The Population Dynamics and Potential Clinical Implications of Phenotypic Resistance to Antibiotics

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Latsis Symposium 2015, Drug Resistance and the Future of Disease Control, Zurich July 4, 2015

Google Hangouts

Overview

I. An unbiased (never) perspective on antibiotics and the resistance problem

***II. In vitro,* i*n plastico*, and i*n silico* (modeling) studies of the pharmacodynamics (PD), pharmacokinetics (PD), immunological, population and evolutionary dynamics of antibiotic treatment**

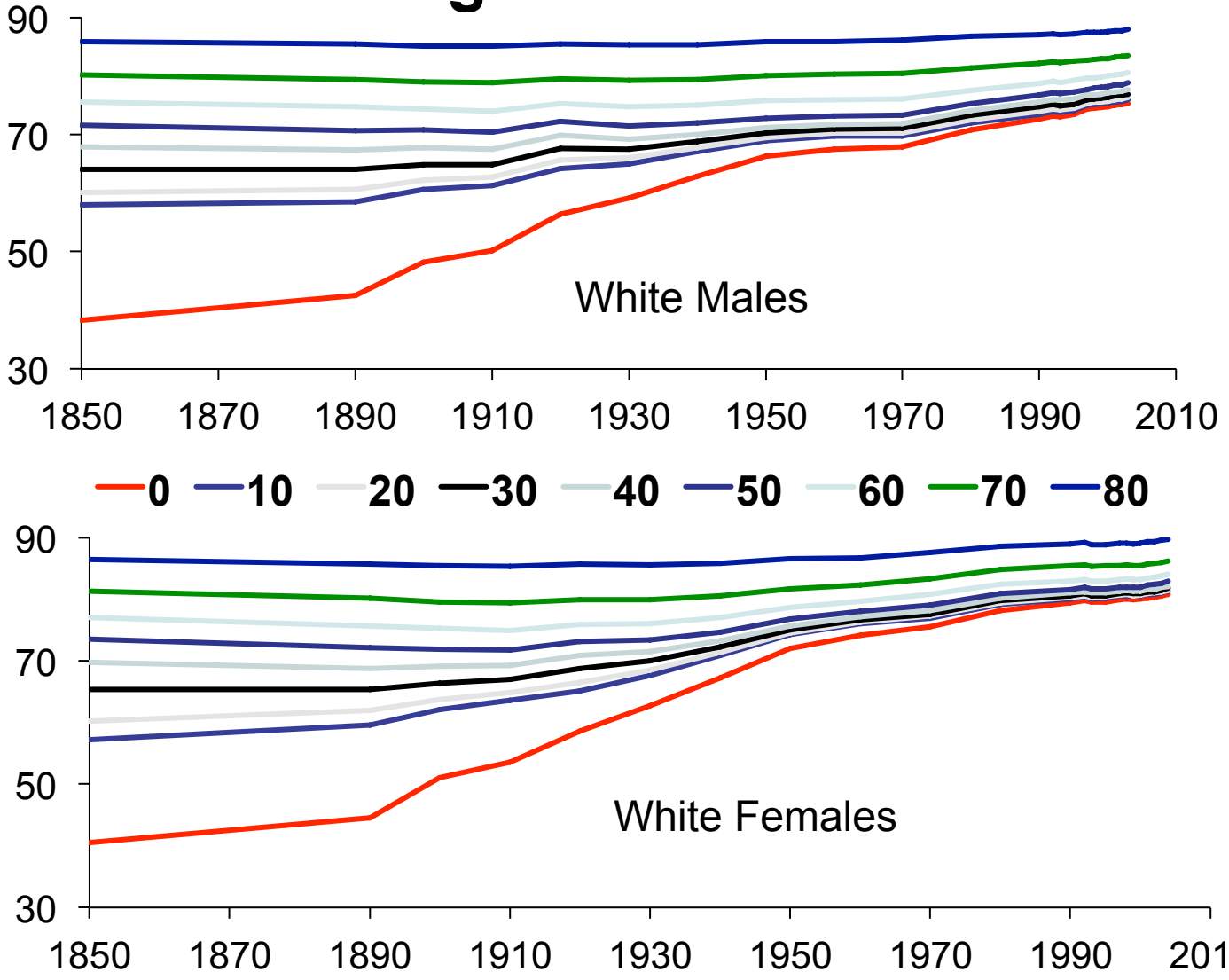
1- The ideal and convenient (to theoreticians as well as experimentalist) world of planktonic bacteria frolicking about in iiquid culture all with equal access to everything good and bad).

Bacteriostatic antibiotics and persistence

2- Dealing with the inconvenient reality of bacteria in physical structured habitats:

Biofilms, colonies

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 .](http://www.dhhs.gov)

Ten Leading Causes of Death USA 1900

All Races and both Sexes Rates per 100,000

All Causes	1548.1
1- Tuberculosis	174.2
2- Pneumonia and influenza	161.3
3- Heart disease	145.4
4- Diarrhea, enteritis, ulcers	104.9
5-Intrachrania lesions – vascular	103.9
6- Nephritis	90.6
7- Accidents excluding automobile	72.5
8- Cancer	66.3
9- Senility	45.2
10-Bronchitis	39.4

All infections Mostly infections

CDC/NCHS – National Vital Statistics Mortality vol48

Ten Leading Causes of Death USA

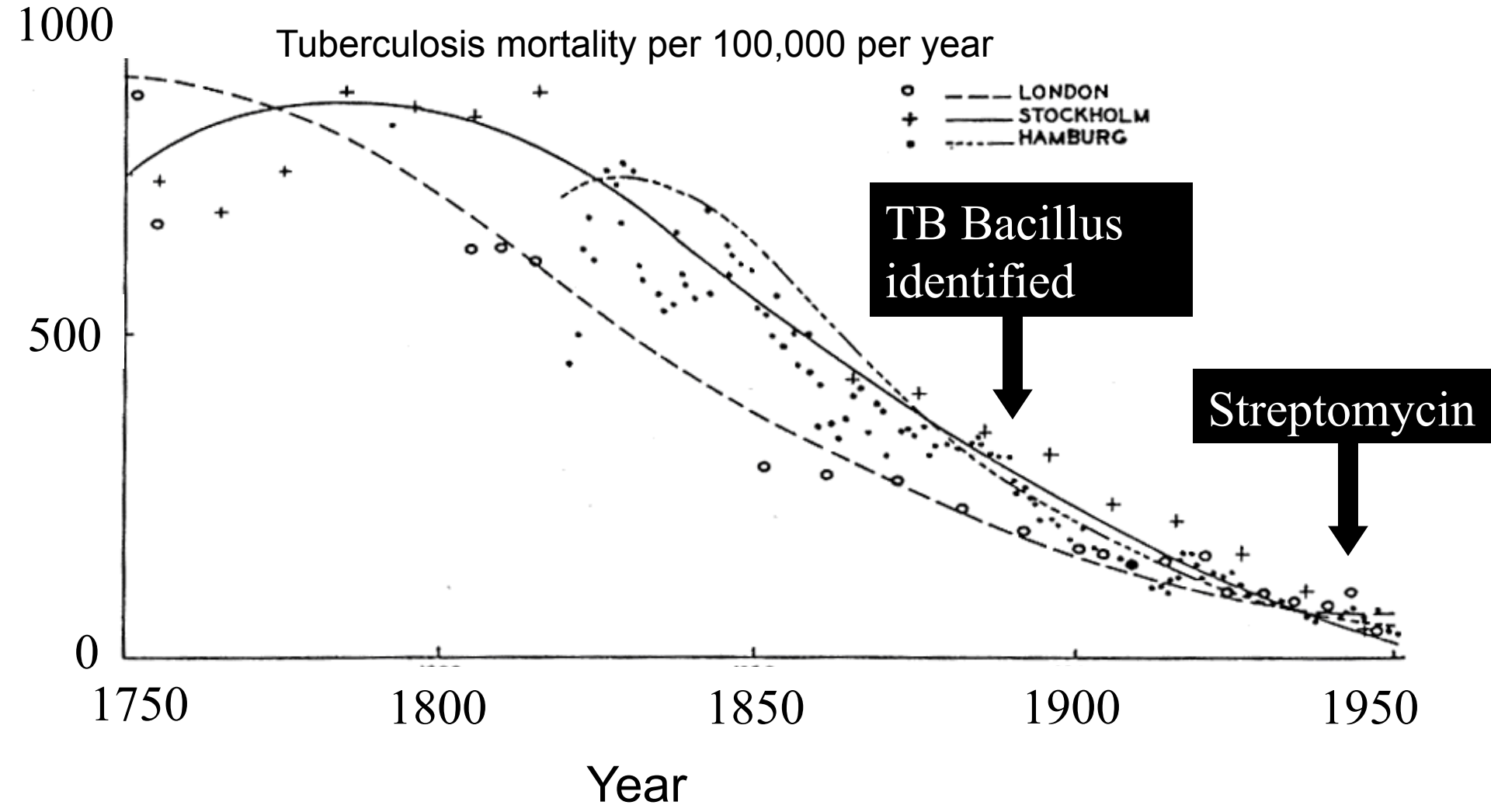
All Races and both Sexes Rates per 100,000

	1980	1998
All Causes	878.3	864.7
1- Heart Disease	336.0	268.2
2 - Malignant neoplasm's (cancer)	183.9	200.3
3 - Cerebrovascular diseases	75.1	58.6
4- Chronic obstructive pulmonary disease	46.6 (5)	41.7
5- Accidents Automobile/Others	23.5/23.2 (4)	16.1/20.1
6- Pneumonia and influenza	24.1	34.0
7- Diabetes mellitus	15.4	24.0
8- Suicide	11.9 (10)	11.3
9 - Nephritis	7.4 (13)	9.7
10- Chronic Liver Disease	13.5 (8)	9.3
All other	95.6	171.4

~ 6% and 7% infection

CDC/NCHS – National Vital Statistics Mortality vol48

Medical intervention played little role in the decline in infectious disease mortality



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.

