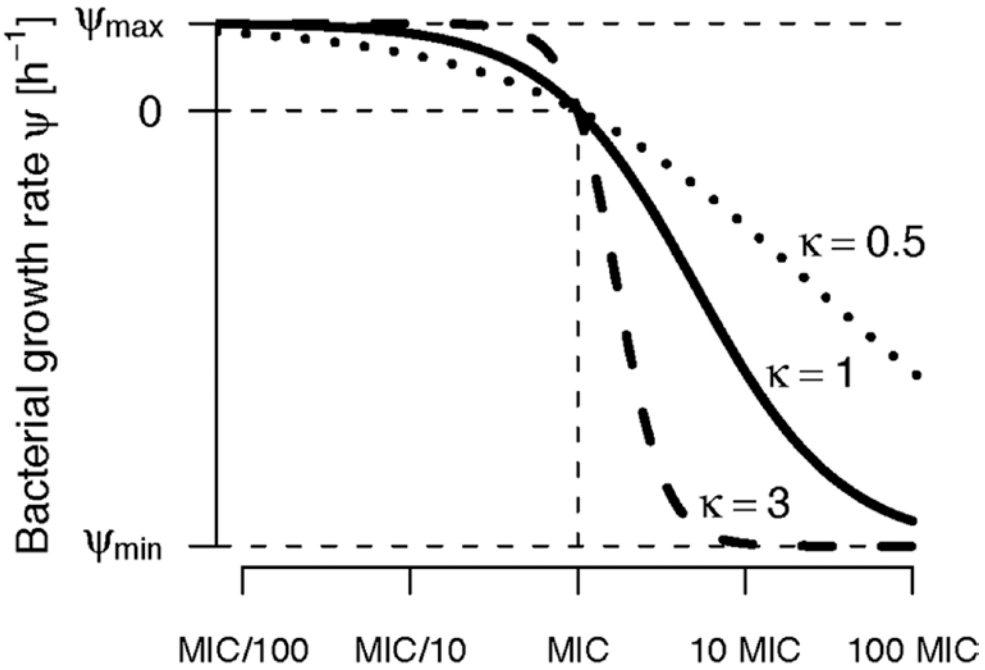
Mycobacterium marinum

"All model systems are wrong, some are useful"

Friendly amendment

PHARMACODYNAMICS: THE HILL FUNCTION APPROACH

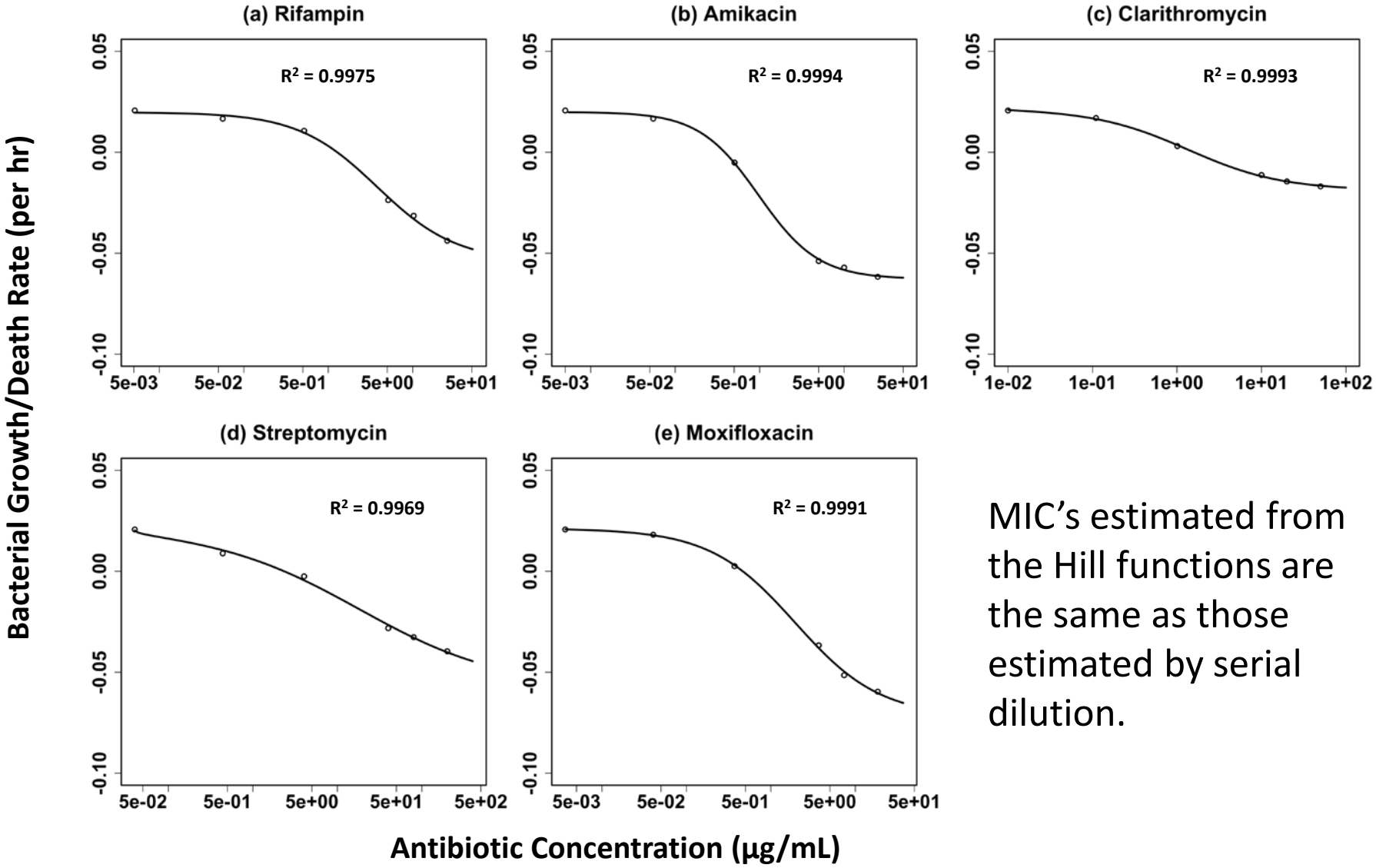
$$\psi(A_i) = \psi_{\max} - \mathrm{H}_i(A_i) \qquad \mathrm{H}_i(A_i) = \left[\frac{\psi_{\max} - \psi_{\min(i)} \left(\frac{A_i}{zMIC} \right)^{\mathrm{K}}}{\left(\frac{A_i}{zMIC} \right)^{\mathrm{K}} - \left(\frac{\psi_{\min(i)}}{\psi_{\max}} \right)} \right]$$



A – antibiotic concentration
ψ_{MAX} – Maximum growth rate
ψ_{MIN} - Minimum growth rate (<0)
zMIC – Minimum inhibitory concentration
k - Hill coefficient, shape parameter

Regoes, R.R. et al. *Antimicro Agents Chemother* **2004**.

FIT OF HILL FUNCTIONS FOR DIFFERENT ANTIBIOTICS – *M. marinum*

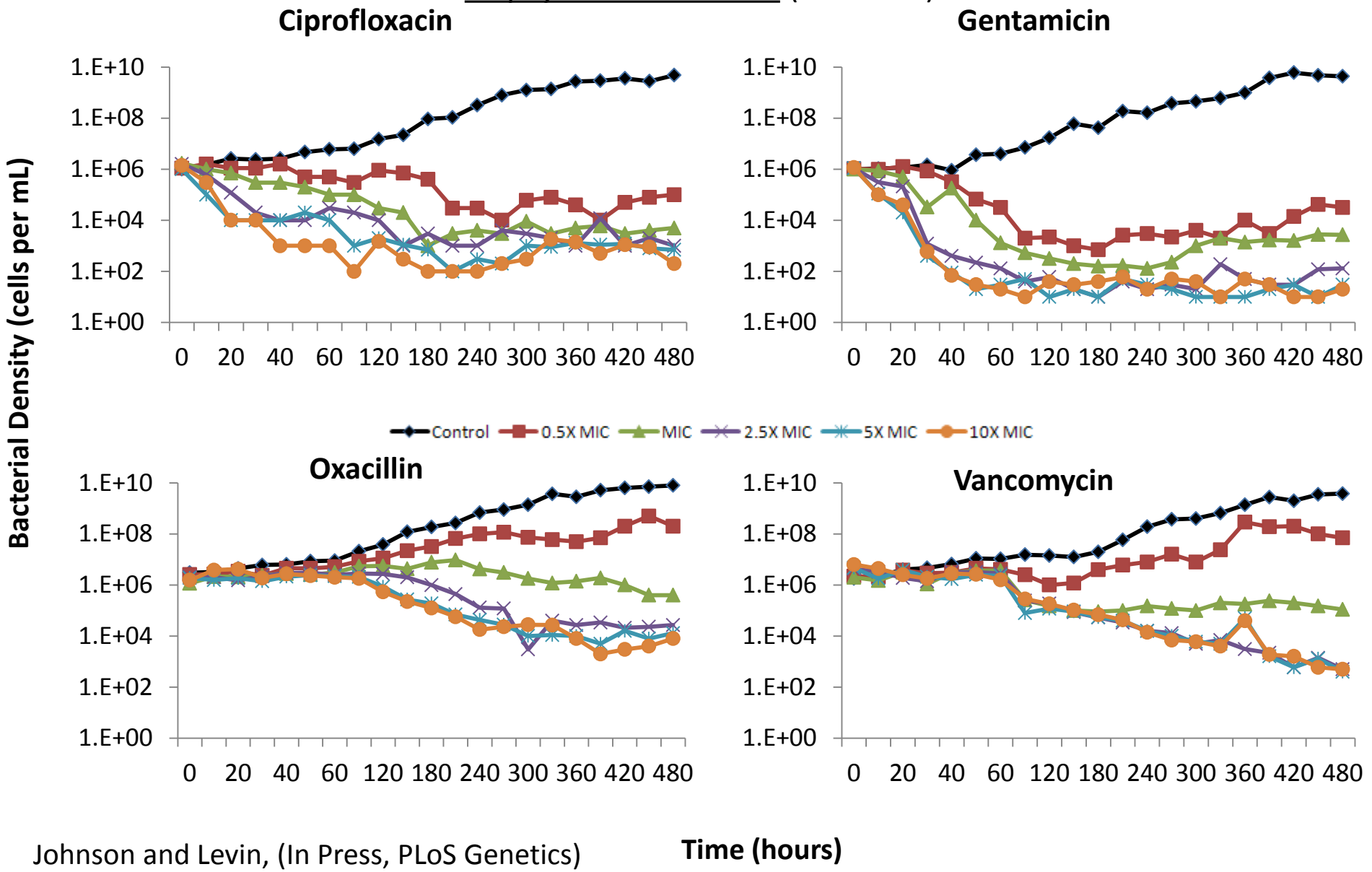


MIC's estimated from the Hill functions are the same as those estimated by serial dilution.

Ankomah, P, and B.R. Levin (2012)*: PLoS Pathog* 8(1): e1002487. doi:10.1371/journal.ppat.1002487

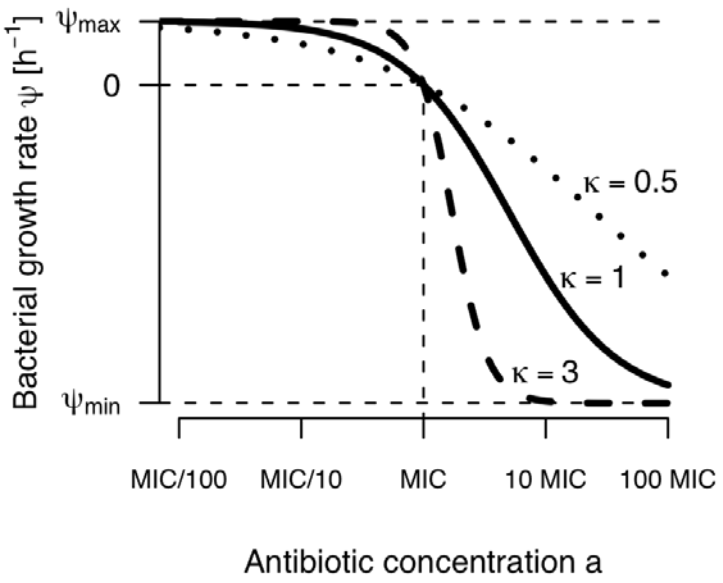
PERSISTENCE – PHENOTYPIC RESISTANCE

Staphylococcus aureus (Newman)

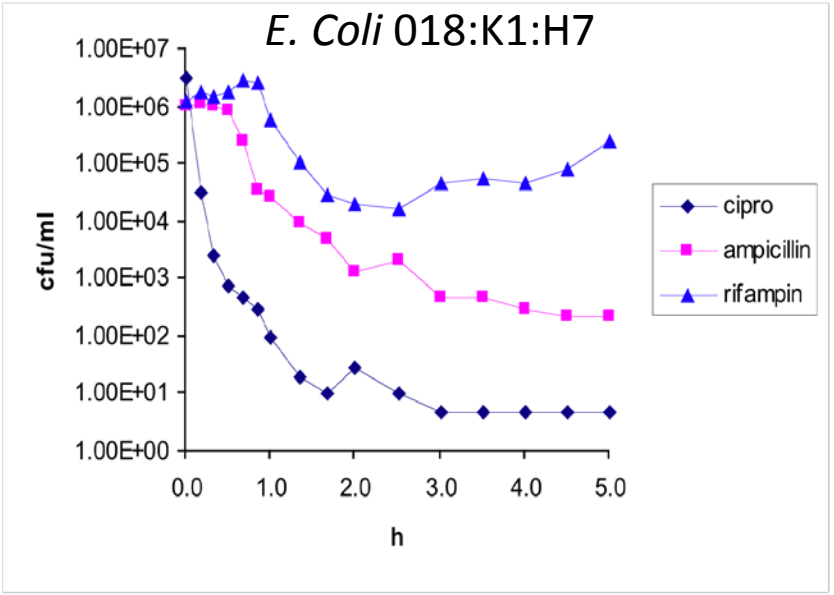


Johnson and Levin, (In Press, PLoS Genetics)

There's more to antibiotic pharmacodynamics than MICs



Shape



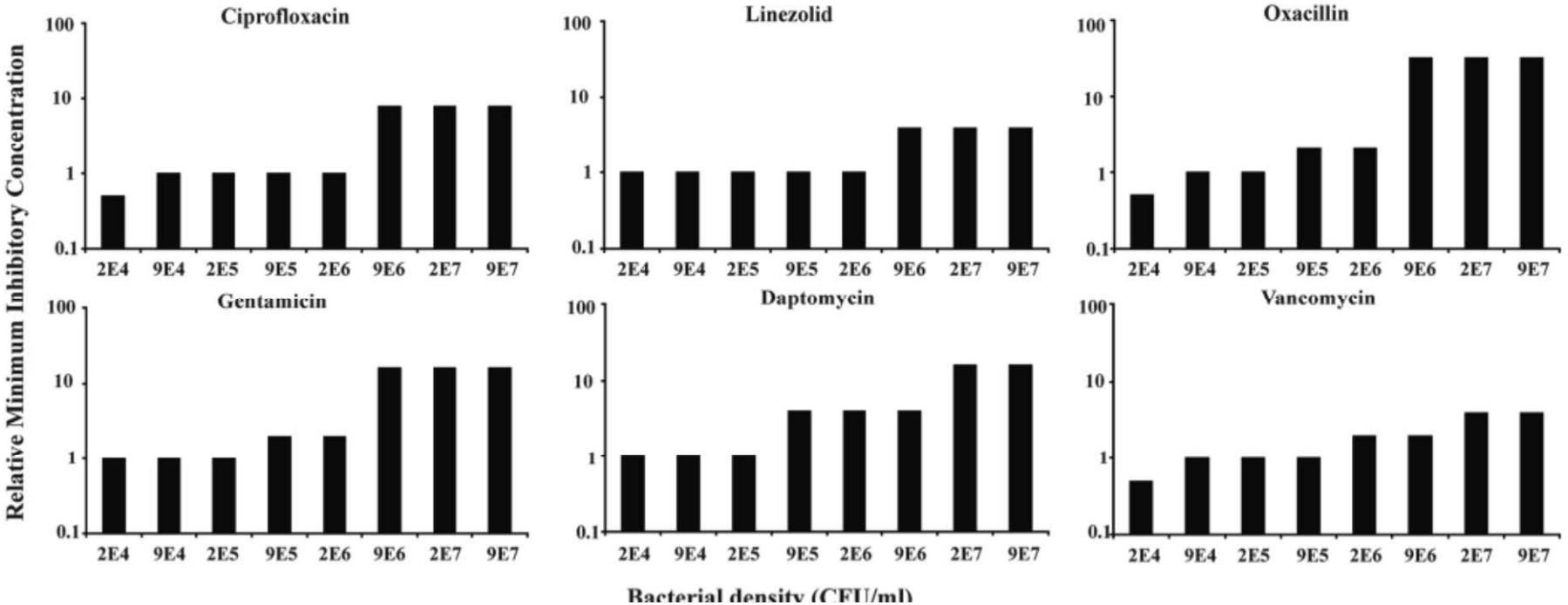
Persistence

Regoes, R., C. Wiuff, R. Zappala, K.N. Garner, F. Baquero and B.R. Levin 2004 Pharmacodynamic functions: a multi-parameter approach to the design of antibiotic treatment regimens. *Antimicrobial Agents and Chemotherapy 48: 3670-3676*