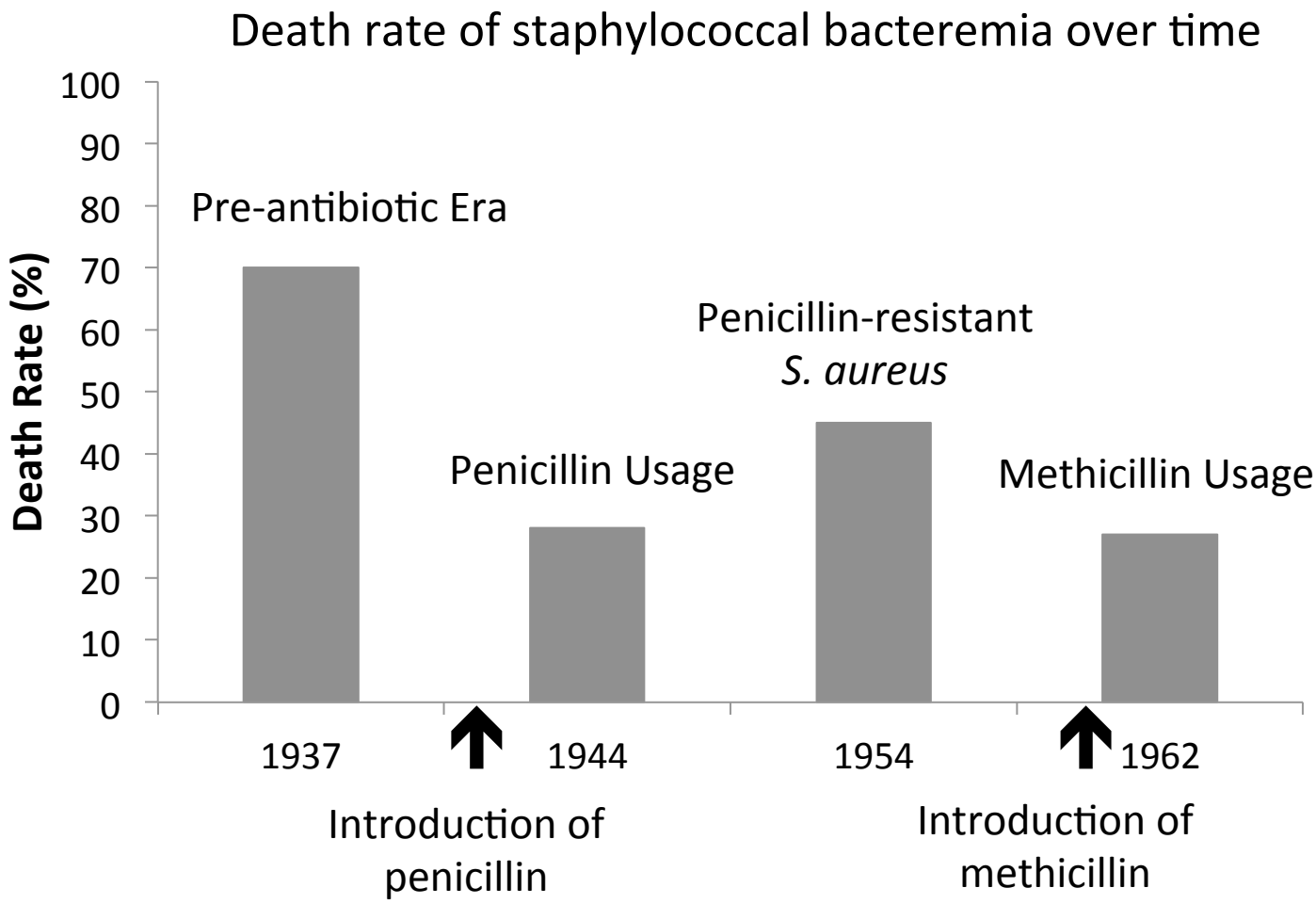
Antibiotics are an Effective Medical Intervention
(or at least have been)

Mortality rates of patients with bacteremic *pneumococcal* pneumonia

Treatment	No.	% mortality
Symptomatic[1]	356	80
Specific Serum[1]	100	45
Penicillin[1] (1940s)	333	17

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

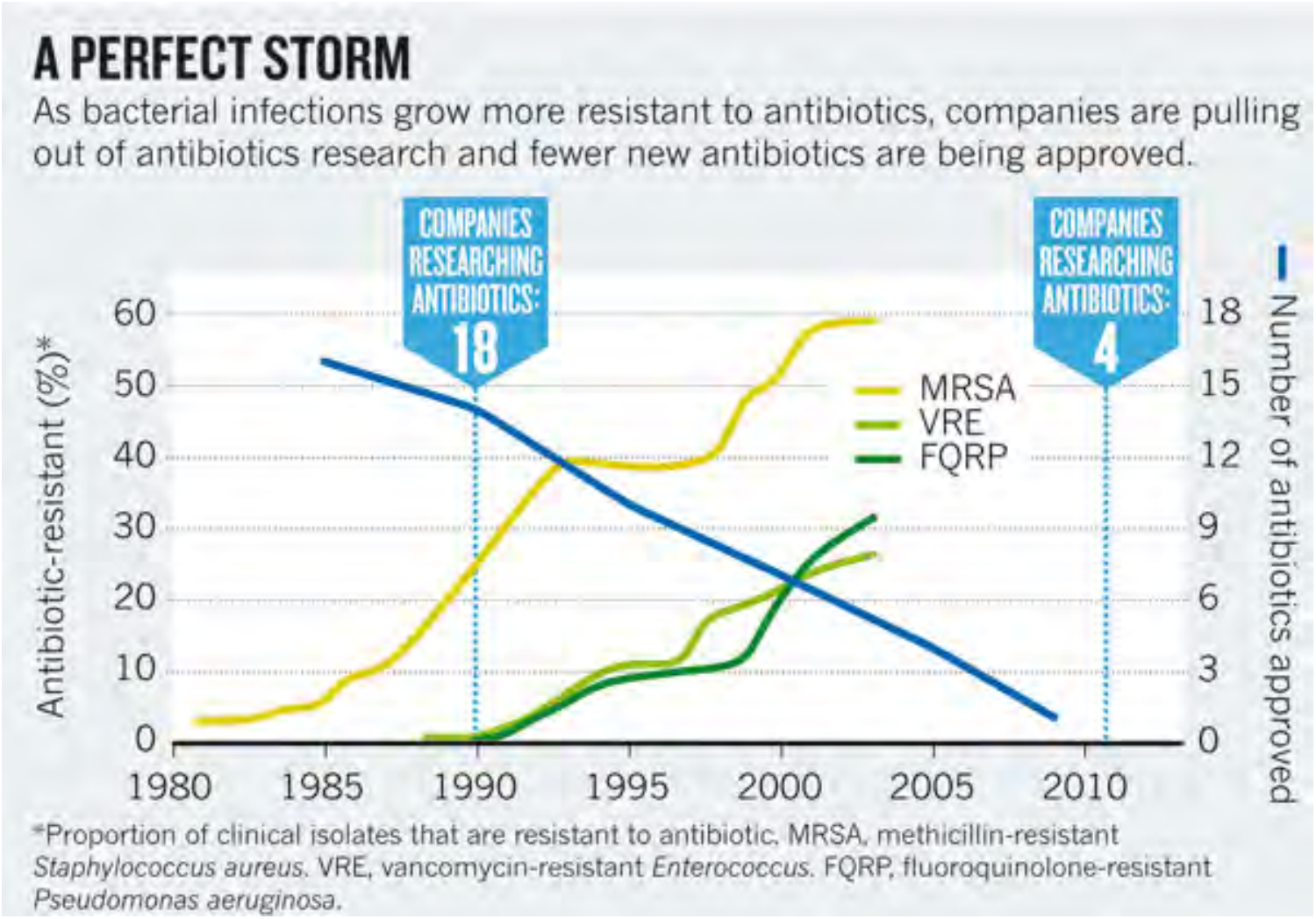
Antibiotic Resistance is Problem and not Just a Career Opportunity



- Antibiotic resistance affects treatment outcomes
- Treatment failure occurs in the absence of resistance

Adapted from: Rubin et al. (1999) *Emerg. Infect. Dis.*

The Ascent of Resistance and Introduction of New Antibiotics



Cooper, M.A., David, S. *Nature*, 2011

Treatment Failure is not Just due to Resistance

Mortality rates of patients with bacteremic pneumococcal pneumonia

Treatment	% mortality
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Treatment Failure is not Just due to Resistance

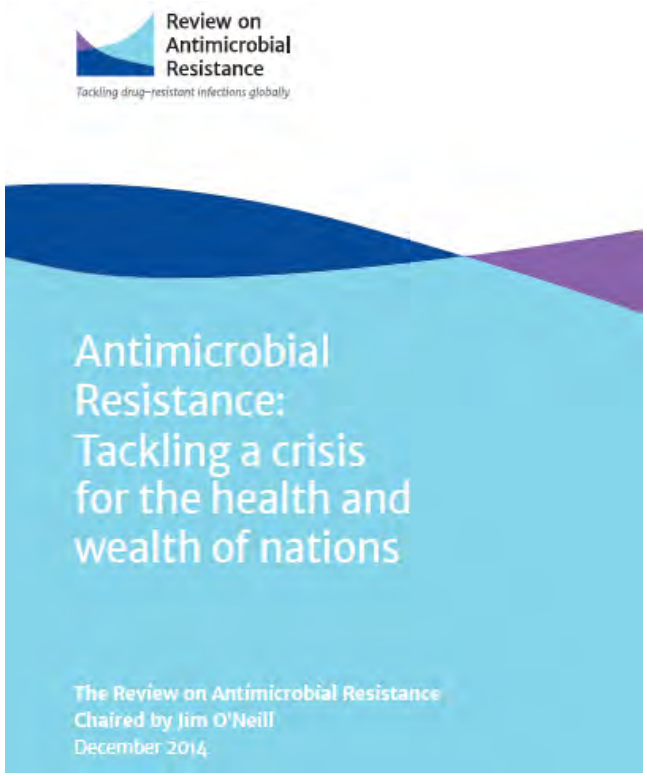
Mortality rates of patients with bacteremic pneumococcal pneumonia

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.

What is the real magnitude of the resistance problem?



The Contribution of Agricultural Use of Antibiotics to Resistance in Humans

Almost certainly has contributed to the origins of resistance genes and genetic elements in bacteria, including those that are commensal and pathogenic to humans.

Conjecture: has contributed little to the spread of resistance in humans. Human use and over use is responsible for that.

The Netherlands observation: Highest rate of antibiotic use in animal husbandry in Europe and the lowest rates antibiotic use and lowest frequencies of resistance.

A Couple of Major Failings of the Antibiotic Resistance Enterprise”

The focus on mortality: The vast majority of antibiotic use is for the treatment (or prevention) of normally self-limiting bacterial infections (or thought to be bacterial infections). How much does resistance increase the morbidity and term of these infections?

The consequences of inadequate access to antibiotics: Lots of whining about contribution of “over-the-counter” use of antibiotics to resistance, with little formal consideration of the positive clinical effects.

The *Ec*LF PK/PD Antibiotic Treatment Collective

Mantra:

Antibiotics do more than select for resistance.

Pretentious goal

To develop antibiotic treatment regimes that minimize morbidity and maximize the rate of clearance of infections whilst minimizing the rate of ascent and dissemination of resistance bacteria.

The *Ec*LF PK/PD Antibiotic Treatment Collective 2014/2015

Pierre Ankomah

Howie Weiss Bruce Levin Tracey Okawa Ingrid McCall
James Dickey Veronique Perrot Justin Kim Young
Jason Jeong Rayshawn Holmes Jeny Concepcion-Acevedo

The PD/PD Antibiotic Treatment Collective 2014/2015

Fernando Baquero
Ramón y Cajal Hospital
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Roland Regoes
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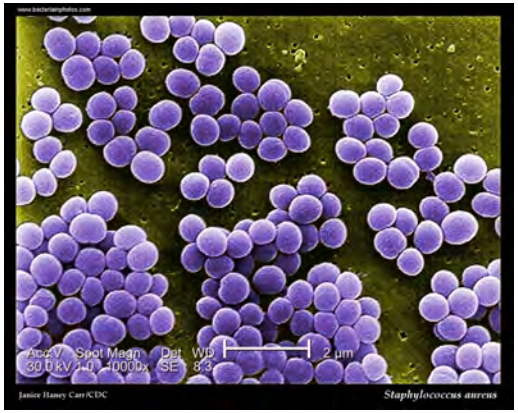
Nathanaël Hozé
ETH Zurich

Klas Udekw
Univ. of Stockholm

Cathrine Friberg

Hanne Ingmer



Staphylococcus aureus

University of Copenhagen

Conjecture: most antibiotic treatment failure is not due to resistance, at least not yet.

- **Host Factors**
  - Age
  - Underlying Disease
  - Improper or inadequate Immune Response

- **Non-inherited/Phenotypic Resistance**
  - Persistence
  - Biofilms
  - Abscesses

Levin, B.R., and Rozen, D.E. (2006) *Nat. Rev. Micro*.

The PD and PK, Immunological Population Dynamics of Antibiotic Treatment of Planktonic Bacteria in Mass Culture

Why are Bacteriostatic Antibiotics as Clinically Effective as Bactericidal?

J Antimicrob Chemother 2015; **70**: 382–395
doi:10.1093/jac/dku379 Advance Access publication 28 September 2014

Journal of Antimicrobial Chemotherapy

Bacteriostatic versus bactericidal antibiotics for patients with serious bacterial infections: systematic review and meta-analysis

Johannes Nemeth[1]*†, Gabriela Oesch[2]† and Stefan P. Kuster[1]†

[1]Division of Infectious Diseases and Hospital Epidemiology, University Hospital and University of Zurich, Zurich, Switzerland;
[2]University Children's Hospital, Zurich, Switzerland

"Conclusions: The categorization of antibiotics into bacteriostatic and bactericidal is unlikely to be relevant in clinical practice if used for abdominal infections, skin and soft tissue infections and pneumonia. Because we were not able to include studies on meningitis, endocarditis or neutropenia, no conclusion regarding these diseases can be drawn."

Awesome Collaborators

Fernando Baquero

Ingrid C. McCall

Magnificent Student Assistants

Jason Jeong

Justin Young Kim

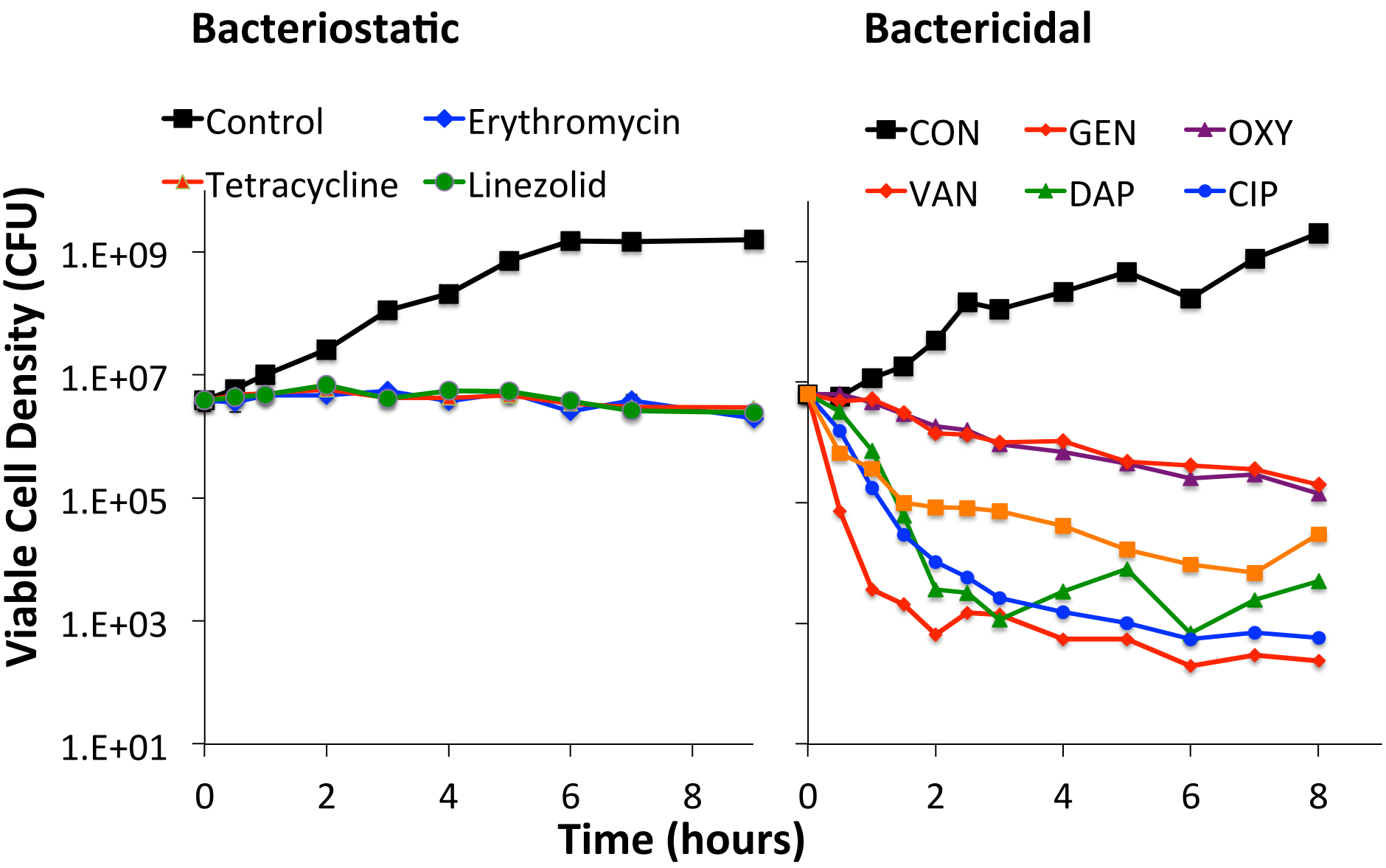
Bacteria, Media and Antibiotics

Bacteria - *Staphylococcus aureus* (Newman's Best)

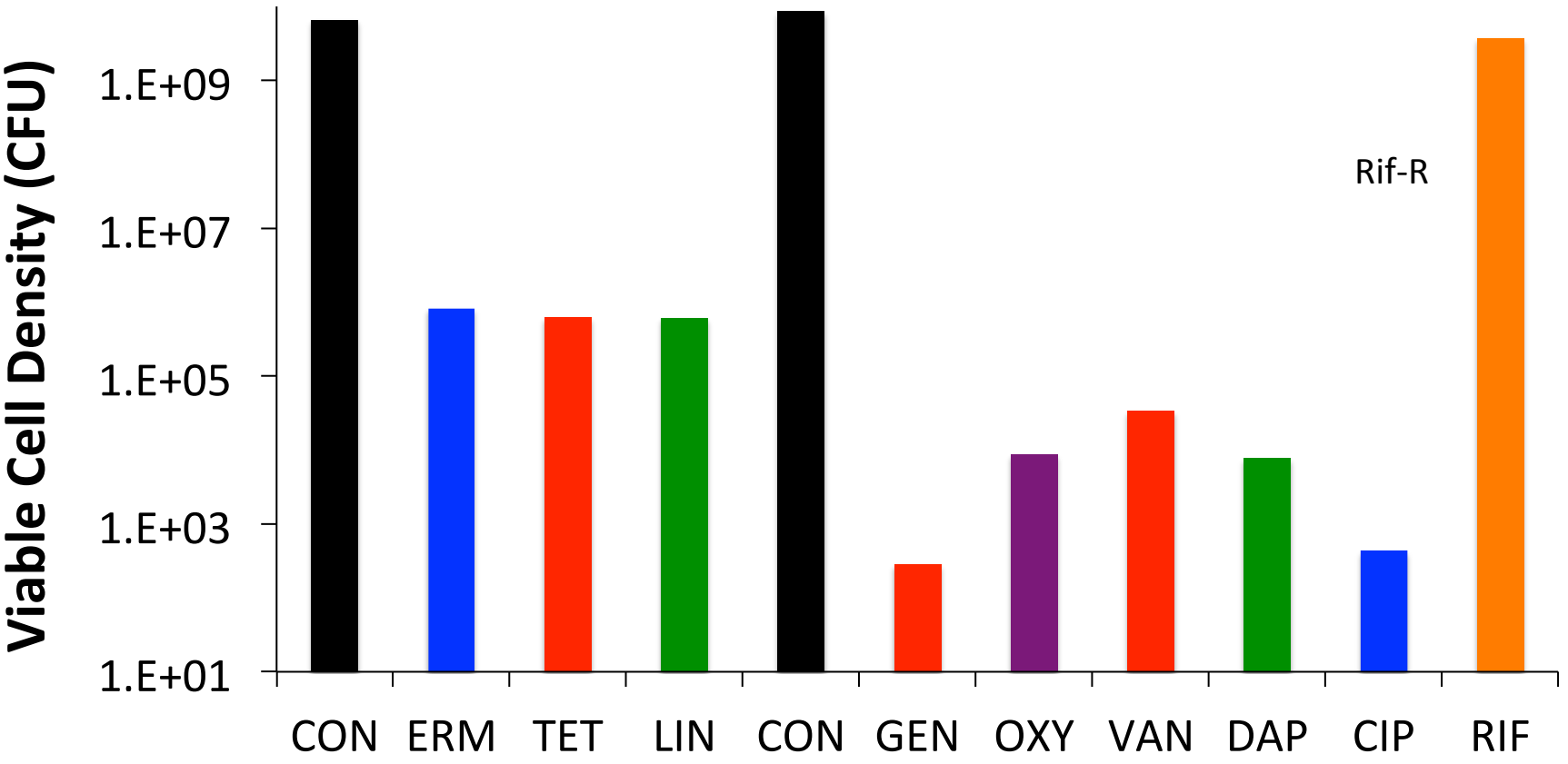
Media: Liquid Cation-adjusted Muller Hinton Broth (MHII)
Agar – Lysogeny Broth (LB)

Antibiotic	MIC µg/ml	Antibiotic	MIC µg/ml
Tetracycline	0.5	Oxacillin	0.30
Erythromycin	0.5	Vancomycin	1.6
Linezolid	1.1	Daptomycin	1.6
Gentamicin	0.8	Rifampin	0.002
Ciprofloxacin	0.16		