Wiuff, R. M. Zappala, R. R. Regoes, K. N. Garner, F. Baquero, B. R. Levin 2005 Phenotypic tolerance: antibiotic enrichment of non-inherited resistance in bacterial populations. *Antimicrobial Agents and Chemotherapy* 49: 1483-1494

MICs increase with density

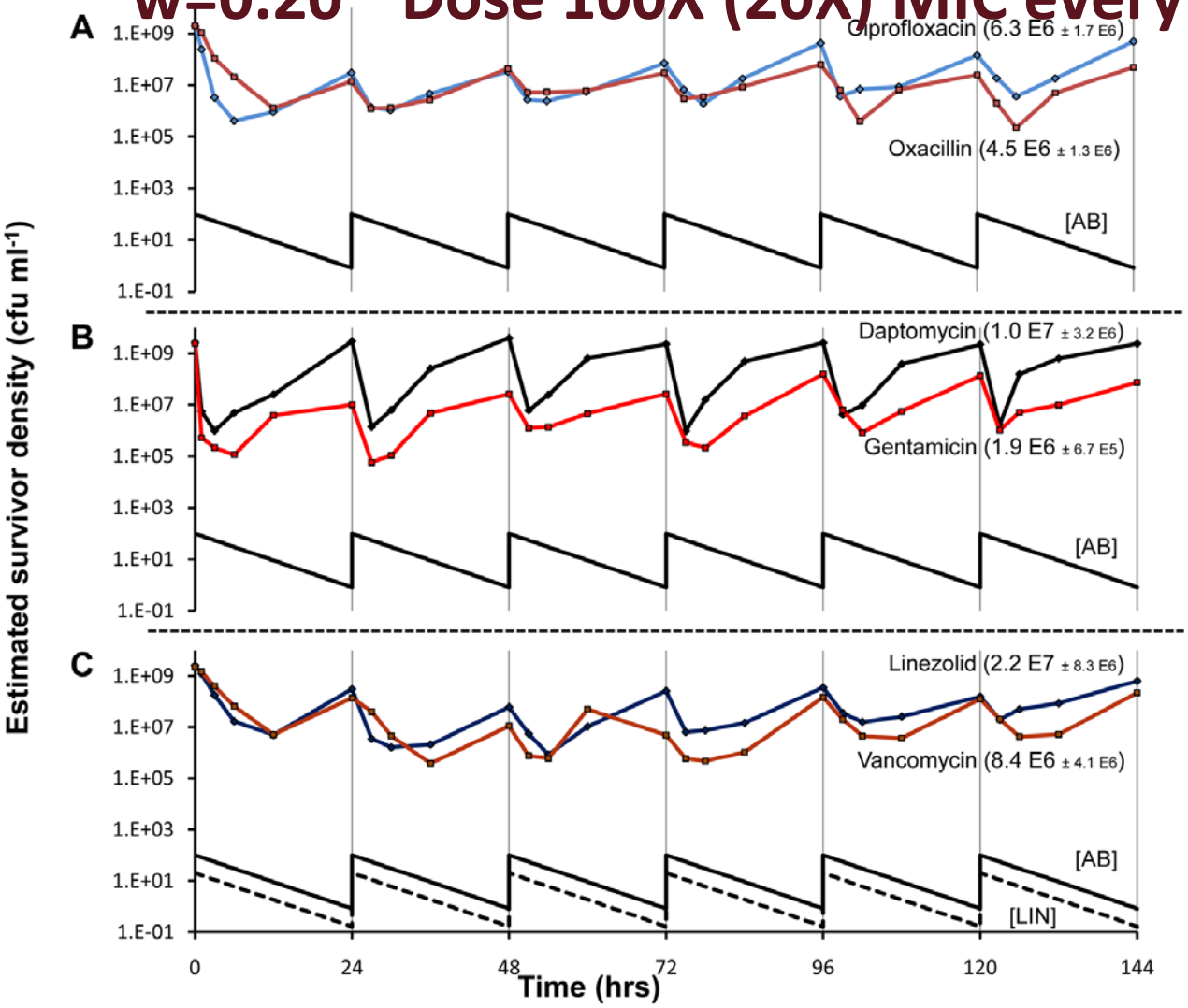
Staphylococcus aureus PS80



Estimated MICs relative to the MIC at $2x10^5$ with different inoculum densities. These estimates were obtain from CFU data; when the viable cell density at 18 hours was approximately equal to that in the initial inoculum

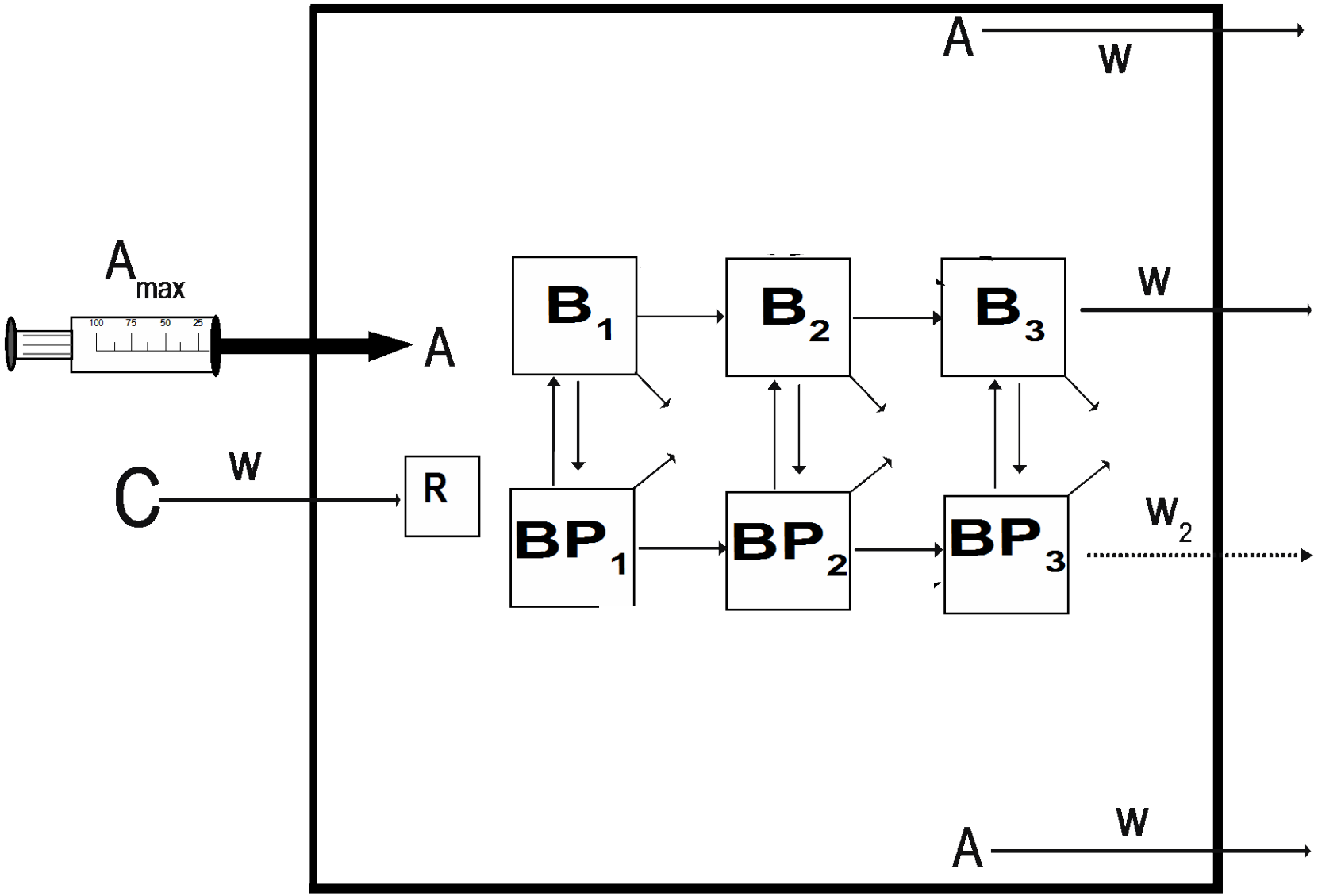
Udekwu, K, N. Parrish, P. Ankomah, F. Baquero and BR Levin (2009) Functional Relationship Between Cell Density and the Efficacy of Antibiotics. Journal of Antimicrobial Chemotherapy, 163:745-757.

Chemostat Treatment Experiments
w=0.20 Dose 100X (20X) MIC every 24 hours



Udekwu, K.I. and B.R. Levin (2012). *Staphylococcus aureus* in continuous culture: a tool for the rational design of antibiotic treatment protocols. PLoS One July 2012 | Volume 7 | Issue 7 | e38866

A MODEL FOR ANTIBIOTIC TREATMENT (NO HOST DEFENSES)



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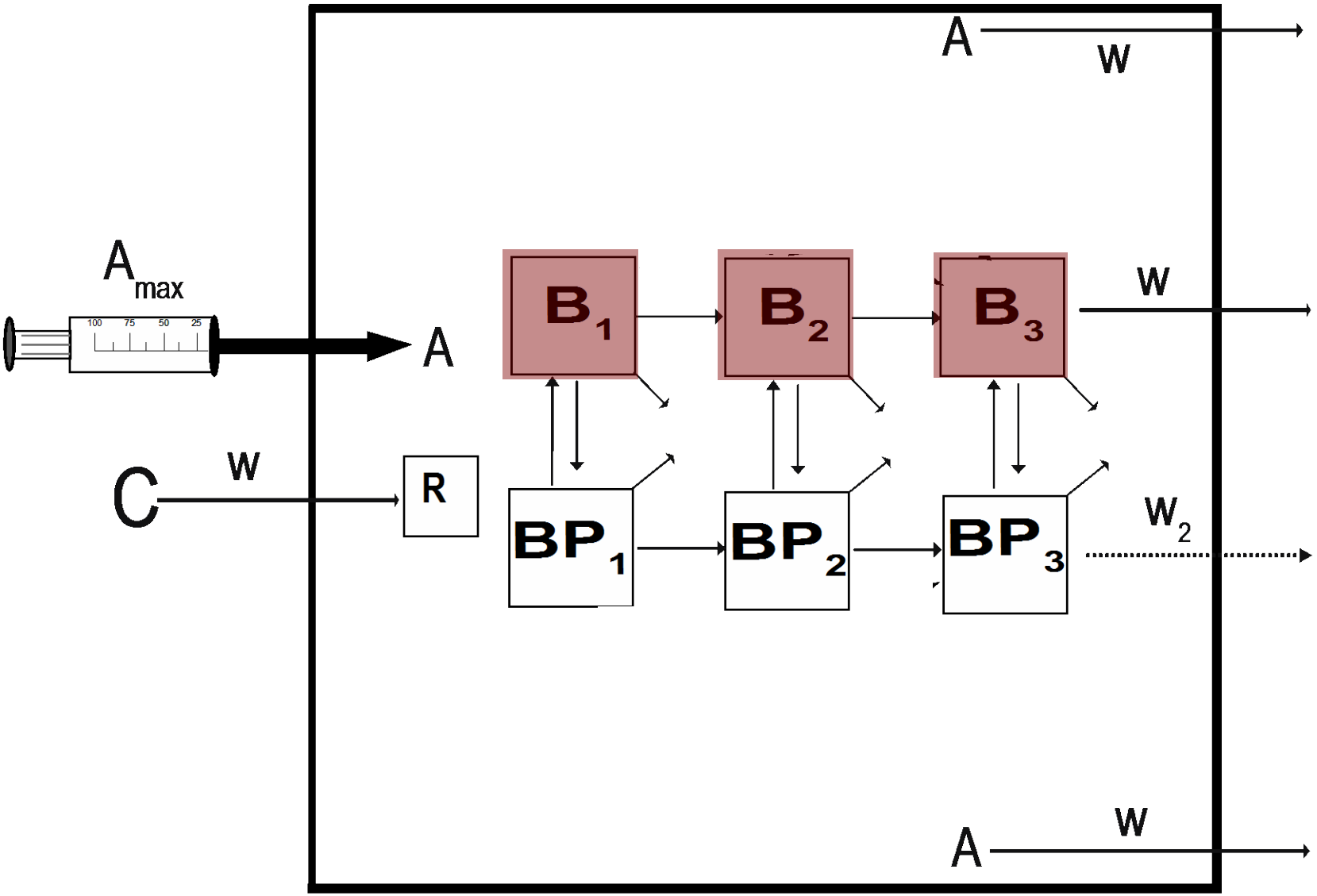
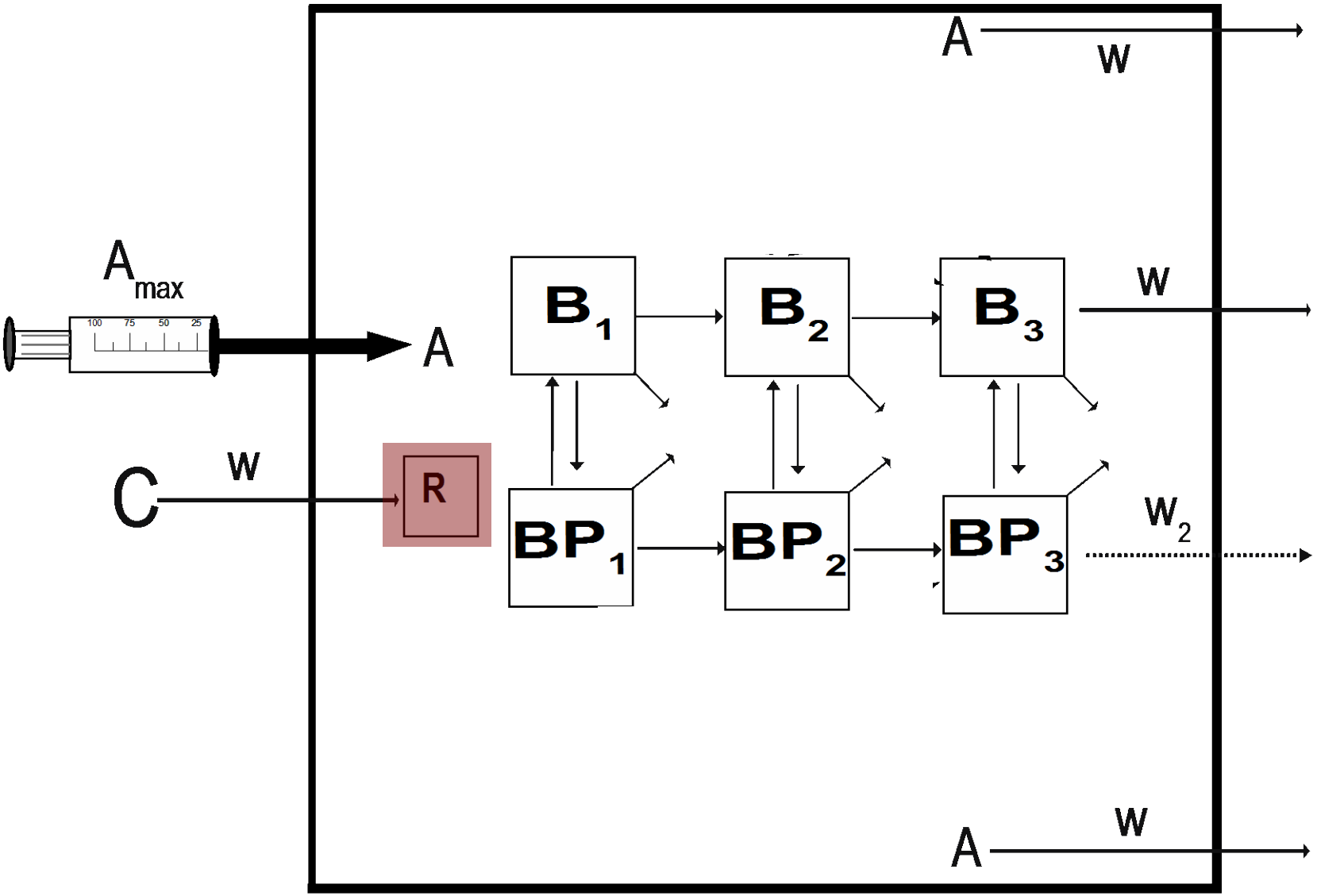
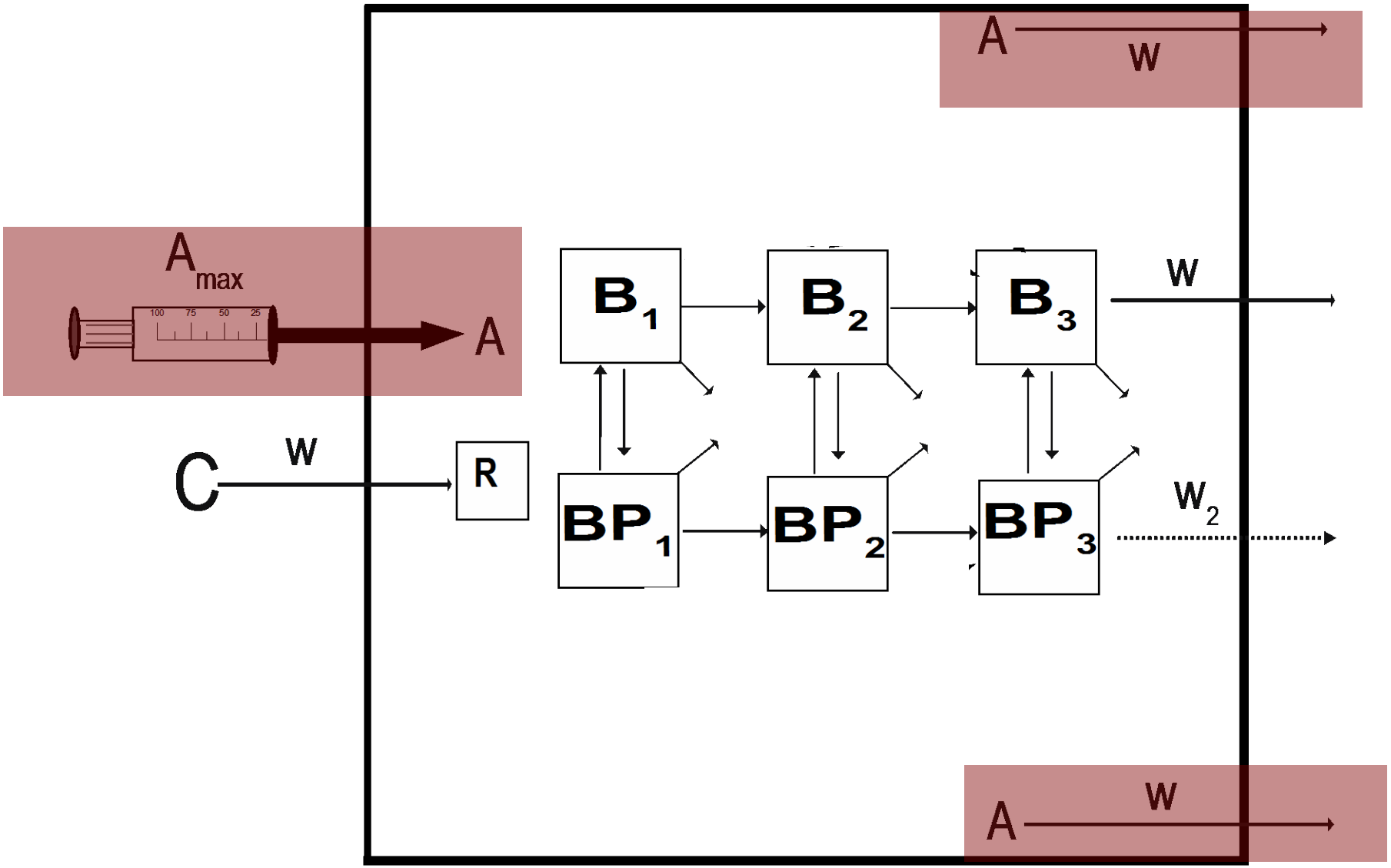


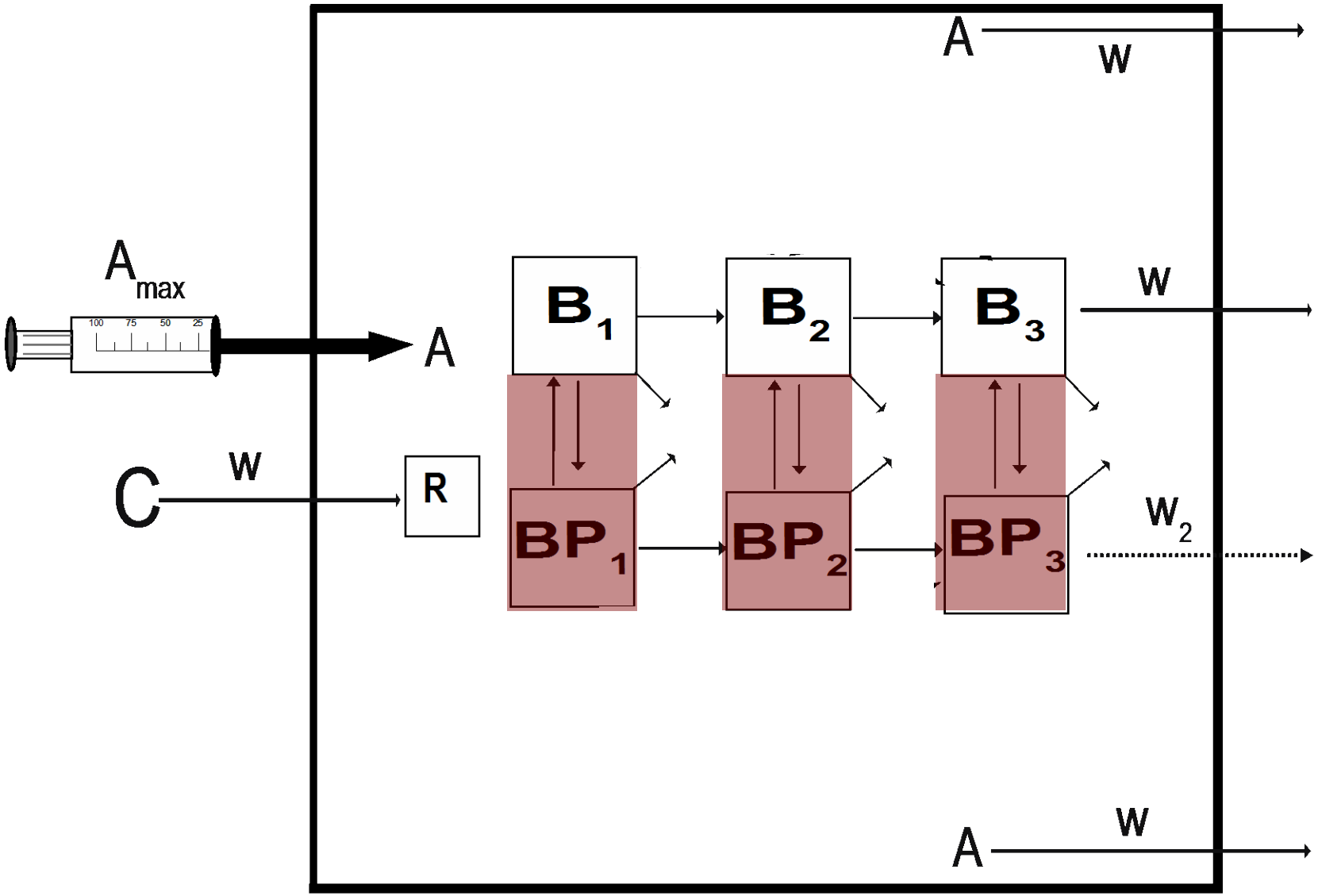
THE MODEL: RESOURCE-MEDIATED GROWTH



THE MODEL: PD AND PK



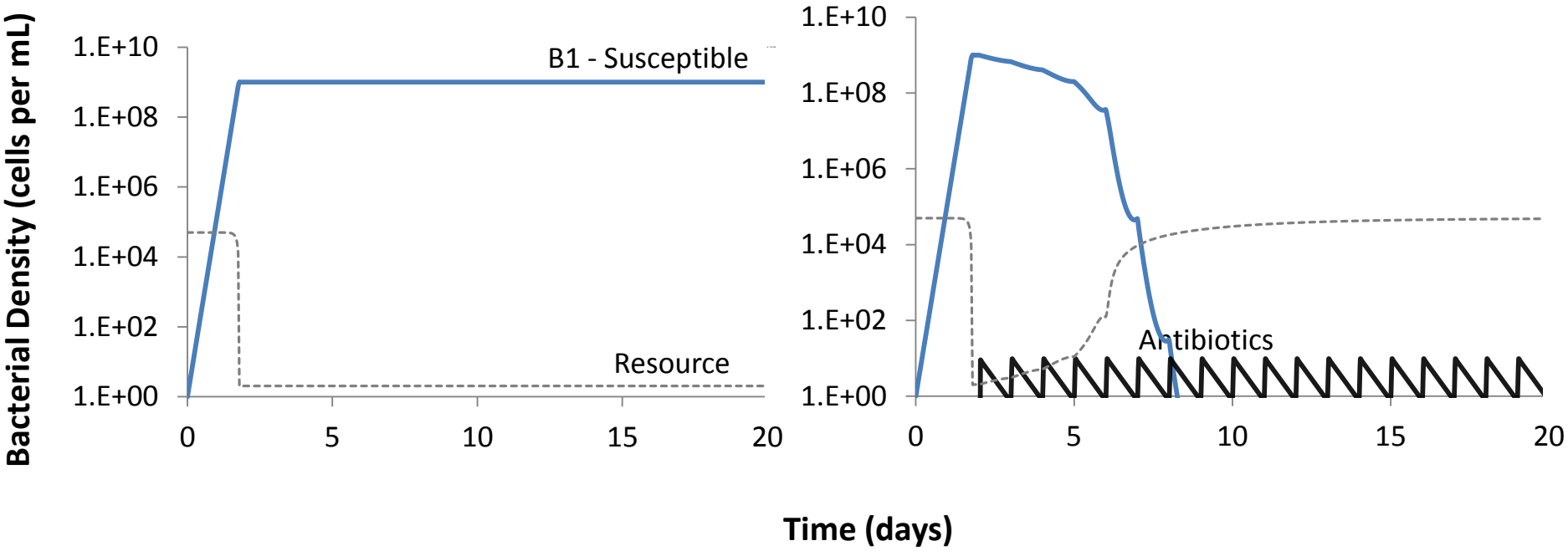
THE MODEL: PHENOTYPICALLY-RESISTANT SUBPOPULATIONS



Simulation Results

BACTERIAL POPULATION DYNAMICS

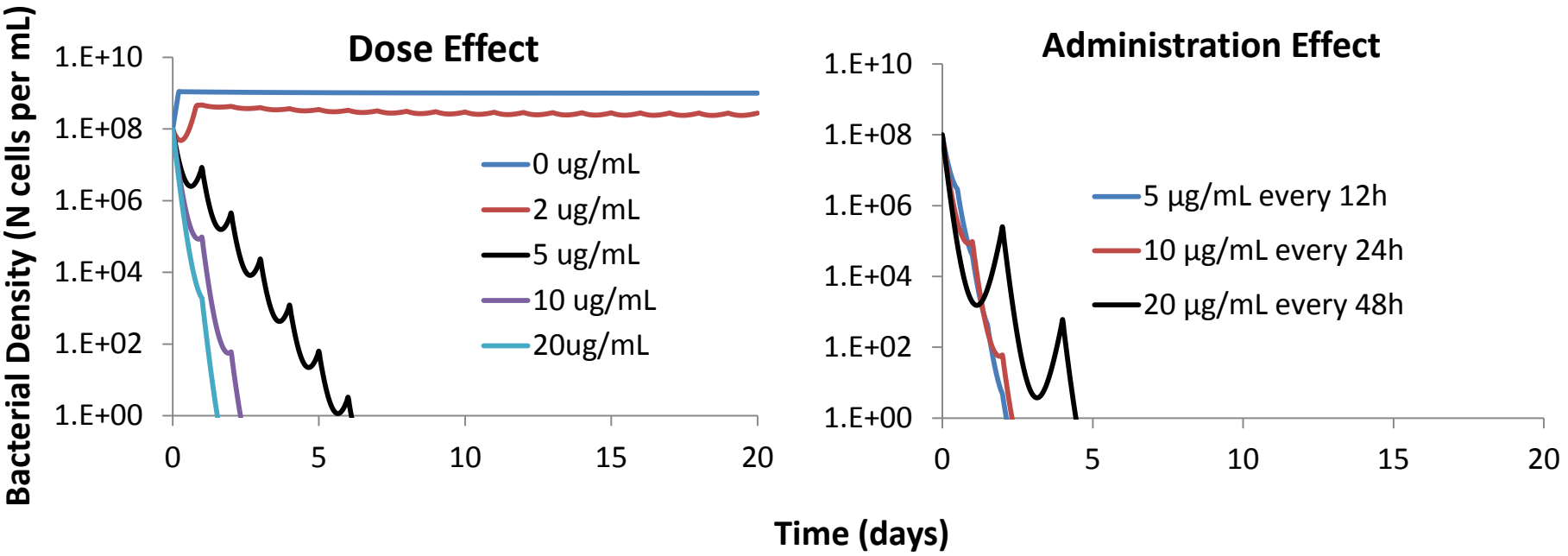
a. No Persisters, No Resistant Bacteria



- Bacterial Growth can be resource-limited
- Treatment commences at high bacterial densities