Time Kill Experiments 10X MIC

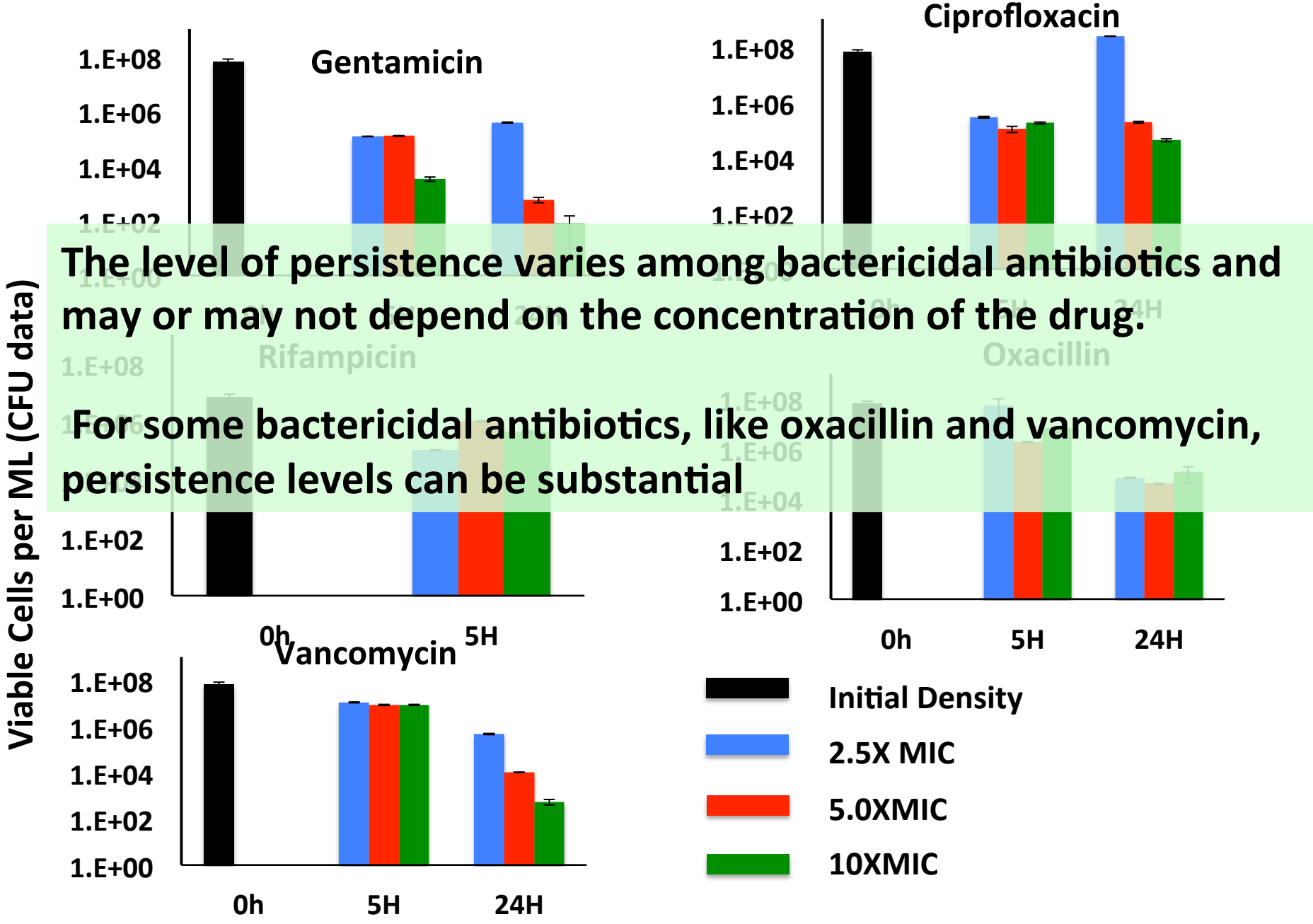


Viable Cell Density at 24 Hours (Persisters)



The fraction of "persisters" produced by bacteriostatic drugs exceeds that of bactericidal. The latter, however, varies among drugs.

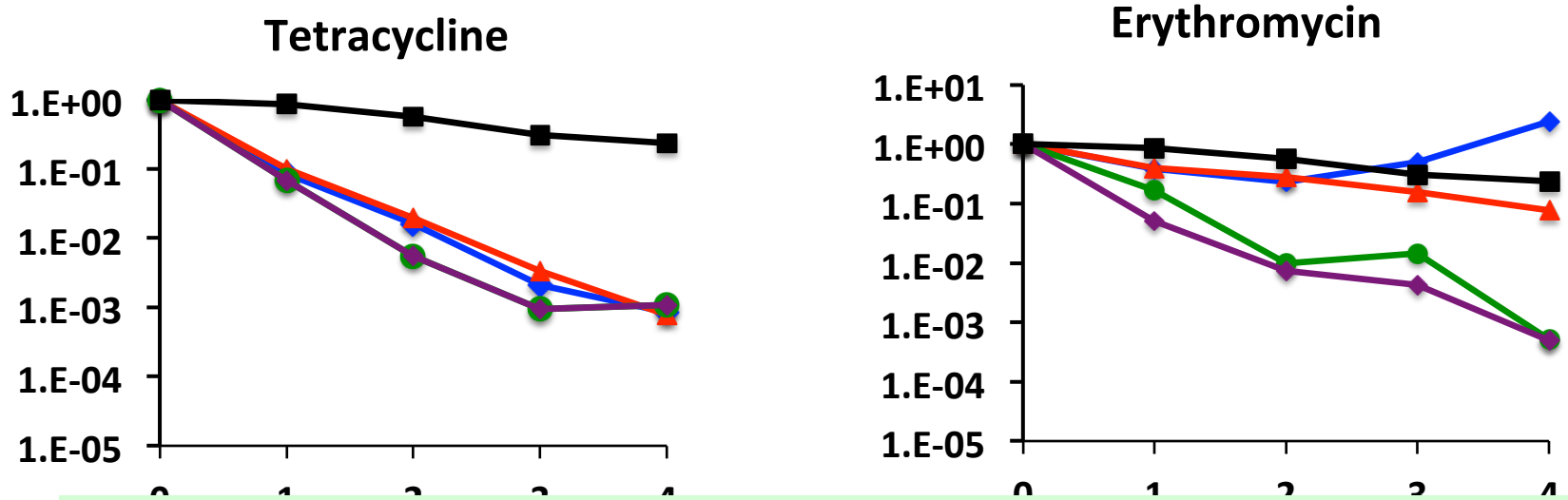
Persistence Levels



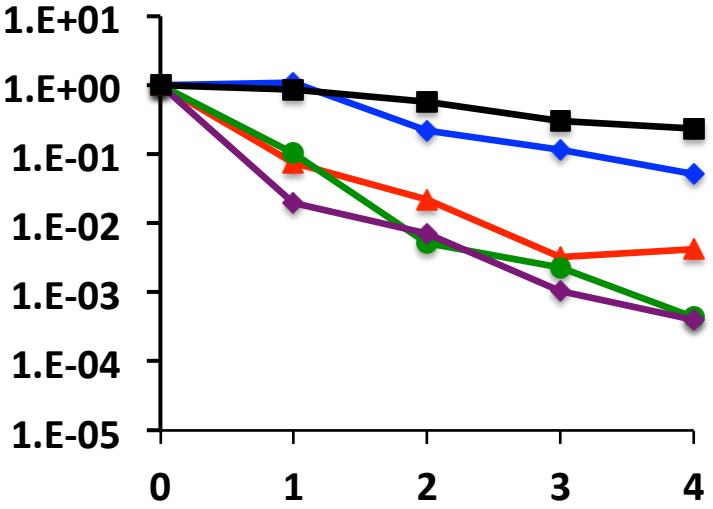
The level of persistence varies among bactericidal antibiotics and may or may not depend on the concentration of the drug.

For some bactericidal antibiotics, like oxacillin and vancomycin, persistence levels can be substantial

Relative Survival of *S. aureus* Exposed to Bacteriostatic Drugs



Bacteriostatic antibiotics are somewhat bactericidal



Density (CFU) Relative to Day 0

Time (days)