EFFECT OF DOSE AND ADMINISTRATION FREQUENCY

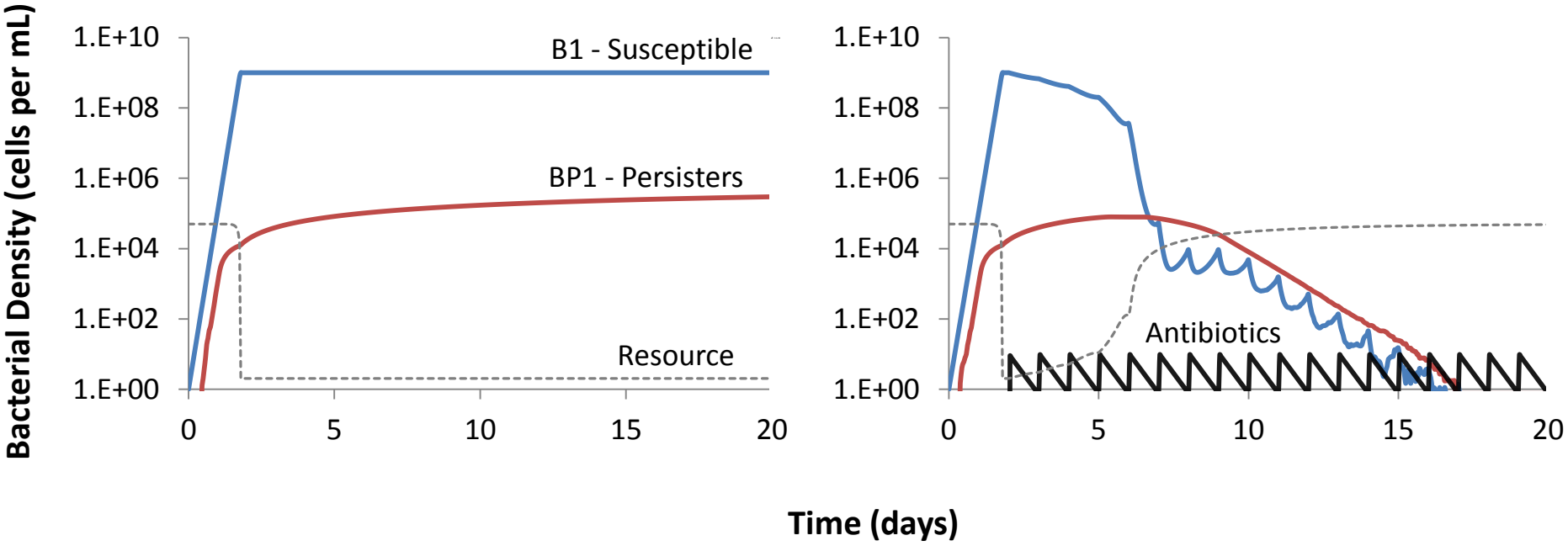
a. No Persisters, No Resistant Bacteria



- Increasing dose increases rate of clearance
- Increasing frequency of treatment does likewise (PK effect)
- Effects of increasing dose plateau (Hill Function Phenomenon)

BACTERIAL POPULATION DYNAMICS

b. Persisters, No Resistant Bacteria



- Persisters can substantially impact cidal dynamics

EFFECT OF DOSE AND ADMINISTRATION FREQUENCY

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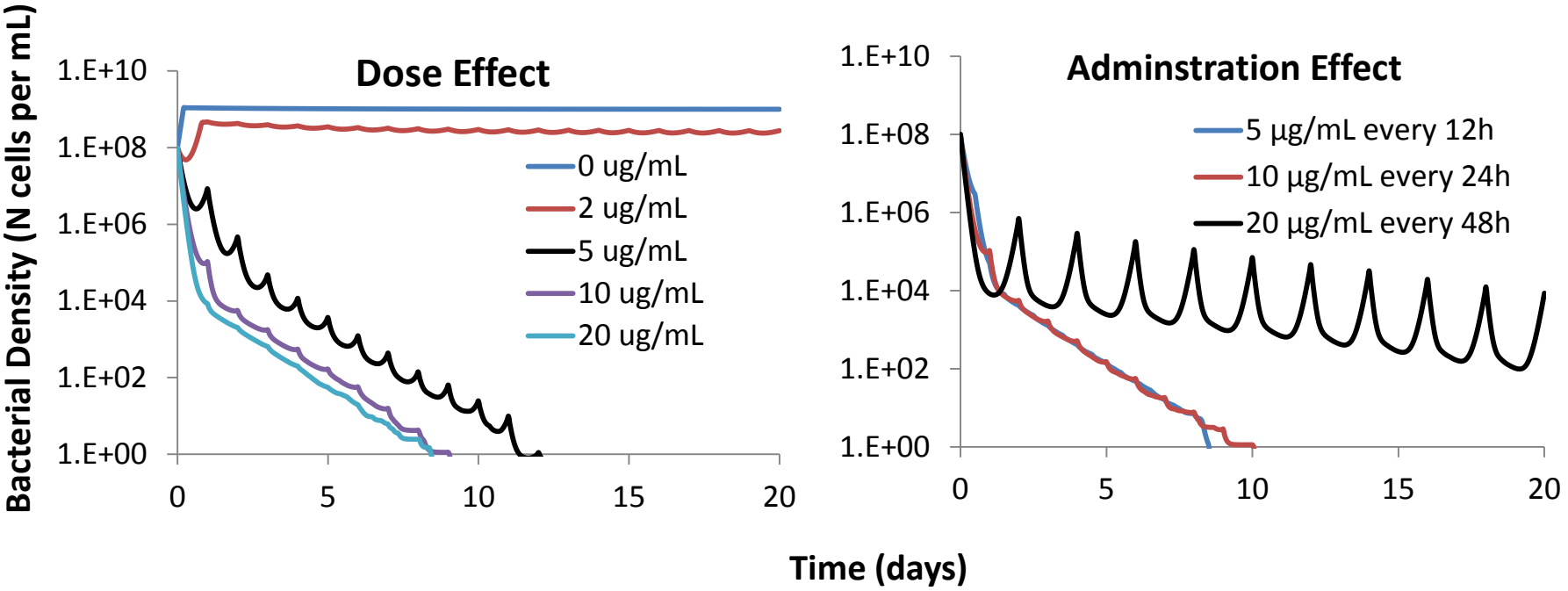
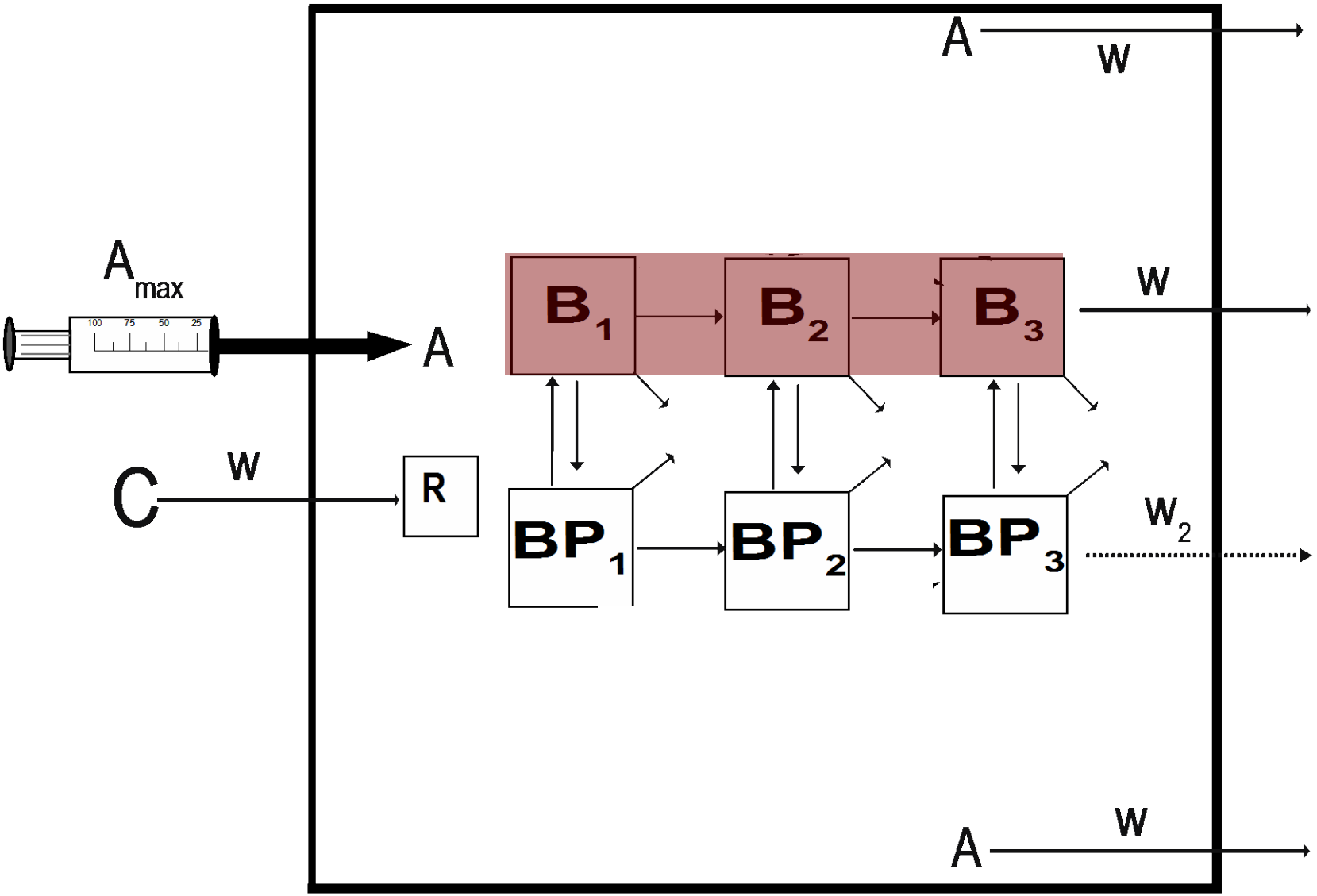


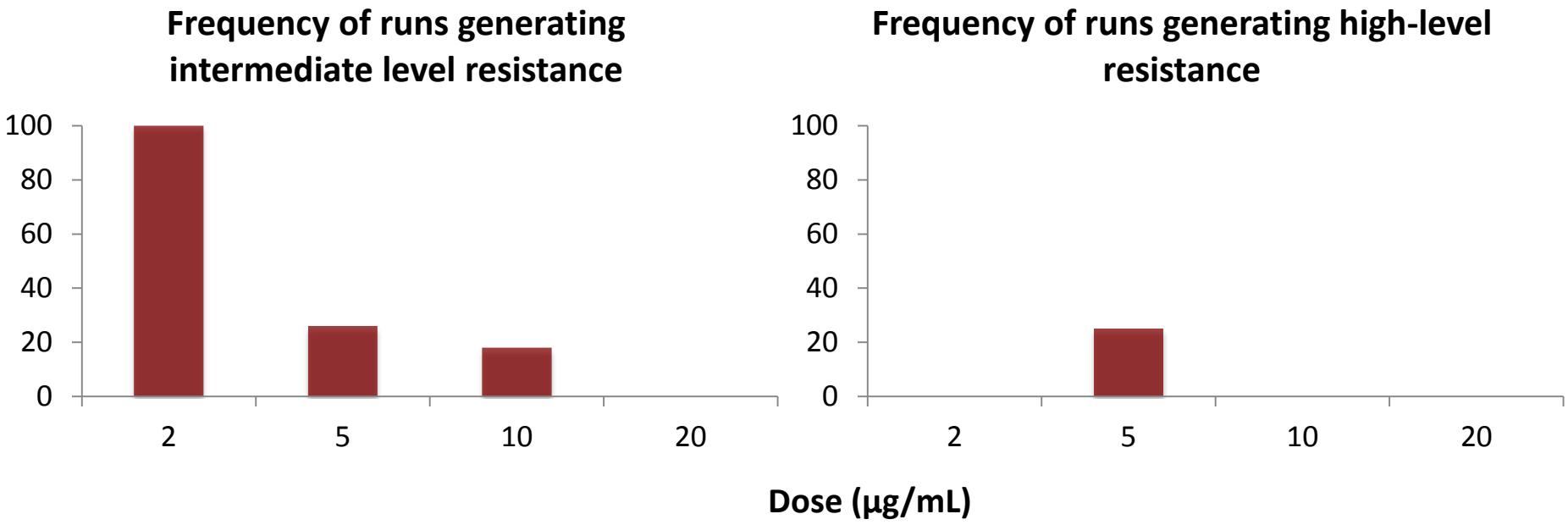
- Persisters increase the time to clearance
- Administering doses at certain frequencies can substantially lengthen the term of therapy

ACQUIRED RESISTANCE



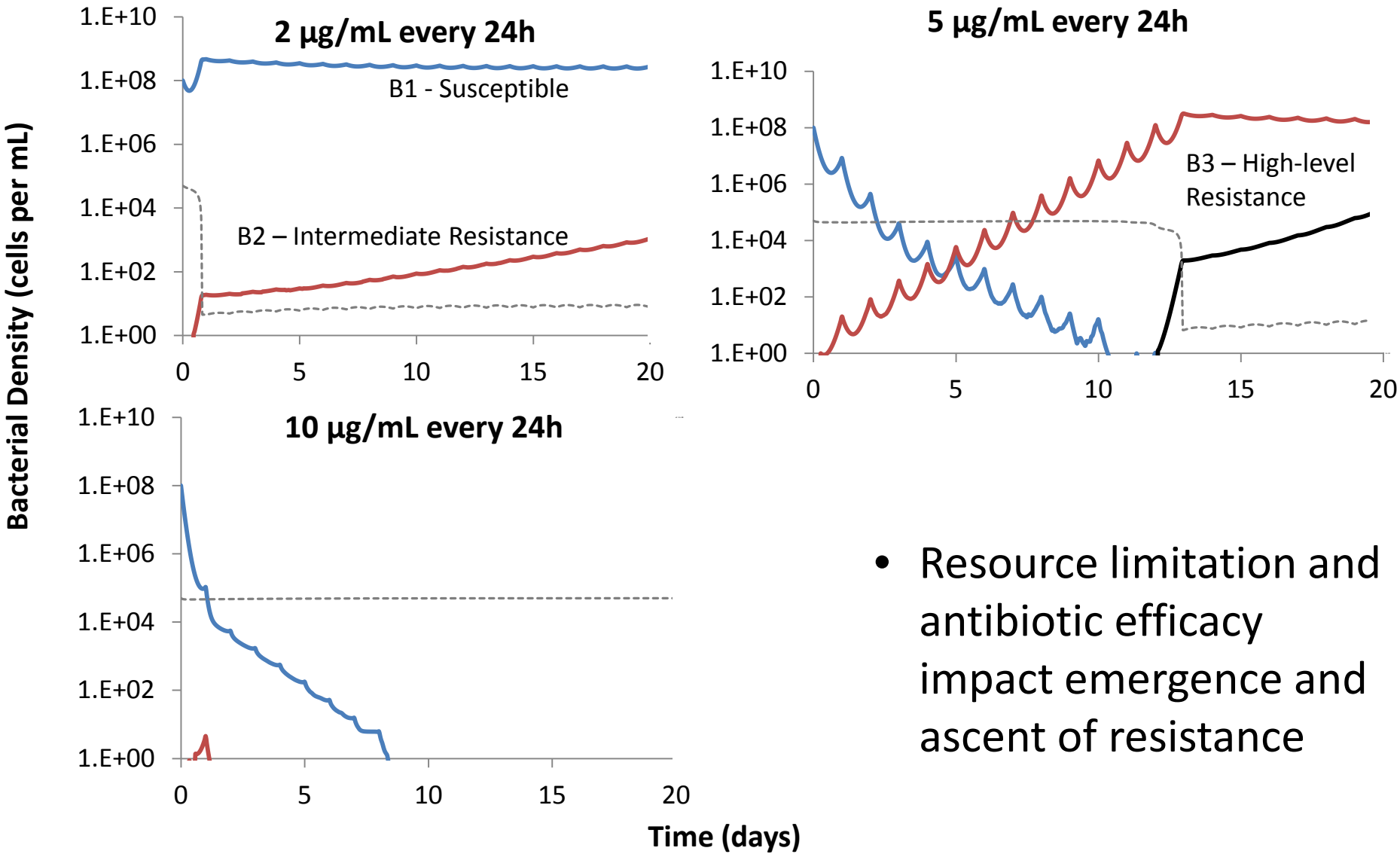
DOSE EFFECT: ACQUIRED RESISTANCE

Simulation Results –Emergence of Intermediate and High level resistance in 100 independent runs



- Intermediate-level resistance: increasing dose reduces the likelihood of resistance emerging
- High-level resistance: generated by some regimens

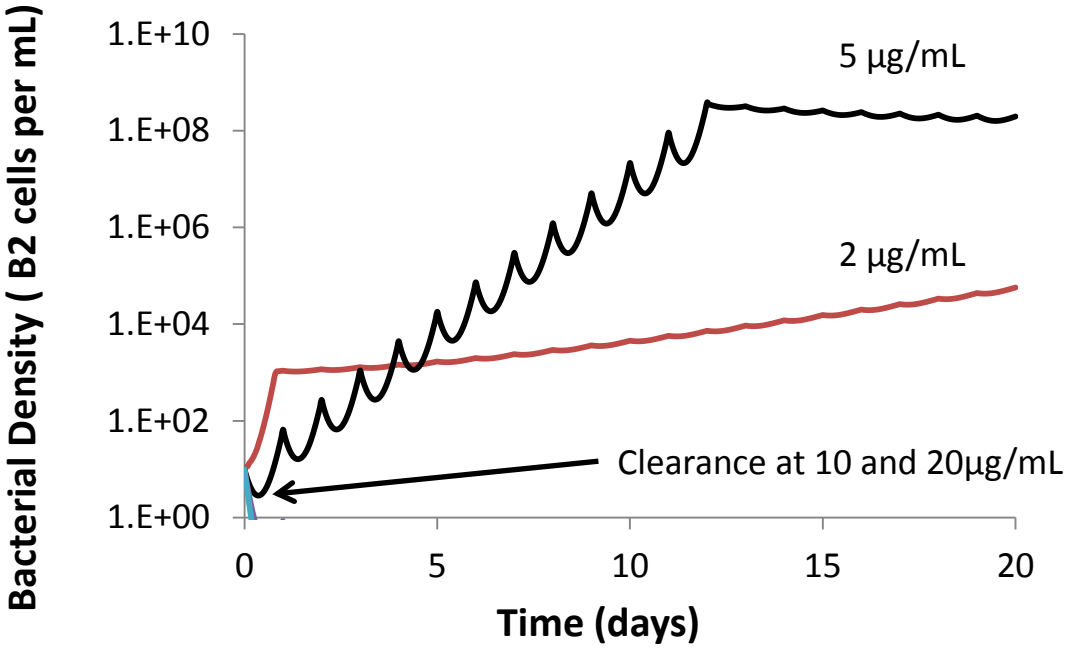
EFFECT OF RESOURCE LIMITATION ON GENERATION OF RESISTANCE



- Resource limitation and antibiotic efficacy impact emergence and ascent of resistance

POPULATION DYNAMICS OF BACTERIA

c. Pre-existing minority population with intermediate-level resistance

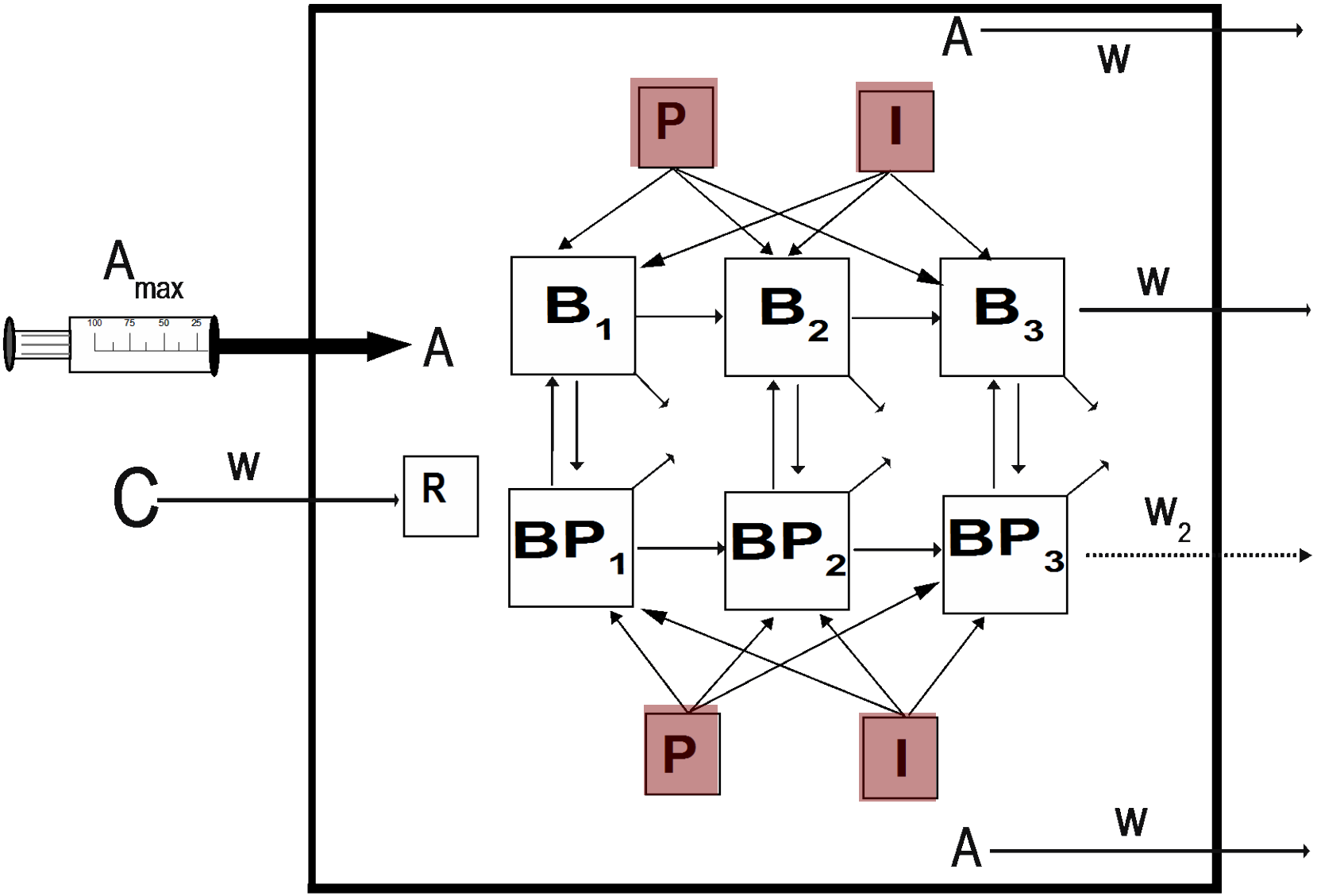


• The dose or the frequency of administration of the drug can prevent the emergence of high-level resistance.

OVERVIEW

- The problem/questions
- Treatment failure and the role of resistance
- The Rational Design Perspective: The PK/PD Mafia and resistance as a continuum
- Population Biologists on the case
- A mathematical model of antibiotic treatment I.
  - Pharmacodynamics meets population dynamics
  - Treatment in the absence of an immune response
- **A mathematical model of antibiotic treatment II.**
  - **Pharmaco- and Population- dynamics meet immunology**
  - **Treatment of self-limiting infections**
  - **Treatment of potentially lethal infections**
- Summary and Conclusions

A MODEL FOR ANTIBIOTIC TREATMENT(+ A HOST IMMUNE RESPONSE)



IMMUNOLOGY AS TWO DIFFERENTIAL EQUATIONS

a. Innate Immune response

$$\frac{dP}{dt} = \eta(P_{MAX} - P)\left(\frac{N}{N + \sigma_P}\right) - \gamma P$$

b. Adaptive immune response

$$\frac{dI}{dt} = \alpha I\left(\frac{N}{N + \sigma_I}\right)$$

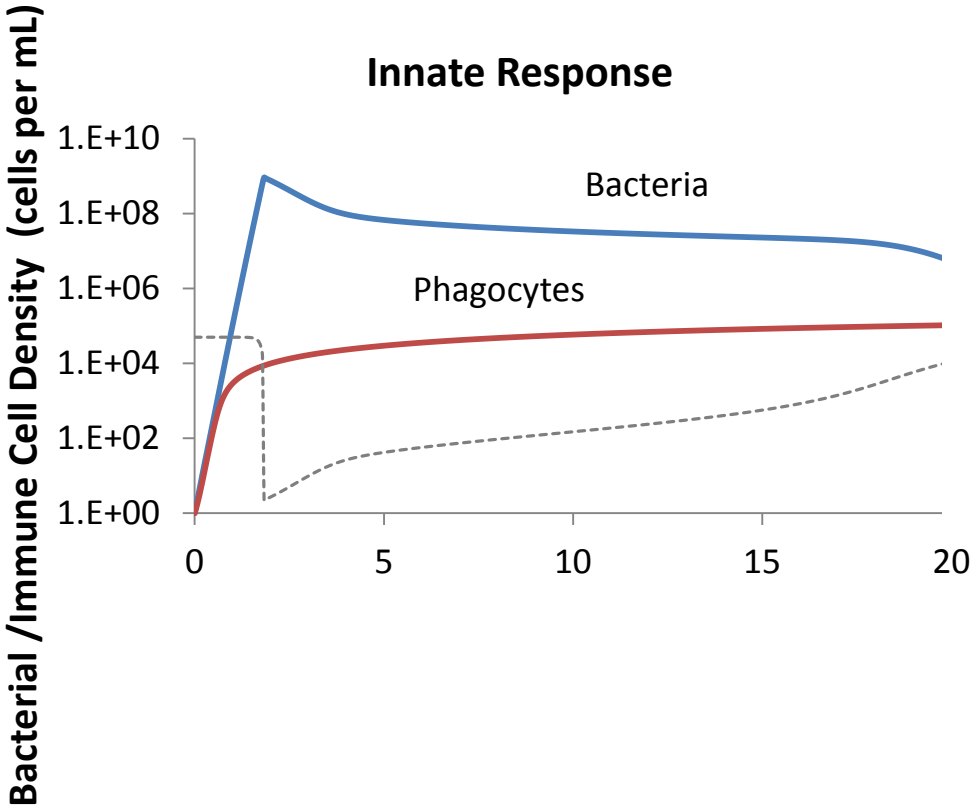
Kochin BF, Yates AJ, de Roode JC, Antia R (2010) PLoS ONE 5(5): e10444