Control No antibiotic

Inoculum

2 X MIC – 4.2×10^5

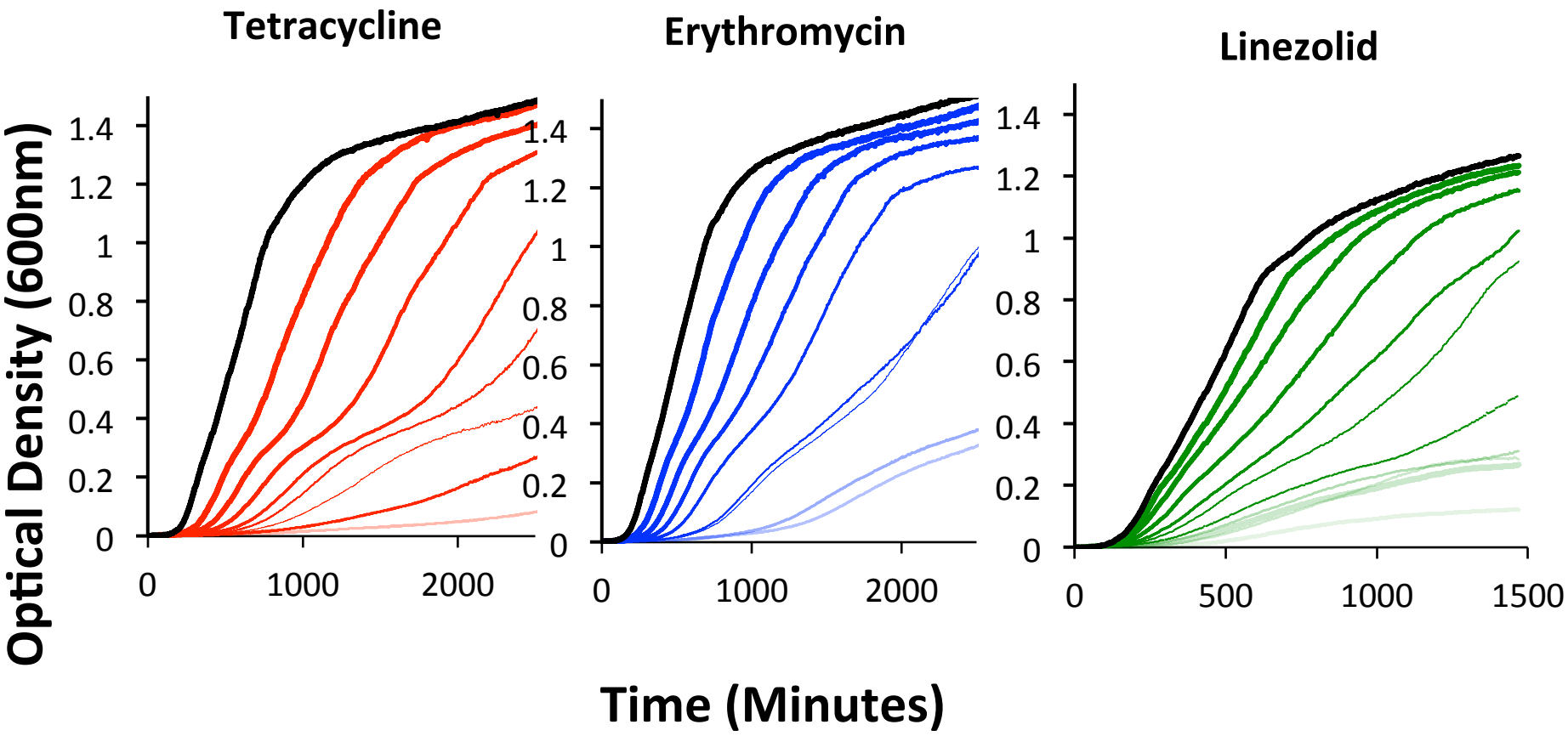
10 XMIC – 4.2×10^5

50 XMIC – 5.2×10^5

10 XMIC – 4.0×10^7

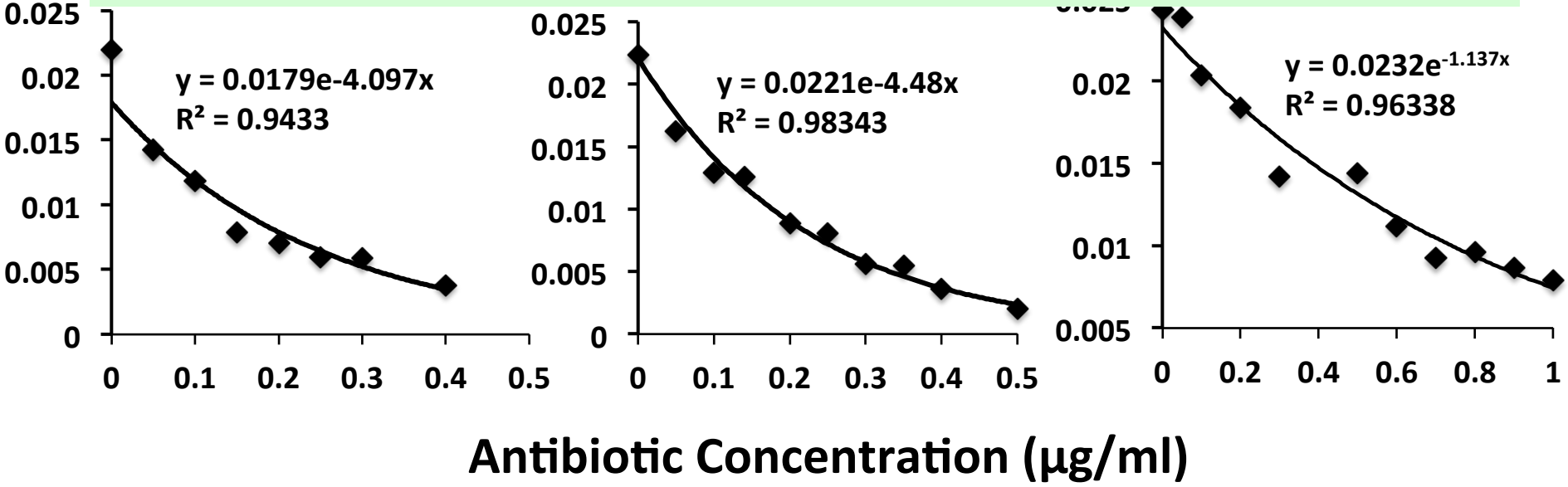
* Means of 3 independent experiments

Sub-MIC Growth Dynamics



Sub-MIC Maximum Exponential Growth Rate (per minute)

At sub-MIC concentrations there is an exponential relationship between the maximum rate of growth of bacteria and the concentration of the antibiotic $\psi(A) = ae^{-bA}$



Antibiotic Concentration (µg/ml)

Antibiotic Concentration-Dependent Growth Rate (Hill Function)

$$\psi(A) = \psi_{MAX} - \left(\psi_{MAX} - \psi_{MIN}\right)\left[\frac{\left(\frac{A}{MIC}\right)^{\kappa}}{\left[\left(\frac{A}{MIC}\right)^{\kappa} - \frac{\psi_{MIN}}{\psi_{MAX}}\right]}\right]$$

Ψ(A) – Rate of growth-death at an antibiotic concentration of A μg/ml)

Ψ_{MAX} – Maximum rate of growth per hour

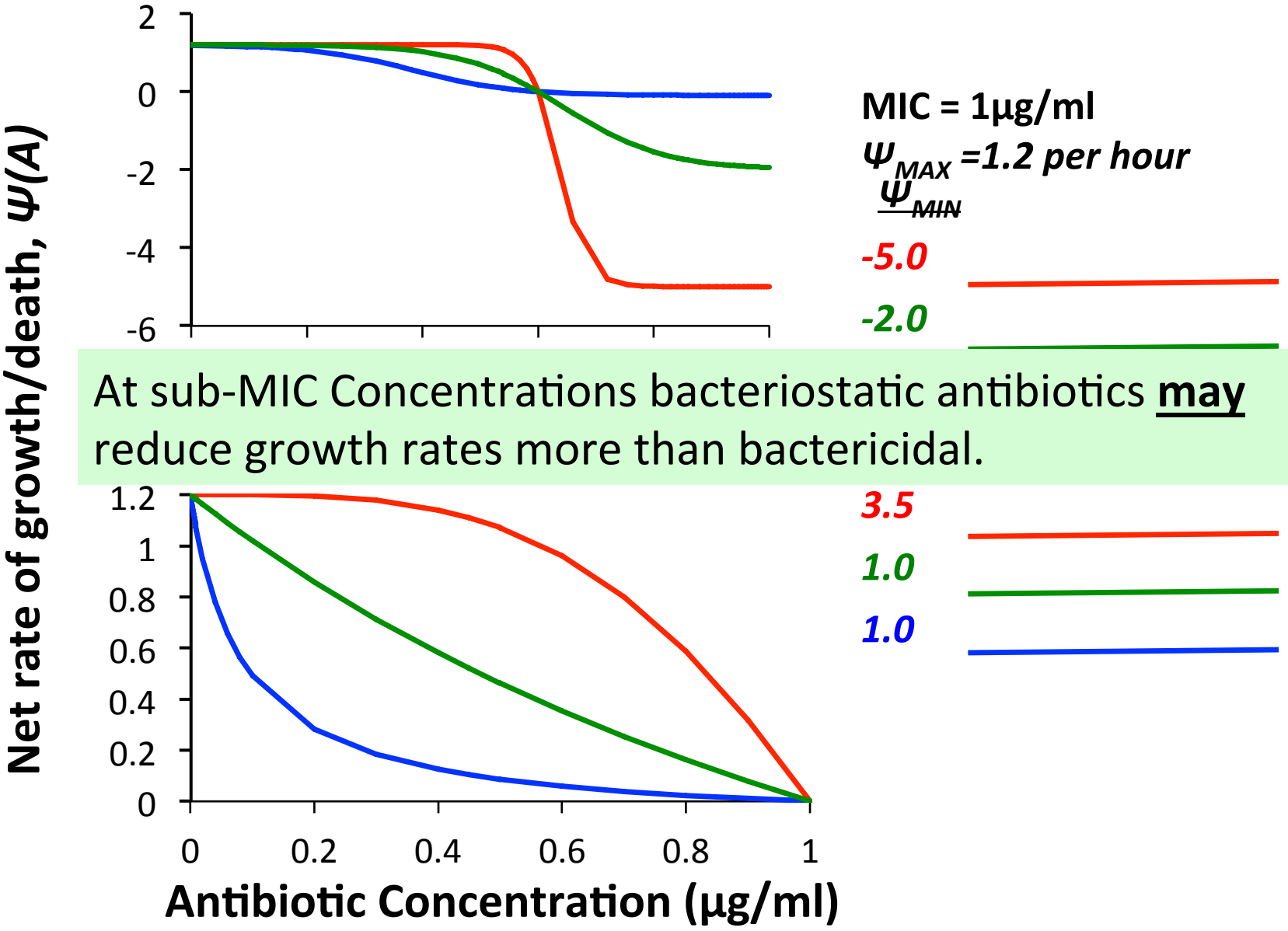
Ψ_{MIN} – Maximum rate of growth (Maximum kill rate) per hour

κ- Hill Coefficient (a shape parameter)