Antia, Levin, May. (1994) Am Nat: 457-472

(i) Antibiotic Treatment of a Self-Limited (Non-Lethal) Infection

POPULATION DYNAMICS OF BACTERIA

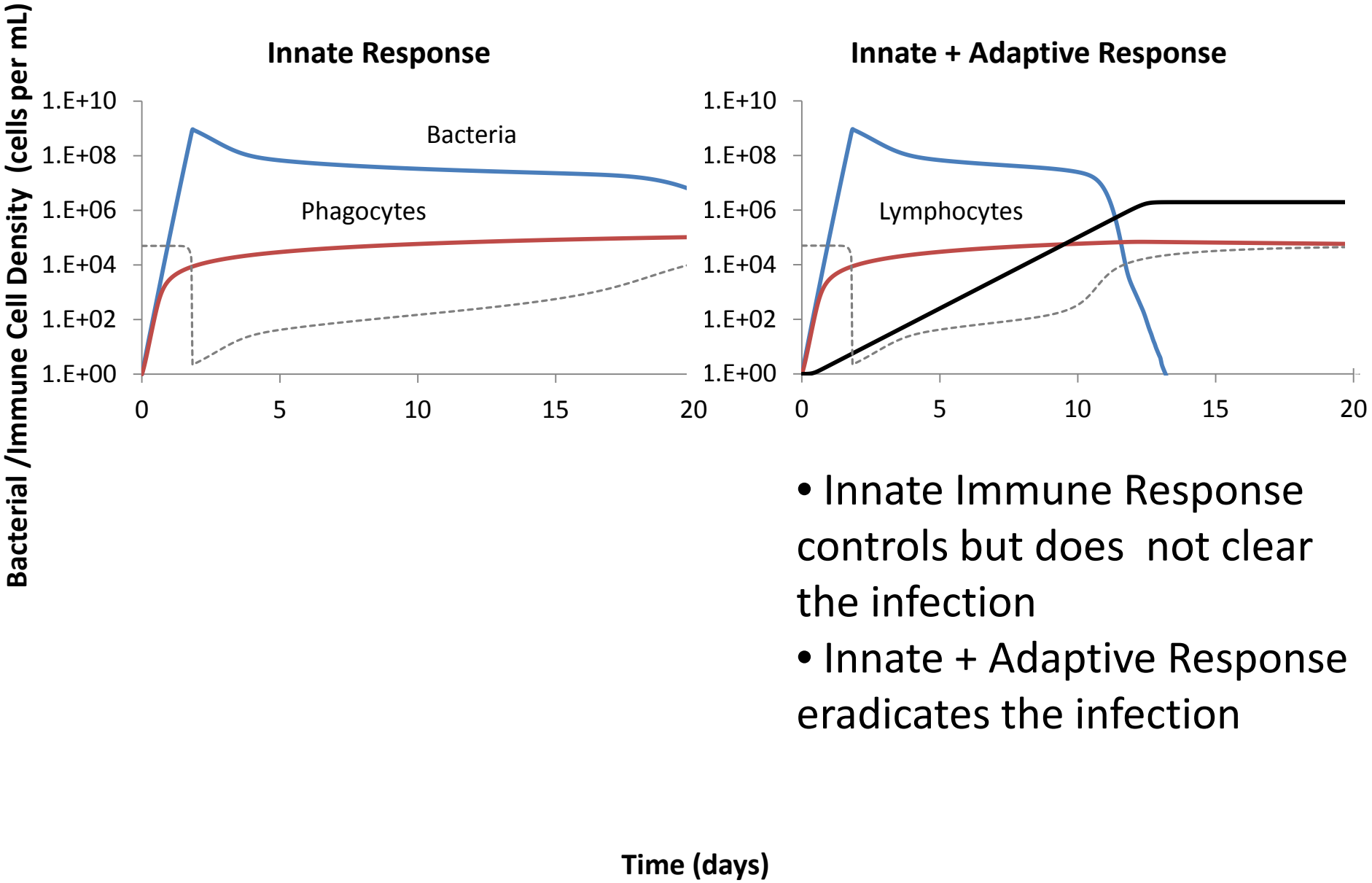
a. No Resistant Bacteria



- Innate Immune Response controls but does not clear the infection

POPULATION DYNAMICS OF BACTERIA

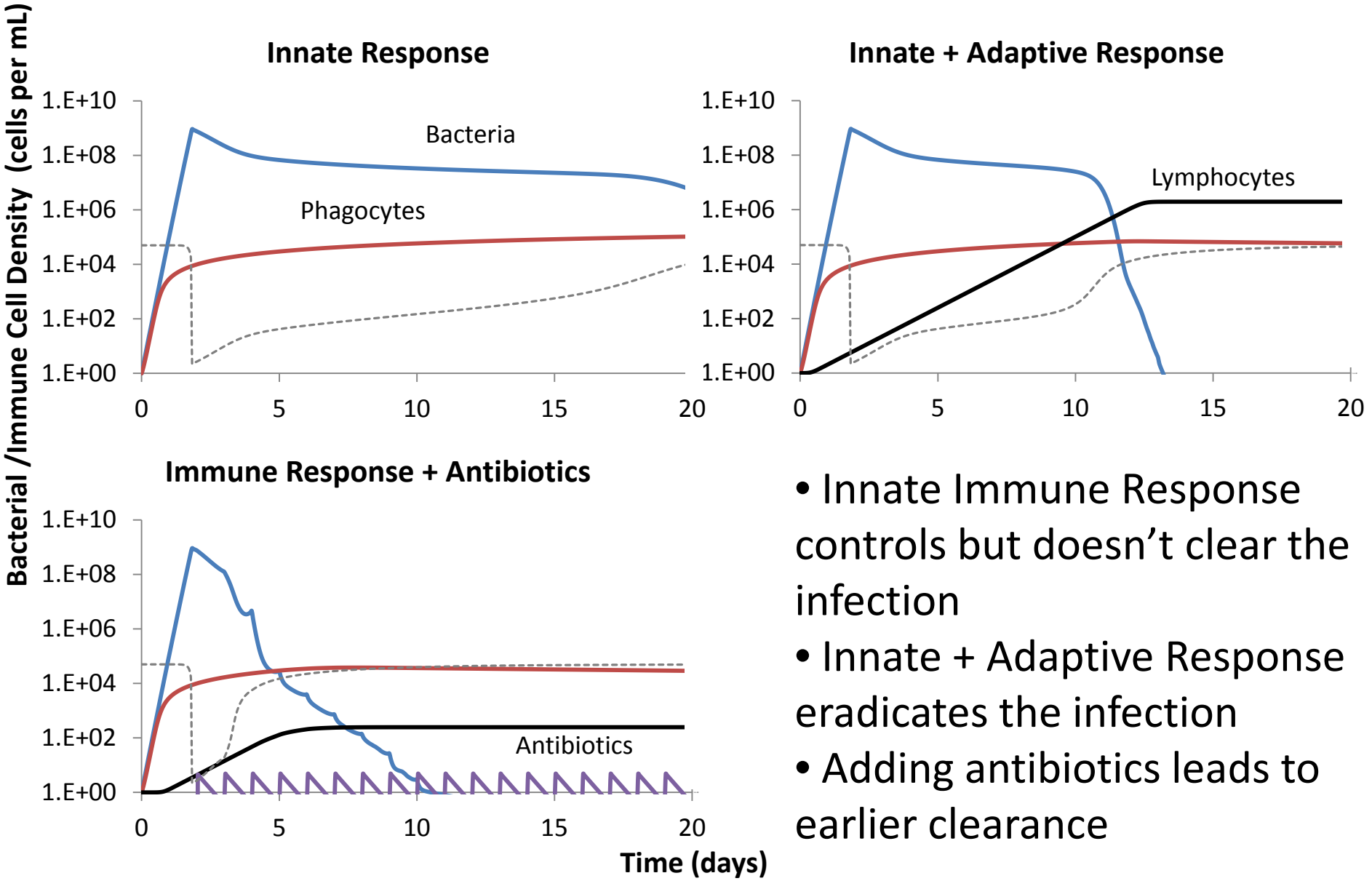
a. No Resistant Bacteria



- Innate Immune Response controls but does not clear the infection
- Innate + Adaptive Response eradicates the infection

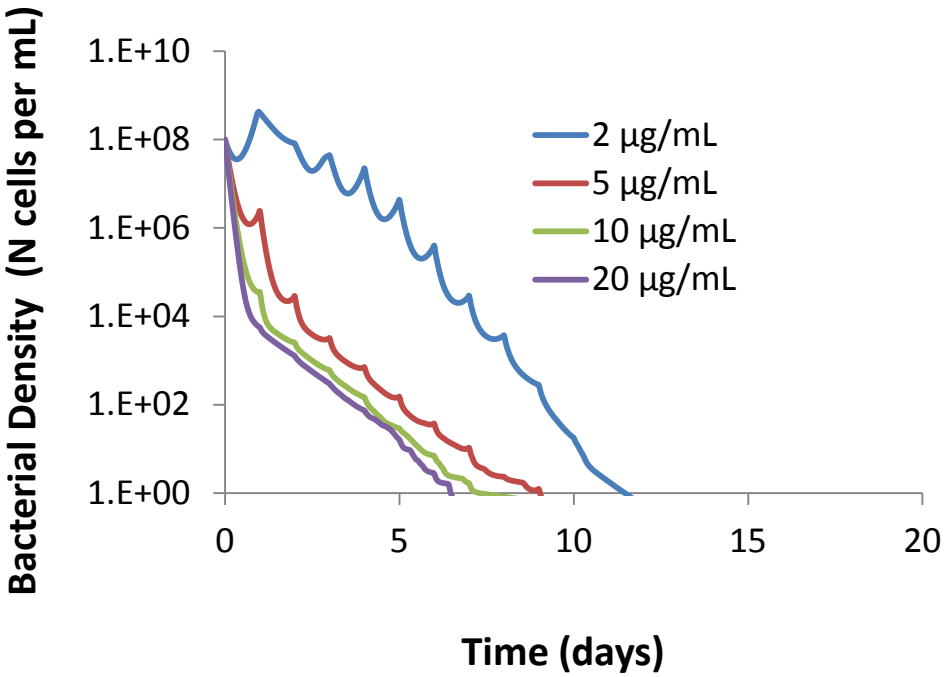
POPULATION DYNAMICS OF BACTERIA

a. No Resistant Bacteria



- Innate Immune Response controls but doesn't clear the infection
- Innate + Adaptive Response eradicates the infection
- Adding antibiotics leads to earlier clearance

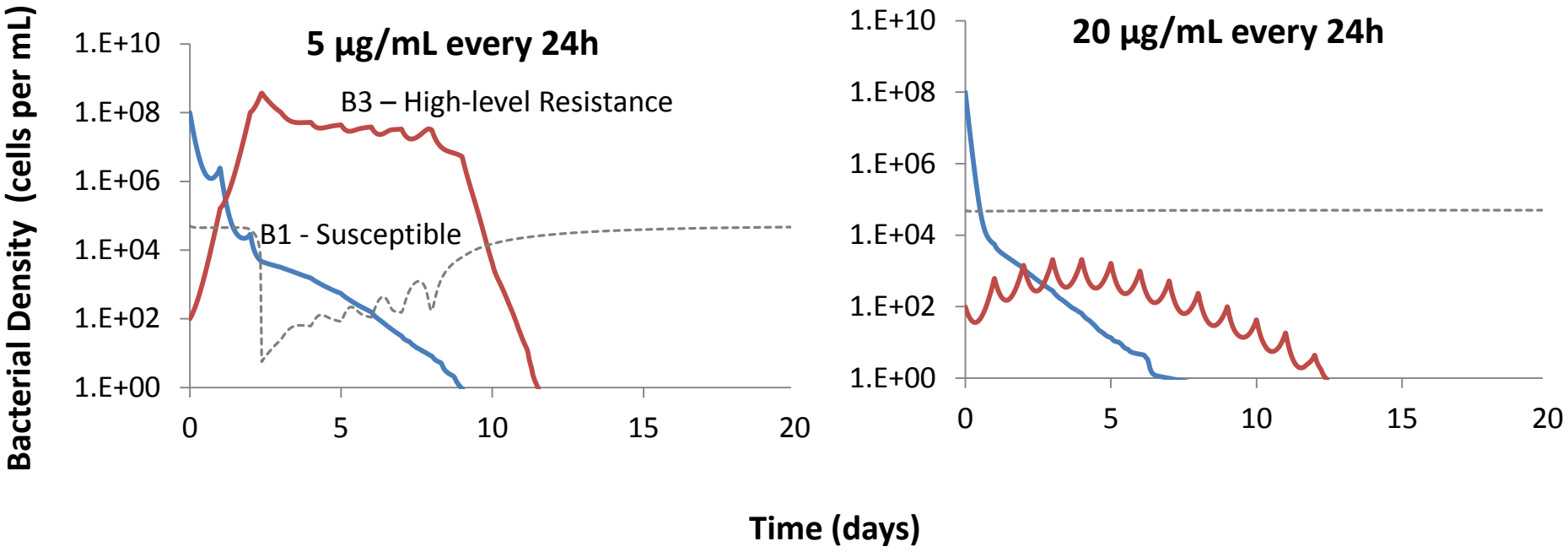
ANTIBIOTIC DOSE EFFECT



- Increasing dose decreases the time to clearance
- The effect of increasing dose on the rate of clearance declines with increasing drug concentrations. ➔ More is only marginally better

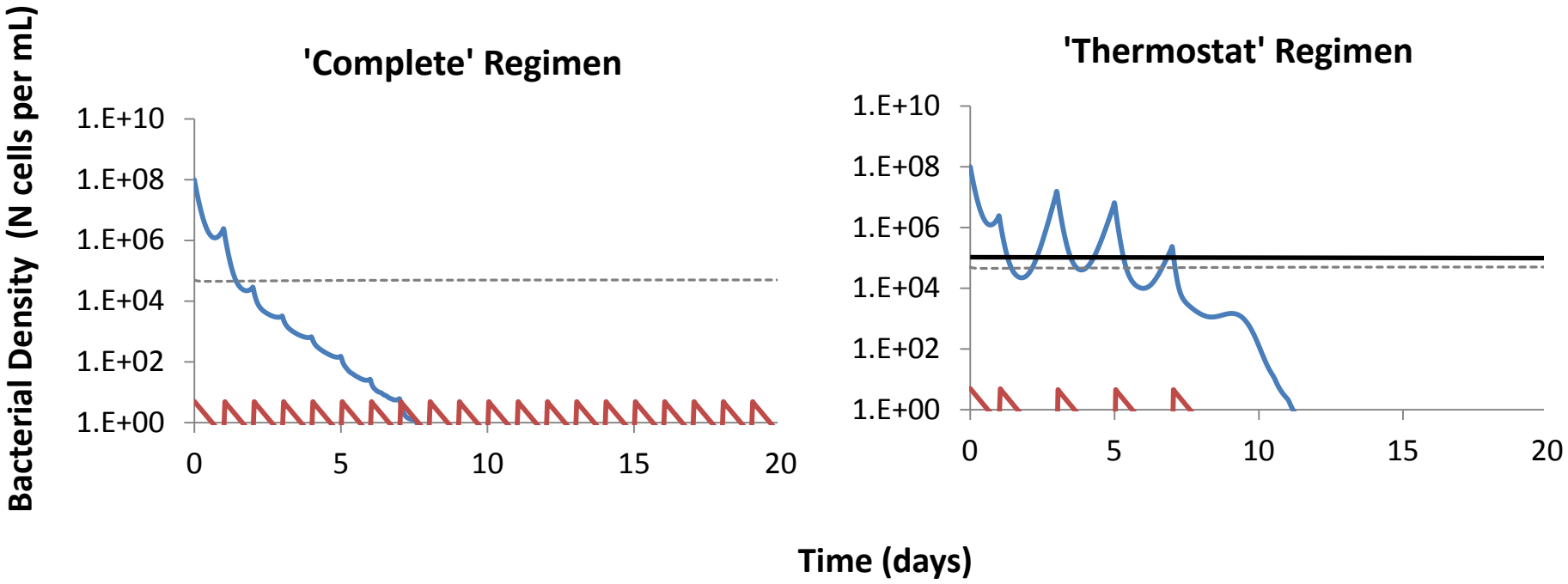
ANTIBIOTIC DOSE EFFECT

b. Pre-existing minority population with high-level resistance



- High doses can prevent ascent of resistant mutants

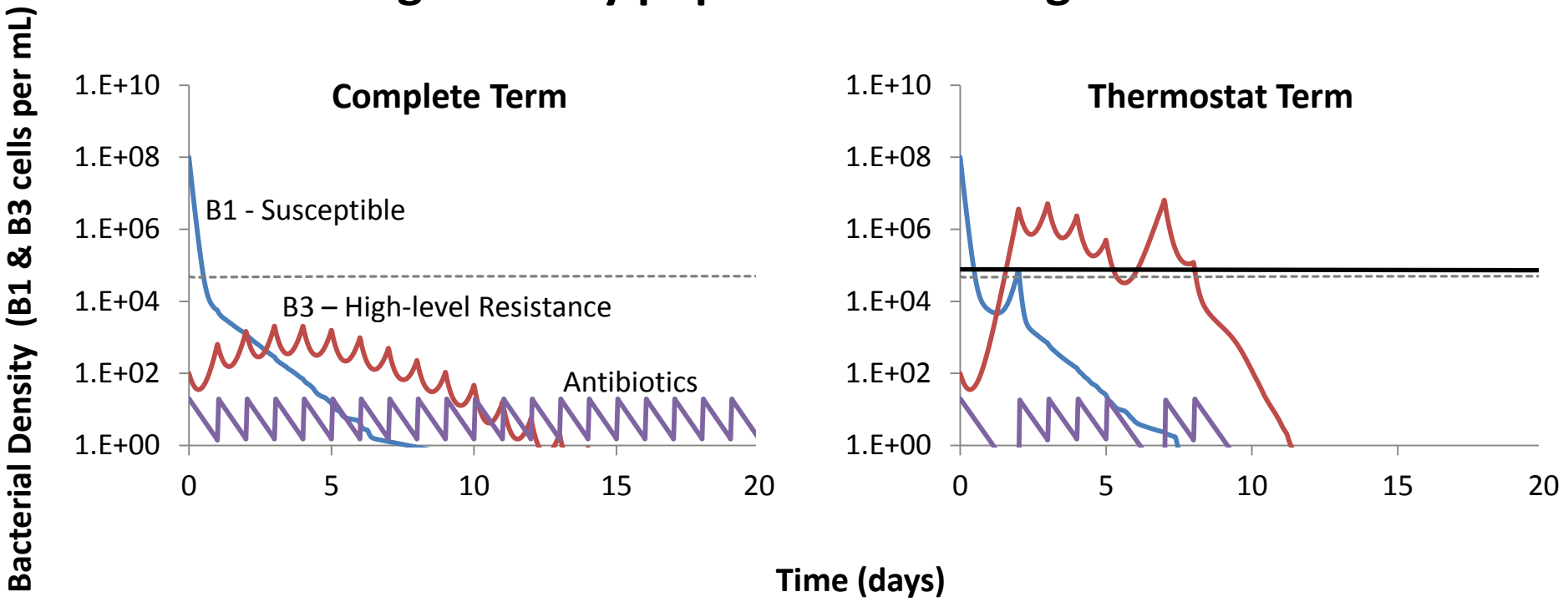
TERM of ADMINISTRATION



• Thermostat Non-Compliance – people stop taking drugs when the density of bacteria fall below some level
– Increase the time to clearance

POPULATION DYNAMICS OF BACTERIA

b. Pre-existing minority population with high-level resistance



• Thermostat Non-adherence to an antibiotic treatment regime could lead to (temporary) ascent of high-level resistance

(ii)

Antibiotic Treatment of an infection that would be lethal in the absence of intervention

TREATMENT FAILURE: IT'S NOT JUST ABOUT RESISTANCE

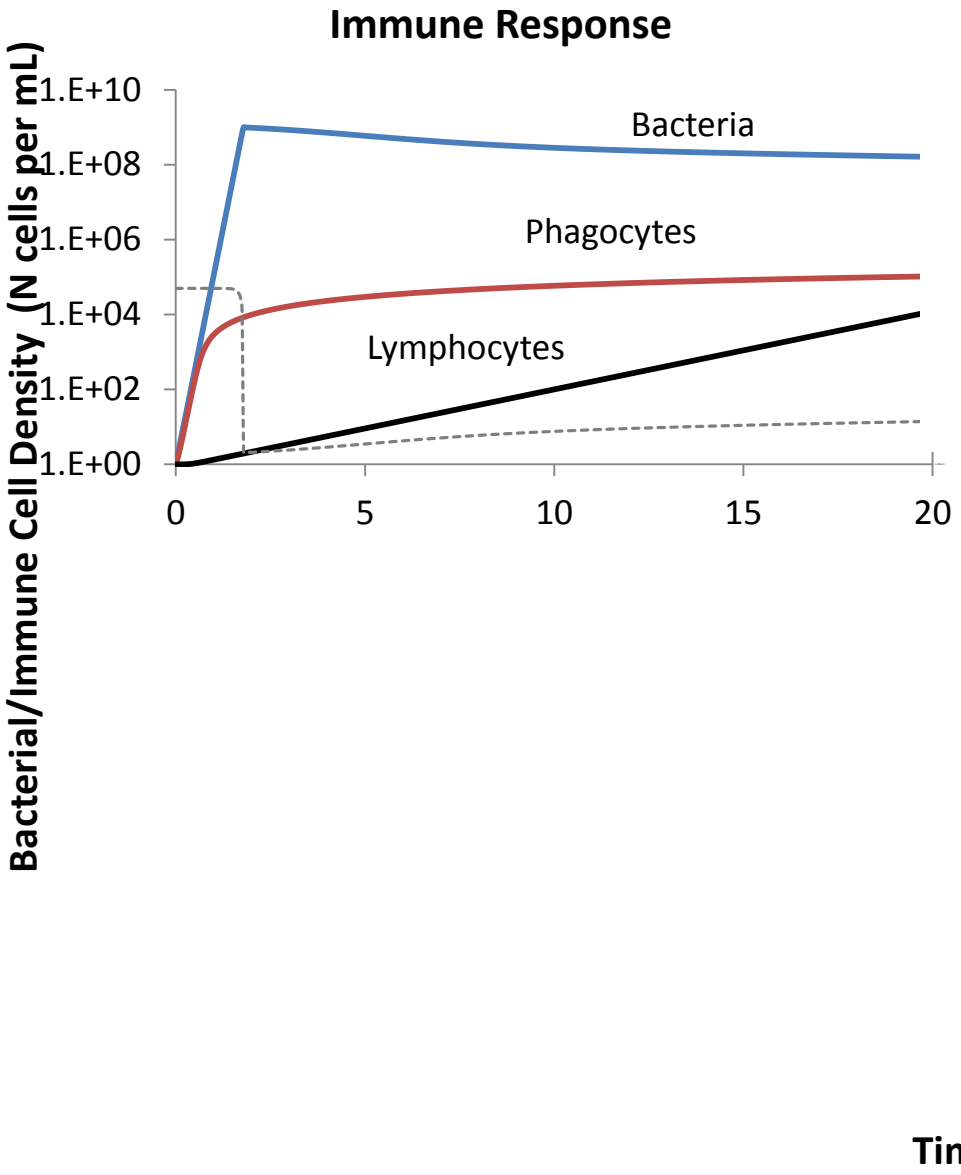
Mortality rates of patients with bacteremic pneumococcal pneumonia

Treatment	% mortality
Symptomatic[1]	80
Specific Serum[1]	45
Penicillin[1] (1940s)	17

[1]M. Finland. Clinical Pharmacology and Therapeutics 13:469-511, 1972.

POPULATION DYNAMICS OF BACTERIA

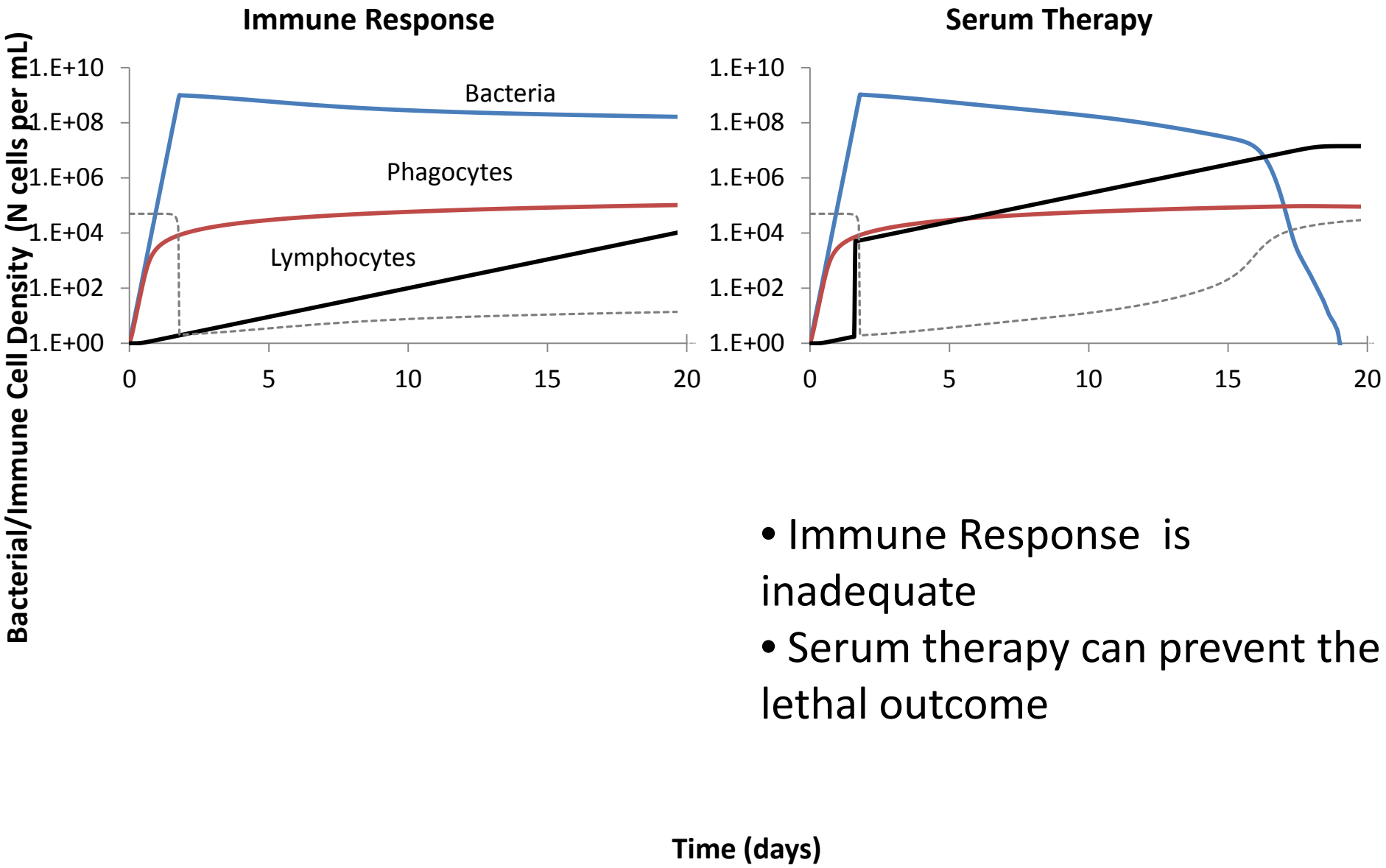
a. No Resistant Bacteria



- Immune Response is inadequate

POPULATION DYNAMICS OF BACTERIA

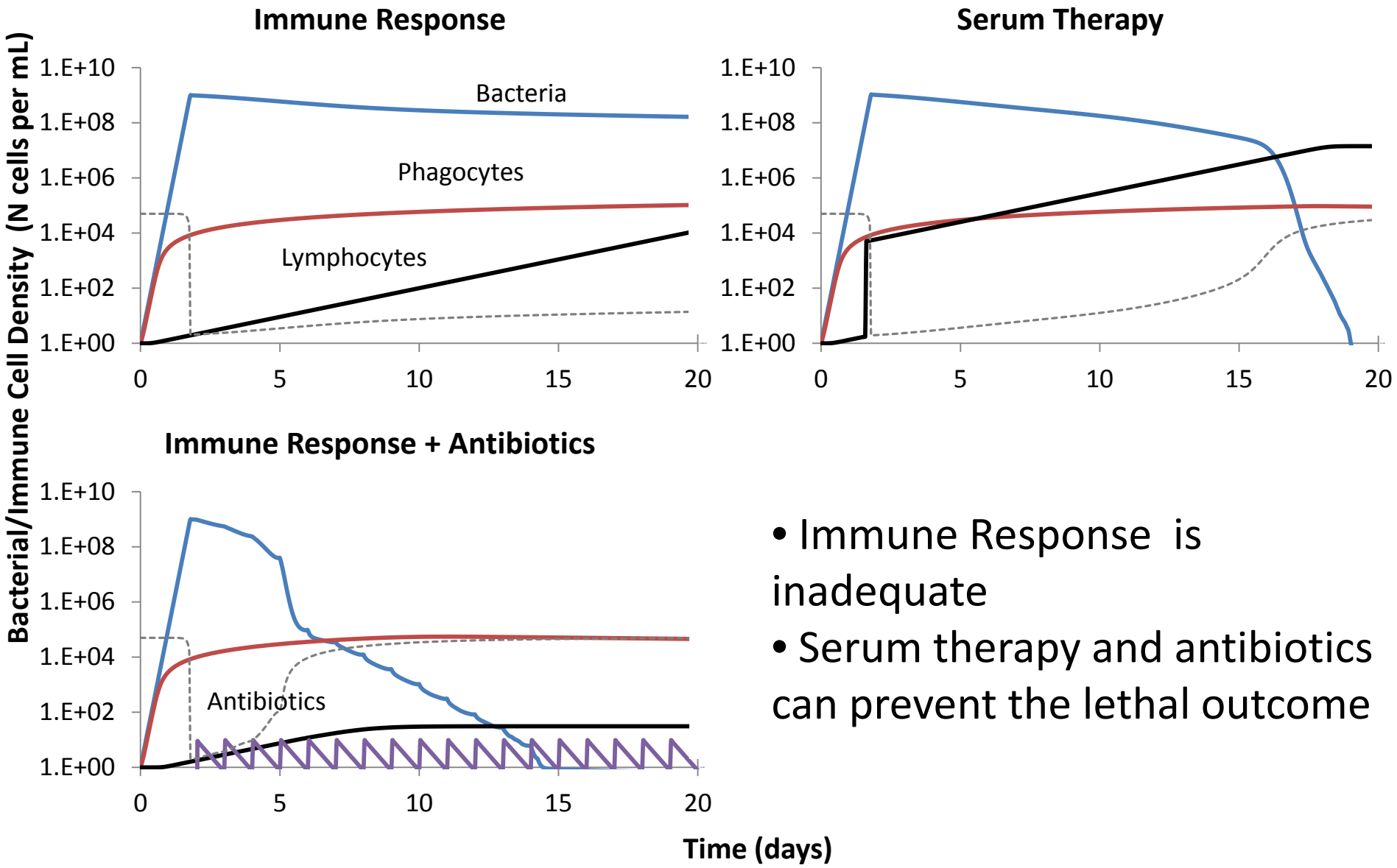
a. No Resistant Bacteria



- Immune Response is inadequate
- Serum therapy can prevent the lethal outcome

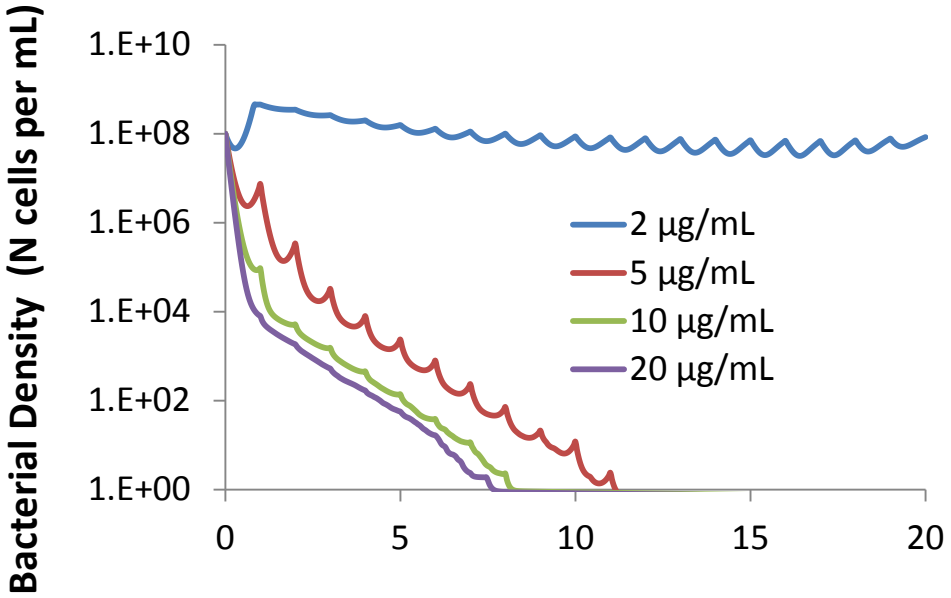
POPULATION DYNAMICS OF BACTERIA

a. No Resistant Bacteria

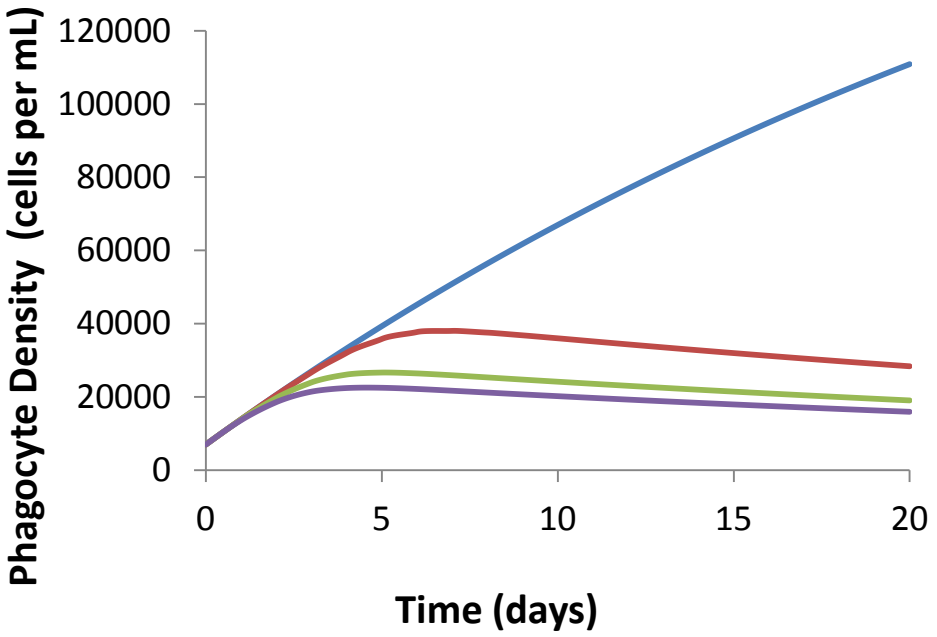


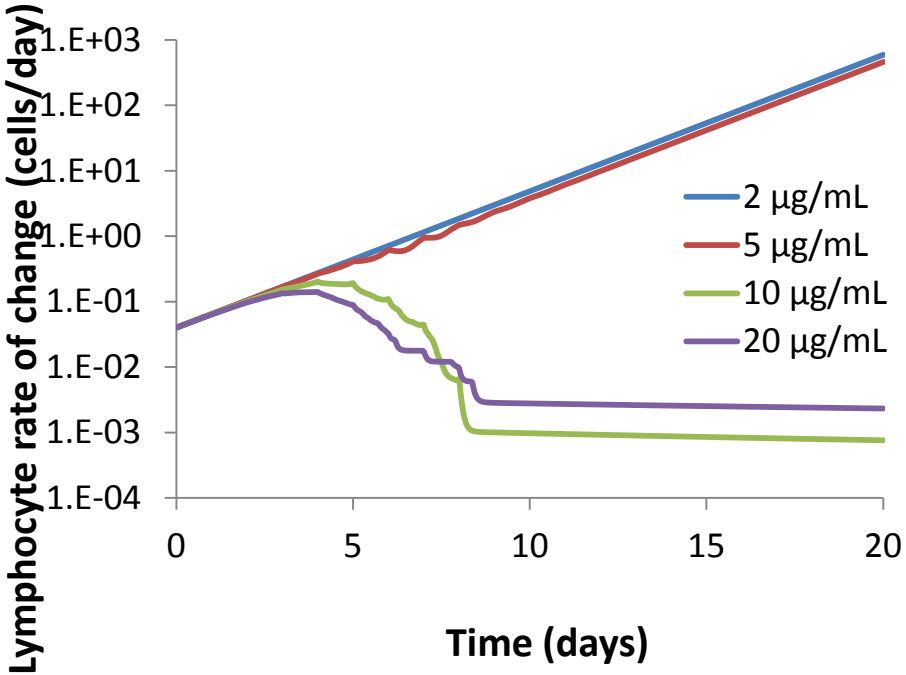
- Immune Response is inadequate
- Serum therapy and antibiotics can prevent the lethal outcome

DOSE AND POTENTIAL IMMUNOPATHOLOGY



- Increasing dose decreases immunopathology (up to a point)





SUMMARY AND CONCLUSIONS

- Phenotypically antibiotic–refractory subpopulations can retard the rate of or prevent clearance

- Rate of clearance increases with the dose of the drug or frequency of its administration

- Higher doses can help mitigate the generation and ascent of resistance

- More need not be better

- A ‘thermostat’ term may increase time to clearance and potentiate ascent of resistance

- Higher doses can help decrease immunopathology

ACKNOWLEDGEMENTS

Bruce Levin, Paul Johnson, Amy Kirby, Nina Walker

NATIONAL INSTITUTES OF HEALTH

PBEE EMORY POPULATION BIOLOGY, ECOLOGY AND EVOLUTION

BURROUGHS WELLCOME FUND

GM 091875

APPENDIX

Where we have been in the Antibiotic PD/PK and Treatment and Resistance Biz

LIPSITCH, M., and B. R. LEVIN, 1997 The population dynamics of antimicrobial chemotherapy. Antimicrob Agents Chemother **41:** 363-373.