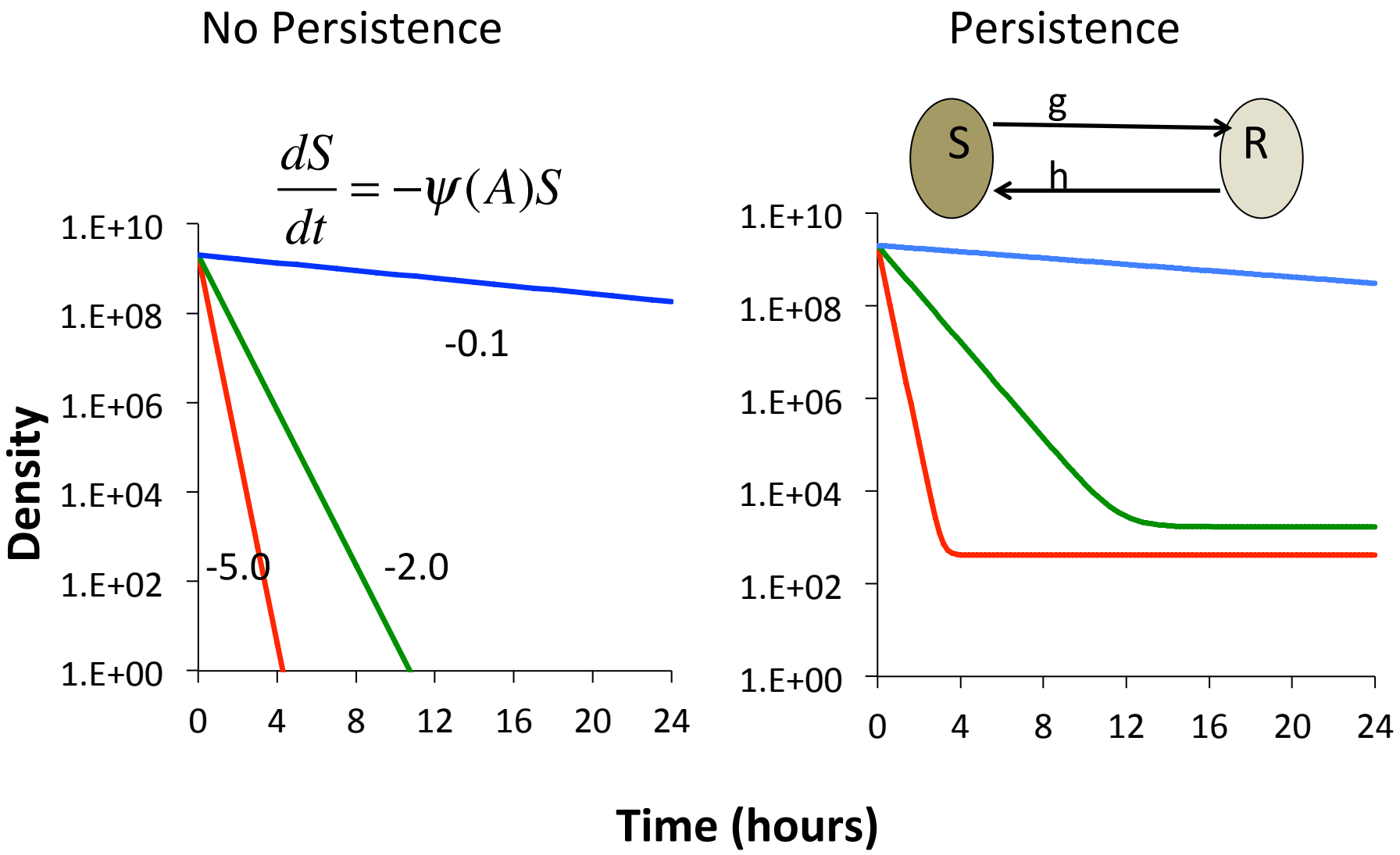
MIC – Minimum inhibitory Concentration

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*

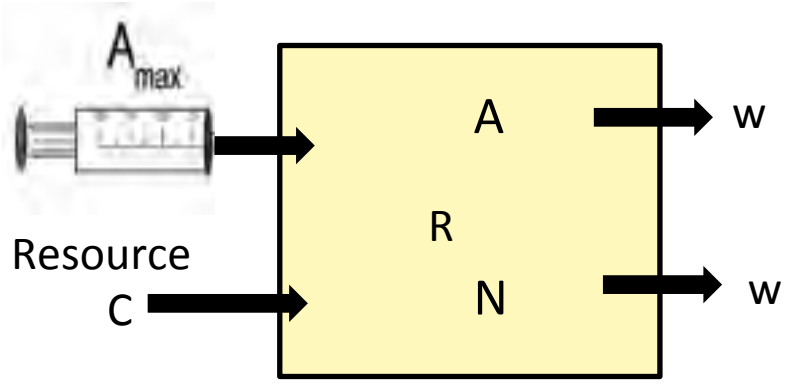
Hill Function Pharmacodynamics



Predicted Time Kill Dynamics Constant Antibiotic Concentration



Simple Model of Antibiotic Treatment



$$\frac{dR}{dt} = w(C-R) - \left(\frac{R}{R+k}\right) e(V_N N)$$

$$\frac{dA}{dt} = -d_A A - wA$$

$$\frac{dN}{dt} = N\psi(A,R)\left(\frac{R}{R+k}\right) - wN$$

Where

$$\psi(A,R) = \left(\psi_{MAX} - (\psi_{MAX} - \psi_{MIN})\left[\frac{\left(\frac{A}{MIC}\right)^{\kappa}}{\left[\left(\frac{A}{MIC}\right)^{\kappa} - \frac{\psi_{MIN}}{\psi_{MAX}}\right]}\right]\right)\left(\frac{R}{R+k}\right)$$

In the simulations $\mathbf{A_{MAX}}$ µg/ml of the antibiotics are added every **Δ** (hours)

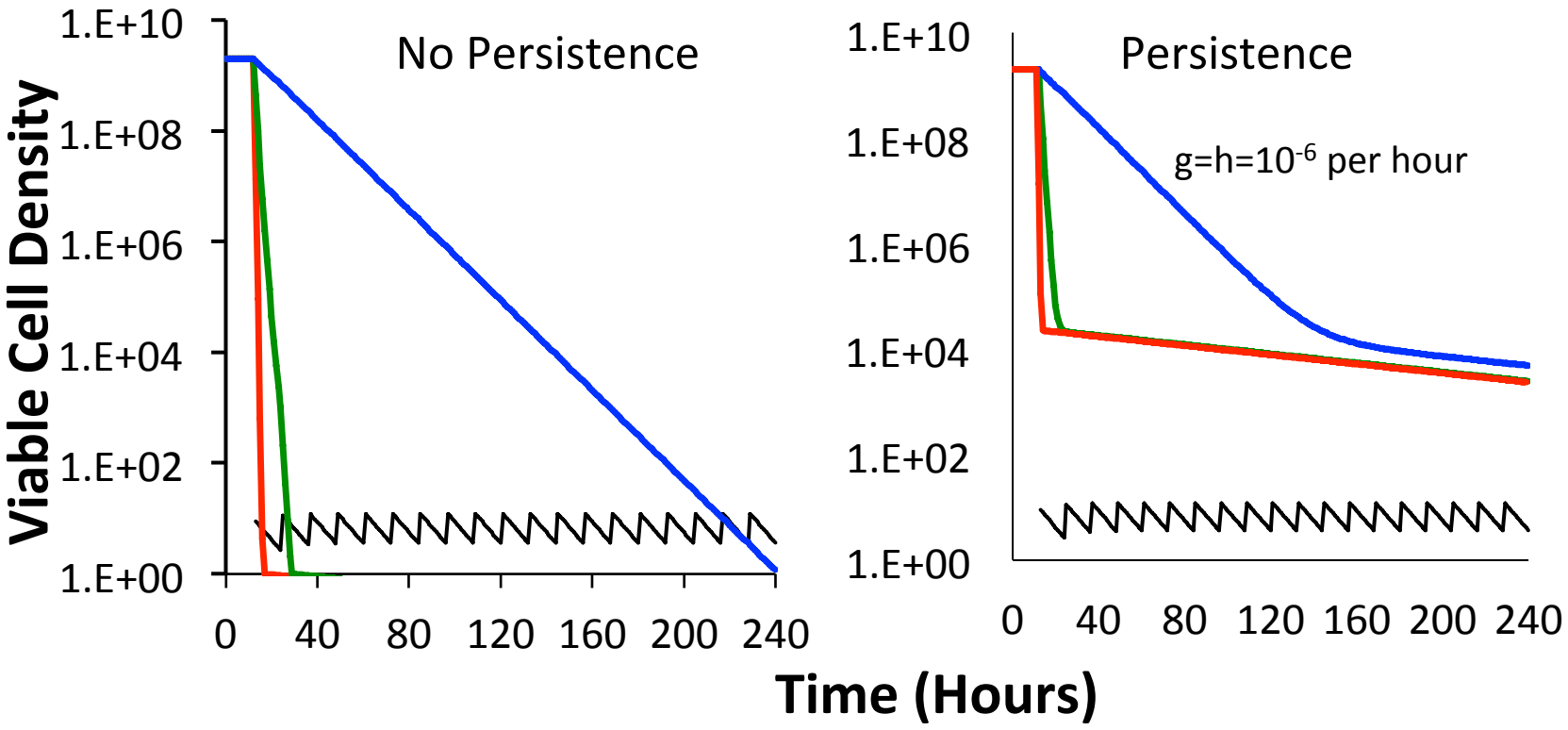
C and R are, respectively the concentration of the limiting resource in the reservoir and in the vessel

e- µg the amount of resource needed to produce a new cell

k –the concentration of the resource where the growth rate is half its maximum value

d_A - decay rate for the antibiotic

Antibiotics as the Sole Mechanisms of Control

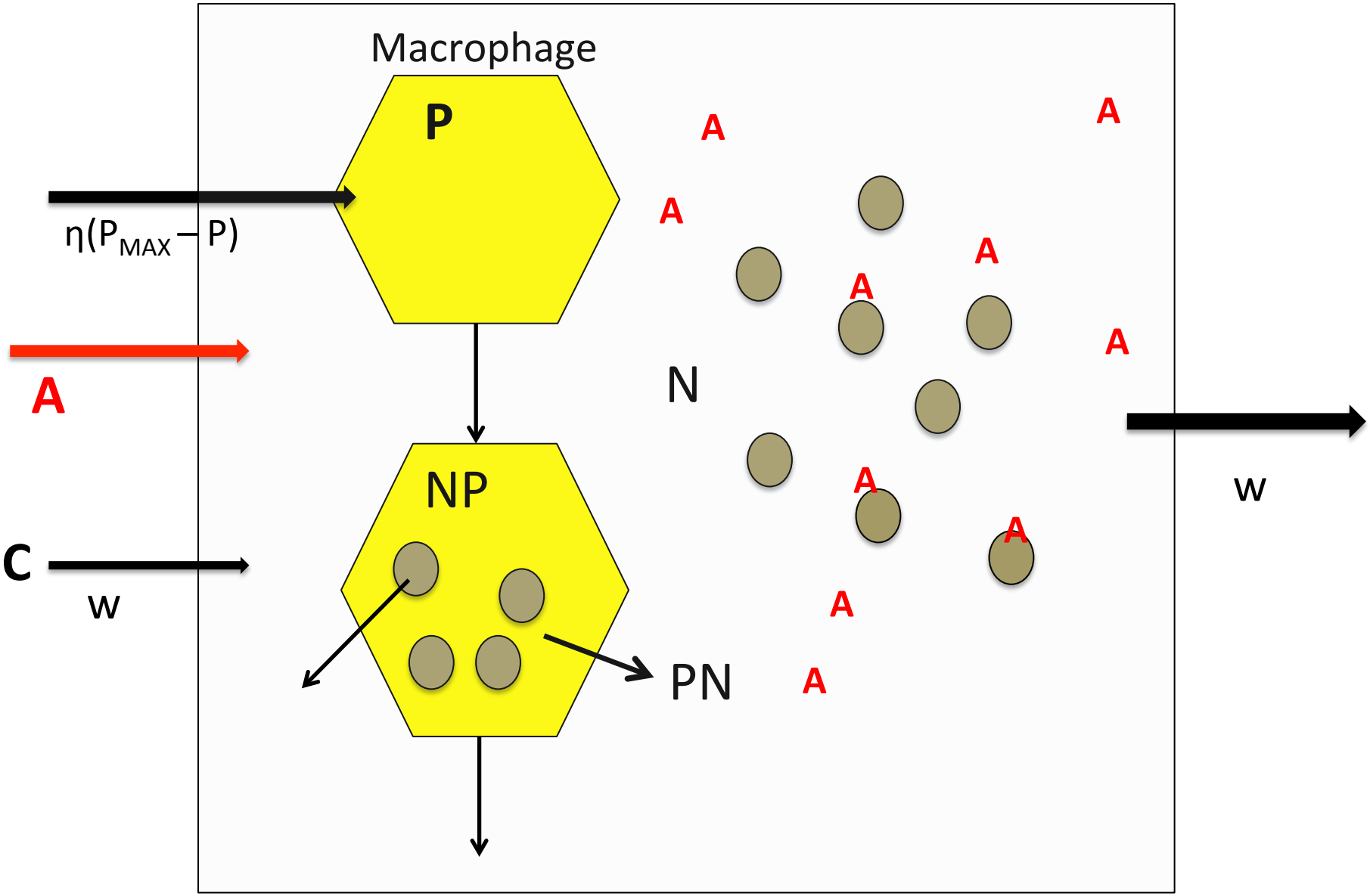


w= 0.1 per hour
C=1000 µg/ml
e = 5×10^{-7} µg per cell
A_{MAX} =10 µg/ml
Δ =12 hours
d_A =0.1 per hour

1- In the absence of persistence, the bacteriostatic antibiotic clears the infection much less rapidly than the bactericidal

2- With persistence, neither the bactericidal or bacteriostatic drug clear the infection