LIPSITCH, M., and B. R. LEVIN, 1998 Population dynamics of tuberculosis treatment: mathematical models of the roles of non-compliance and bacterial heterogeneity in the evolution of drug resistance. Int J Tuberc Lung Dis **2:** 187-199.

NEGRI, M. C., M. LIPSITCH, J. BLAZQUEZ, B. R. LEVIN and F. BAQUERO, 2000 Concentration-dependent selection of small phenotypic differences in TEM beta-lactamase-mediated antibiotic resistance. Antimicrob Agents Chemother **44:** 2485-2491.

REGOES, R. R., C. WIUFF, R. M. ZAPPALA, K. N. GARNER, F. BAQUERO *et al.*, 2004 Pharmacodynamic functions: a multiparameter approach to the design of antibiotic treatment regimens. Antimicrob Agents Chemother **48:** 3670-3676.

WIUFF, C., R. M. ZAPPALA, R. R. REGOES, K. N. GARNER, F. BAQUERO *et al.*, 2005 Phenotypic tolerance: antibiotic enrichment of noninherited resistance in bacterial populations. Antimicrob Agents Chemother **49:** 1483-1494.

LEVIN, B. R., and D. E. ROZEN, 2006 Non-inherited antibiotic resistance. Nat Rev Microbiol **4:** 556-562.

UDEKWU, K. I., N. PARRISH, P. ANKOMAH, F. BAQUERO and B. R. LEVIN, 2009 Functional relationship between bacterial cell density and the efficacy of antibiotics. J Antimicrob Chemother **63:** 745-757.

LEVIN, B. R., and K. I. UDEKWU, 2010 Population dynamics of antibiotic treatment: a mathematical model and hypotheses for time-kill and continuous-culture experiments. Antimicrob Agents Chemother **54:** 3414-3426.

Where we have been in the Antibiotic PD/PK and Treatment and Resistance Biz

Haber, M., B.R. Levin and P. Kramarz (2010) Antibiotic control of antibiotic resistance in hospitals: A simulation study. BMC Infections Disease, 10: 254.

Levin, B. R. (2011) Population geneticists discover bacteria and their genetic/molecular epidemiology Chapter 1 IN Population Genetics of Bacteria: a Tribute to Thomas S. Whittam Editors: Seth T. Walk and Peter C. H. Feng. ASM Press

Ankomah, P, and B.R. Levin (2012) Two-drug antimicrobial chemotherapy: A mathematical model and experiments with *Mycobacterium marinum* (PLoS Pathogens January 2012 | Volume 8 | Issue 1 | e1002487)

Chien, Y-W, B. R. Levin and K. Klugman (2012) The anticipated severity of a "1918-like" influenza pandemic in contemporary populations: the contribution of antibacterial interventions (PloS One Volume 7 | Issue 1 | e29219)

Kirby, A, K. Garner and B.R. Levin (2012). The Relative Contributions of Physical Structure and Cell Density to the Antibiotic Susceptibility of Bacteria in Biofilms. . Antimicrobial Agents and Chemotherapy, 56, 2967-2975

Udekwu, K.I. and B.R. Levin (2012). *Staphylococcus aureus* in continuous culture: a tool for the rational design of antibiotic treatment protocols. PLoS One July 2012 | Volume 7 | Issue 7 | e38866

Johnson, P.T and B.R. Levin (2012) Pharmacodynamics, Population Dynamics and the Evolution of Persistence in *Staphylococcus aureus* PLoS Genetics (In Press)

In mouse-o studies of antibiotic and phage therapy

Research with Renata Zappala, Jim Bull Terry DeRouin and Nina Walker

Renata Zappala MD/PhD

Jim Bull at Breakfast

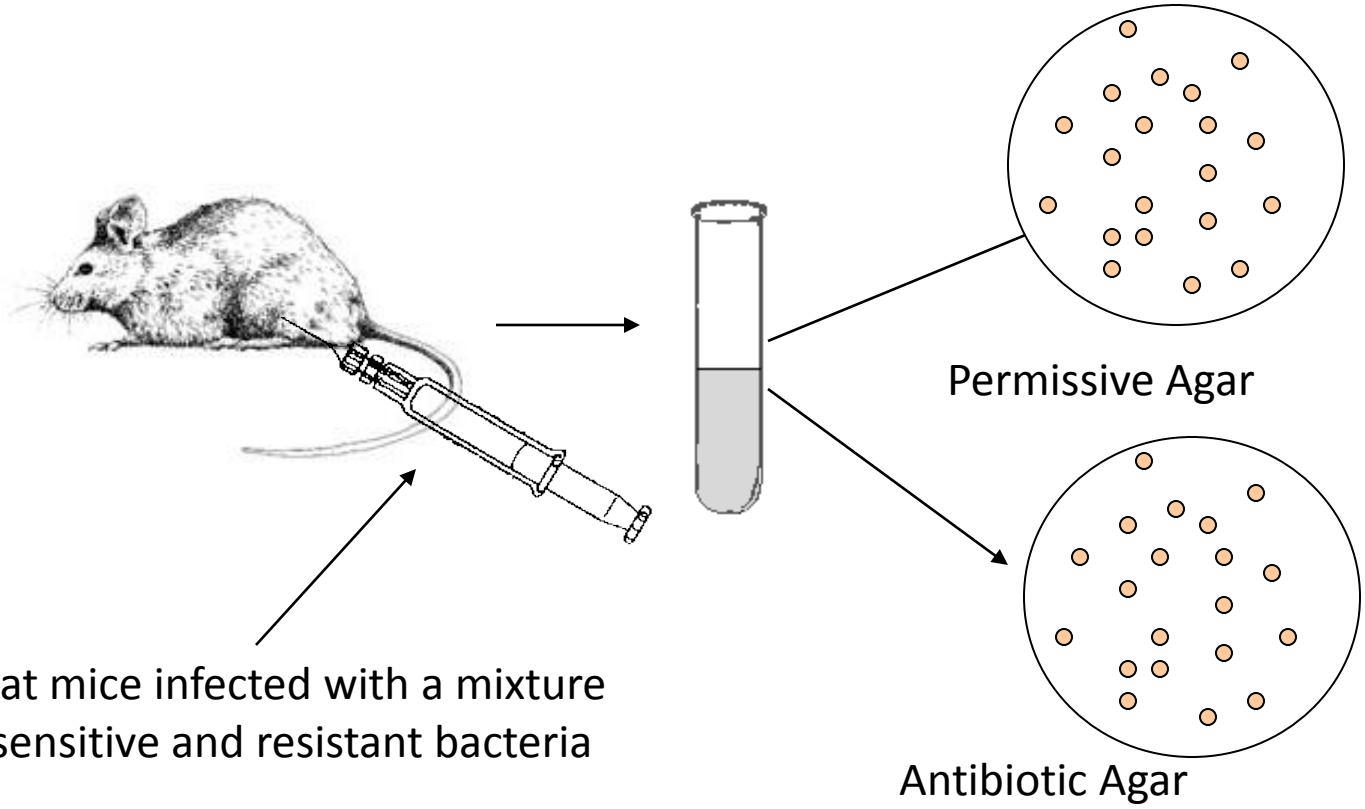
The Motivation - Inspiration

Deaths occurring in groups of 30 mice infected with *E. coli* 018:K1:H7 with different treatments 8 hours after infection

Treatment	No. of Doses	No. Deaths
Extract of E. coli K1	1	28
K1- Specific phage	1	1
Streptomycin	1	29
Streptomycin	8	3
Tetracycline	8	13
Ampicillin	8	26
Chloramphenicol	8	29
Trimethoprin Sulphafurazol	8	26

Smith, H. W. & Huggins, M. B. (1982). Successful treatment of experimental Escherichia coli infections in mice using phage: its general superiority over antibiotics. *J Gen Microbiol* **128**, 307-318.

Resistance Competition Assay (RCA)*



Negri, M. C., Lipsitch, M., Blazquez, J., Levin, B. R., and Baquero, F. (2000). Concentration-dependent selection of small phenotypic differences in TEM beta-lactamase-mediated antibiotic resistance. Antimicrob Agents Chemother *44*, 2485-2491.

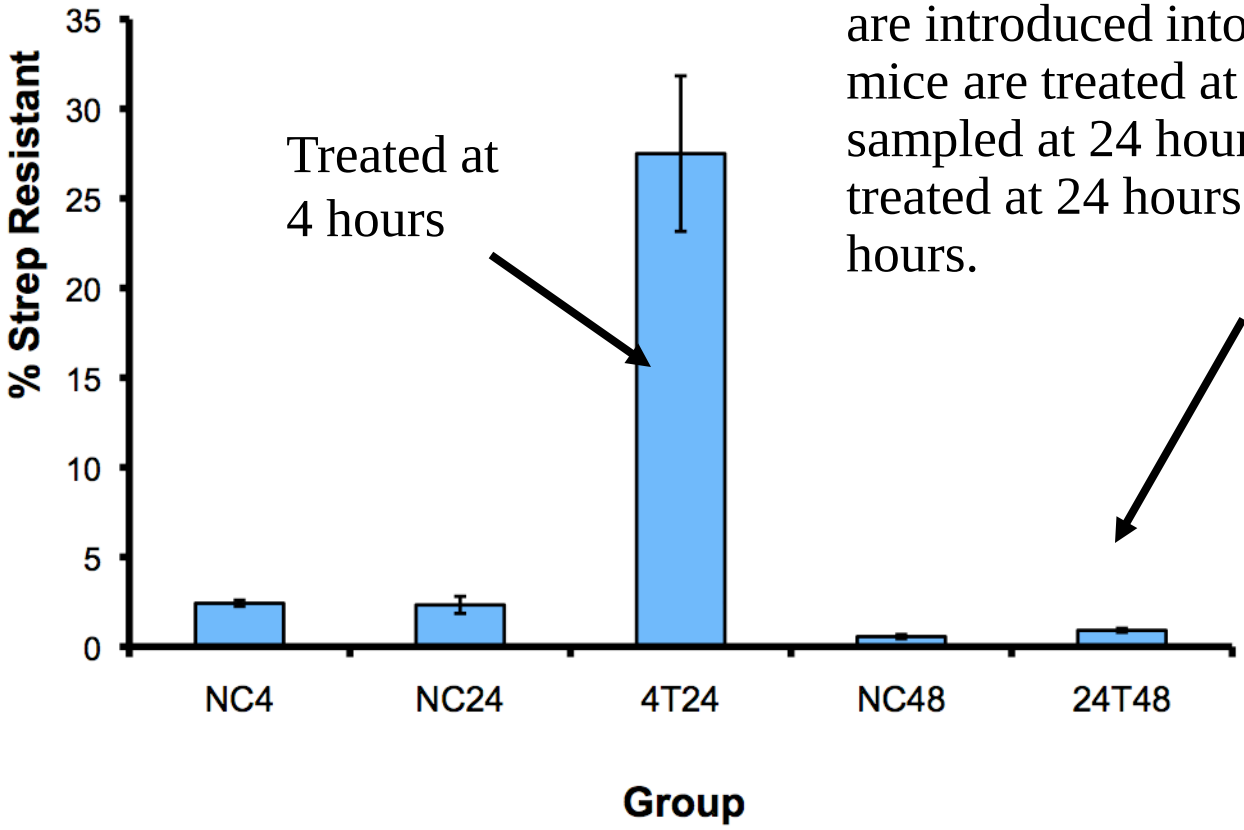
Bull, J. J., Levin, B. R., DeRouin, T., Walker, N., and Bloch, C. A. (2002). Dynamics of success and failure in phage and antibiotic therapy in experimental infections. BMC Microbiol *2*, 35.

Selection for Phage and Streptomycin Resistant *E. coli* K1*

	Frequency of Resistant Bacteria		
	Inoculation	Control	Treated
Immediate Treatment			
H- Phage	0.074	0.044	0.64
W-Phage	0.0025	0.004	0.0071
Streptomycin	0.00017	0.00044	0.38
Treatment at 8 hours			
H- Phage	0.0095	0.0013	0.0065
Streptomycin	0.00012	0.00031	0.00012

Data from Bull et al. (2003) BMC Microbiol *2*, 35.

Resistance competition assay for the efficacy of streptomycin treatment



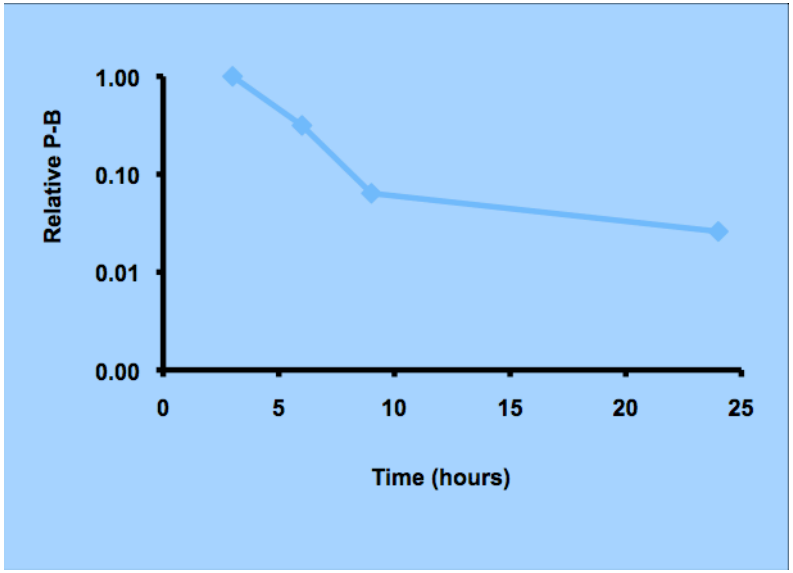
Treated at 4 hours

A mixture of antibiotic sensitive and a low frequency of resistant bacteria are introduced into the thigh. Some mice are treated at 4 hours and sampled at 24 hours and other are treated at 24 hours and sampled at 48 hours.

Treated at 24 hours

Why does the efficacy of treatment decline with the term of the infection?

We introduced cells carrying a single copy of Cm-r plasmid that does not replicate at 37C. After 8 hours, the change in the frequency of cells with that plasmid no longer declined ➔ **the cells were no longer dividing.**



Antibiotics (and phage) are relatively ineffective in killing non-replicating bacteria.

Similar results were obtained by Harry Eagle (1952) studying penicillin treatment of *Streptococcus pneumoneae* infections in laboratory mice.

Eagle, H. (1952). Experimental approach to the problem of treatment failure with penicillin. *American Journal of Medicine* **13**, 389-399.