Model of Macrophage Gobbling and Antibiotic Treatment



Model of Macrophage Gobbling and Antibiotic Treatment

$$\frac{dR}{dt} = w(C-R) - e\left(\frac{R}{R+k}\right)\psi N(A)$$

$$\frac{dA}{dt} = -d_A A - wA$$

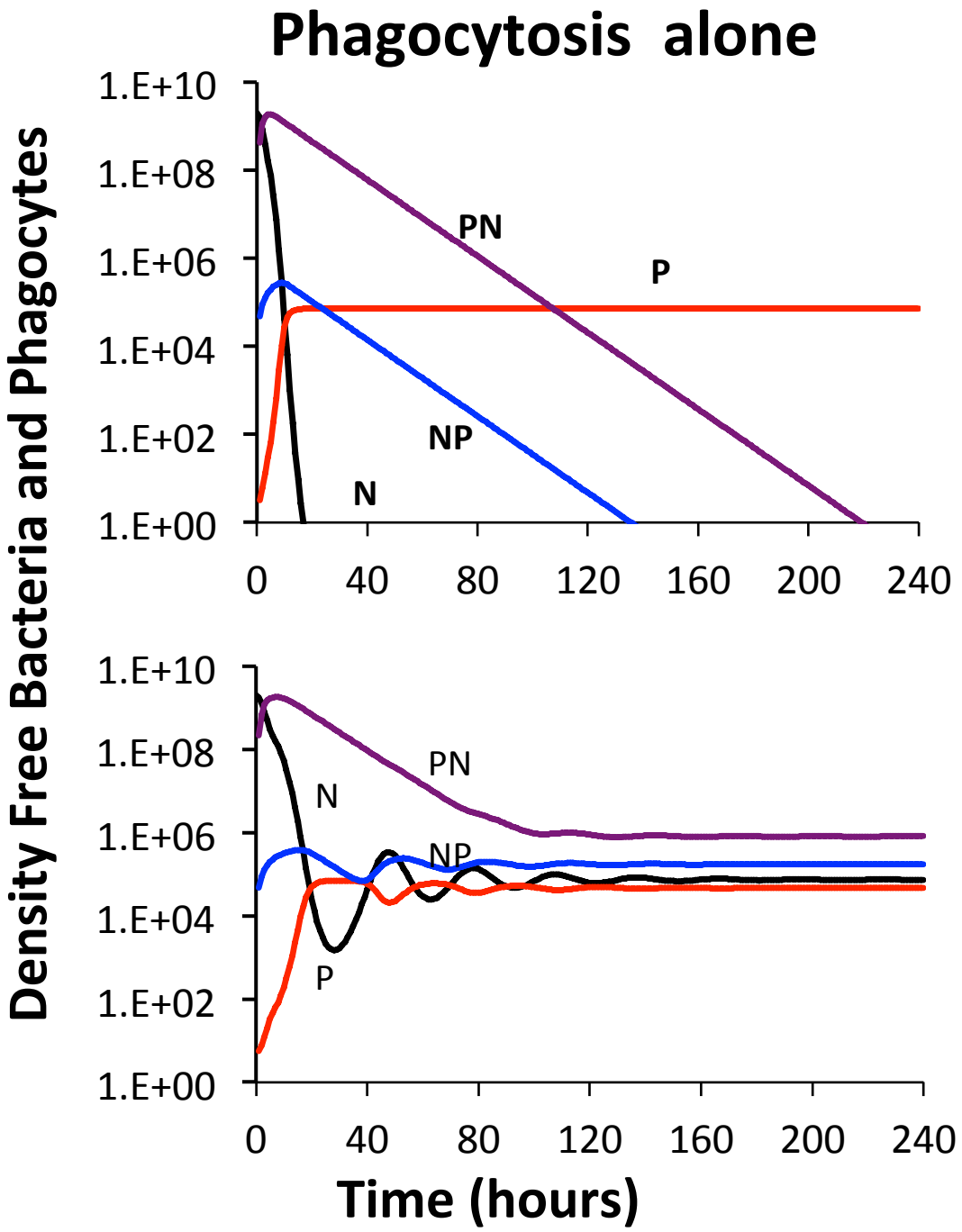
$$\frac{dN}{dt} = \psi(A)N\left(\frac{R}{R+k}\right) - \gamma N(P+P_N) - gN + hN_E - wN$$

$$\frac{dN_E}{dt} = gN - hN_E - \gamma N_E(P+P_N) - wN_E$$

$$\frac{dP}{dt} = \eta(P_{MAX}-P) - \gamma(N+N_E)P - P(d_P+w)$$

$$\frac{dP_N}{dx} = \gamma(N+N_E)P - P_N(d_P+w)$$

$$\frac{dN_P}{dt} = \gamma(N+N_E)(P+P_N) - N_p(\alpha+w)$$



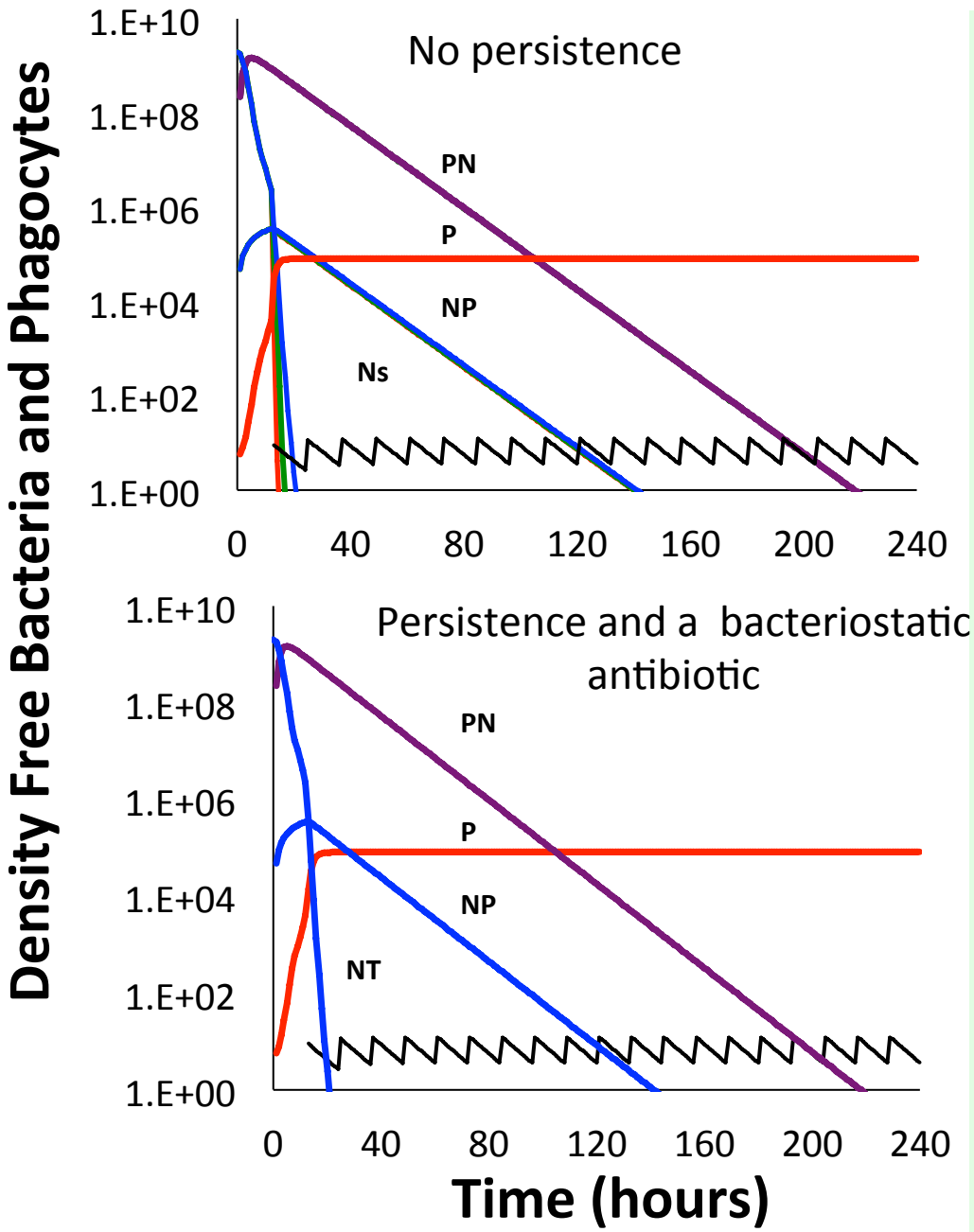
Depending on the parameters, like the rate constant of phagocytosis: Phagocytic leukocytes can:

Clear the infection

Or

Maintain the infection at a density lower than that determined by resources alone.

Phagocytosis and Antibiotic Treatment

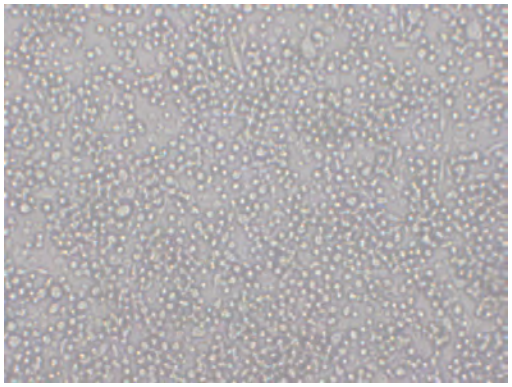


With the phagocytosis parameters in the range for maintenance of the of the bacteria, antibiotics can clear the infection.

There is little difference in the efficacy of the bactericidal and bacteriostatic antibiotics to clear the free bacteria.

With phagocytosis persistence will not preclude the clearance of the infection, even with a bacteriostatic antibiotic

Phagocytosis of Erythromycin treated *S. aureus* Newman



Human monocytic leukemia (THP1) cells in RPMI Media

1- Incubate at 37 C in 95% CO2

2- Treat with PMA to differentia into macrophage and incubate for 72 hours

3- Establish monolayers on the bottoms of 12 well polystyrene plates.

4- Introduce *S. aureus* Neman in RPMI with 10µg/ml Erythromycin

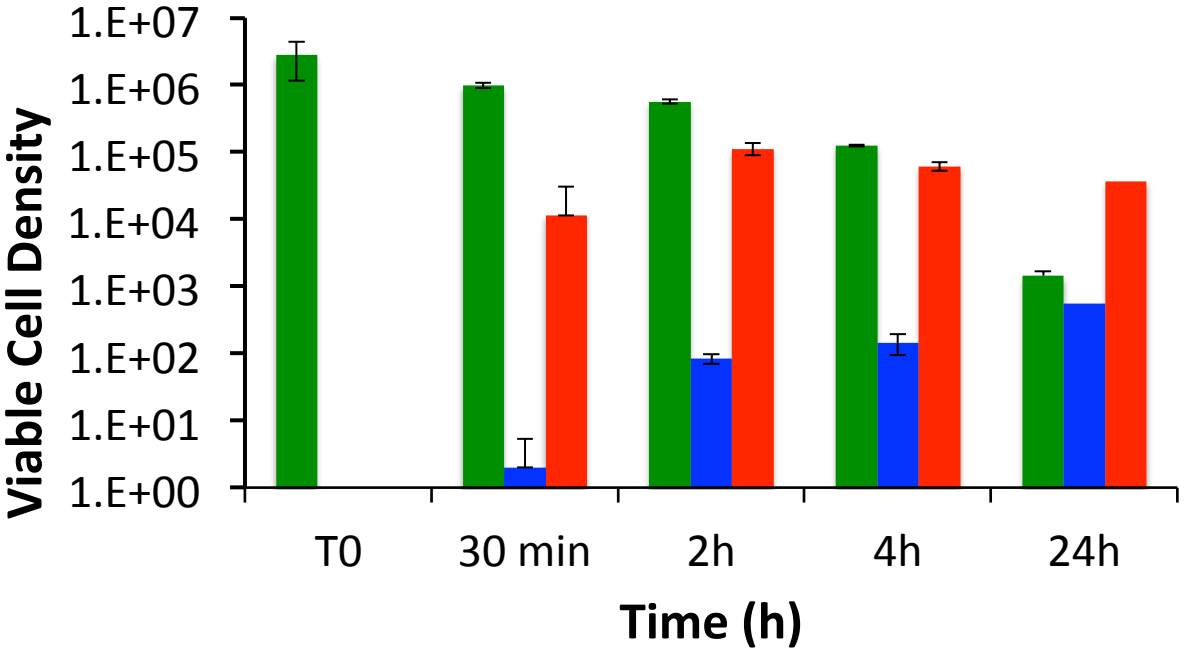
5- Incubate for 2 (or more hours)

6 – Add 200µg/ml Gentamicin to kill extracellular, planktonic bacteria (at the concentrations used, GEN is not antagonistic with ERM). And incubate without shaking.

7 – Wash out the GEN with buffer and by serial dilution and plating estimate the density of viable cells in the suspension.

8 – Add the detergent Nonidet P-40 to release the macrophage - Re-suspend and estimate the densities of bacteria **(PN)**

Rate of loss of free and accumulation of phagocytized *S. aureus*



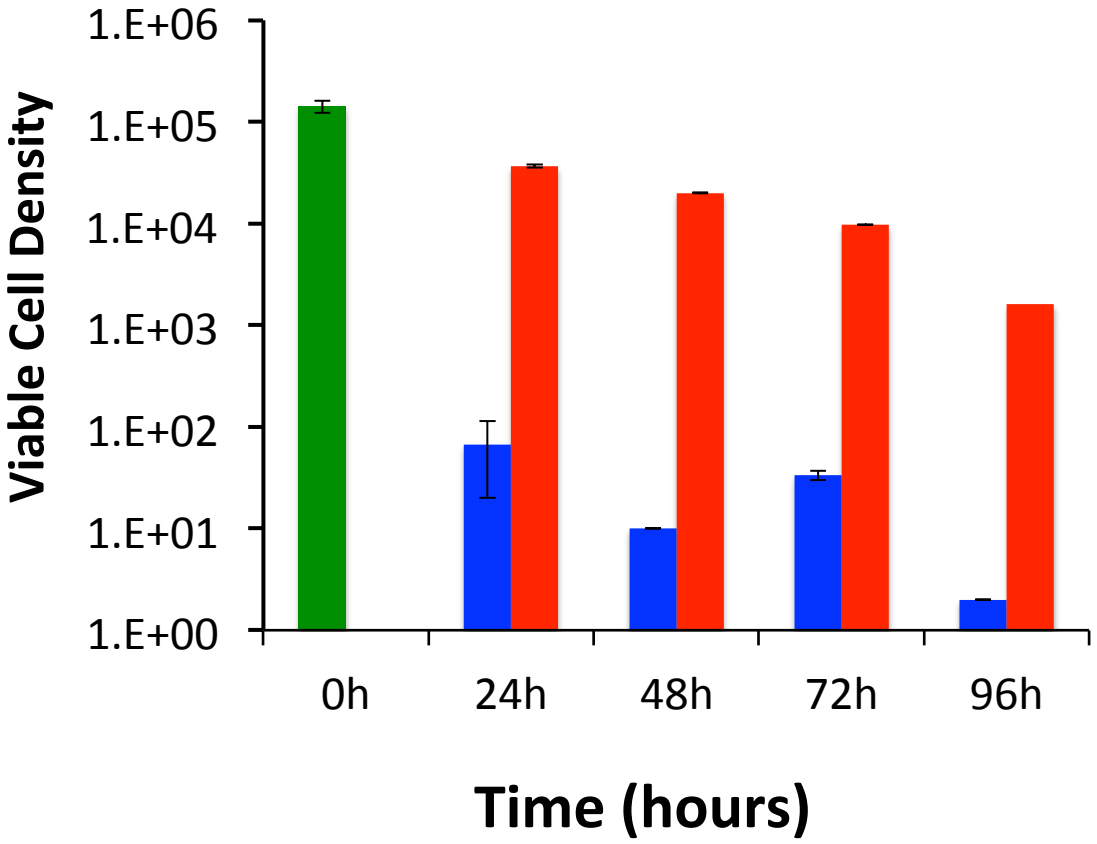
Within 2 hours a substantial fraction of the free bacteria have been gobbled

Free Bacteria

Post Gentamicin Released upon washing

Post Gentamicin Release after detergent treatment (phagocytized)

Survival within the phagocytes



1- By 24 hours a nearly all of the viable, free bacteria have been phagocytized.

2- The *S. aureus* within the macrophage are still viable after 4 days.

“Essentially all models (and model systems) are wrong, some are useful*”

The utility of *in vitro* and *in silico* studies is the generation of testable (and reject-able) hypothesis.

* George Box with (a friendly amendment)

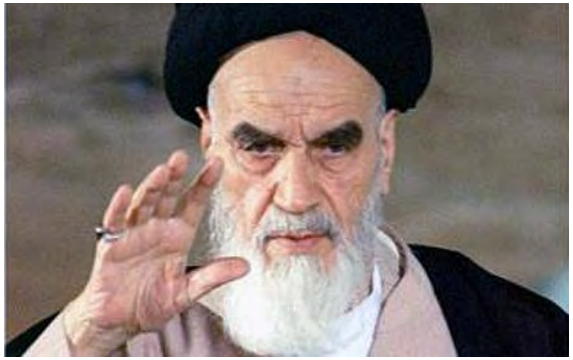
For a broader theoretical consideration of the collaboration of the antibiotics with the innate and adaptive immune system and its implications for acquired resistance see:

Ankomah, P. B.R. Levin 2014. Exploring the collaboration between antibiotics and the immune response in the treatment of acute, self-limiting infections. PNAS 111: 8331-8338

Peter (Pierre) Ankomah

Dr^2 Pierre Ankomah MD/PhD

Defenders of Orthodoxy



Worth Reading as well as Citing

THE LANCET, AUGUST 16, 1913.

Address in Pathology

ON

CHEMOTHERAPEUTICS:

SCIENTIFIC PRINCIPLES, METHODS, AND RESULTS.

Delivered before the Seventeenth International Congress of Medicine

BY WIRKL. GEH. OBER-MED.-RAT PROFESSOR DR. PAUL EHRLICH,

DIRECTOR OF THE ROYAL INSTITUTE FOR EXPERIMENTAL THERAPY, FRANKFURT AM M.

MR. PRESIDENT, LADIES AND GENTLEMEN,—It must be a great pleasure and a special honour for all of us to meet here personally on British soil for a scientific purpose, in order to take part in the great work which will be of benefit to the whole world.

that "Corpora non agunt nisi liquida," then for chemotherapy the principle is true that "Corpora non agunt nisi fixata." When applied to the special case in point this means that parasites are only killed by those materials to which they have a certain relationship, by means of which they are fixed by them. I call such substances "parasitotropic." But I should like immediately to add that there are evident exceptions to this law. So, for instance, we are acquainted with a small series of cases in which the apparent therapeutic results are obtained, although the allied substances in question do not possess parasite-destroying qualities. That is the case in the infiltration of the subcutaneous tissues, which is caused by a kind of yeast (sporotrichosis). Here Block proved that the clinically highly therapeutic iodide of potassium first of all dissolves the cells of the infiltration, whilst the parasites, as such, are not in the first instance attacked. But, in any case, it is safer and better for the development of chemotherapy not to build on the basis of exceptional work, but it is better to start with such substances, which produce the destruction of parasites by fixation.

Now it has been assumed in different quarters that some

A conjecture and pretentious recommendation

We conjecture that the widely assumed therapeutic superiority of bactericidal antibiotics over the so-called “bacteriostatic” drugs that has become almost a “mantra” for the clinical application of antibiotics and the development of new drugs, are a reflection of this failure to consider the role of host immune system in the clearance in bacterial infections.

We recommend that rather than focus solely on the pharmacodynamics and pharmacokinetics of antibiotics, consideration should be given to the action of these drugs in combination with the host's immune defenses.

2- Antibiotic (and phage) treatment of physically structured populations of bacteria

The Great Bio Film

Starring

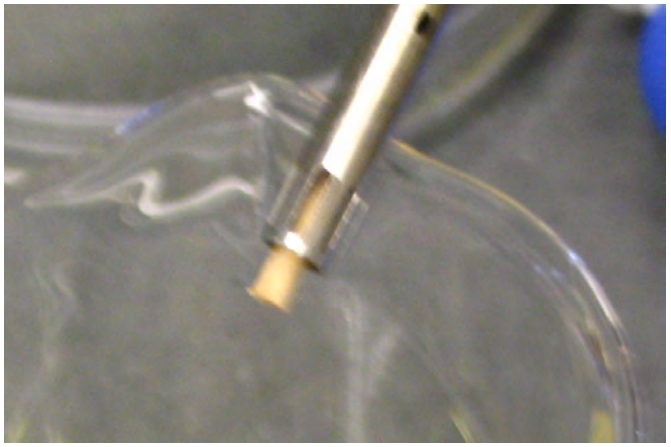
Kim Garner

Amy Kirby

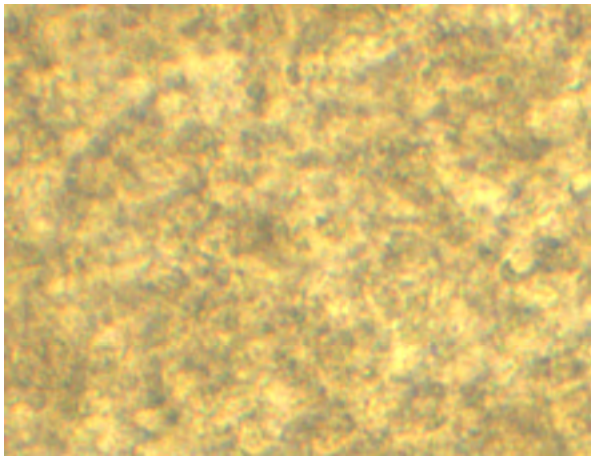
An *Ec*LF production with a cast of billions

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

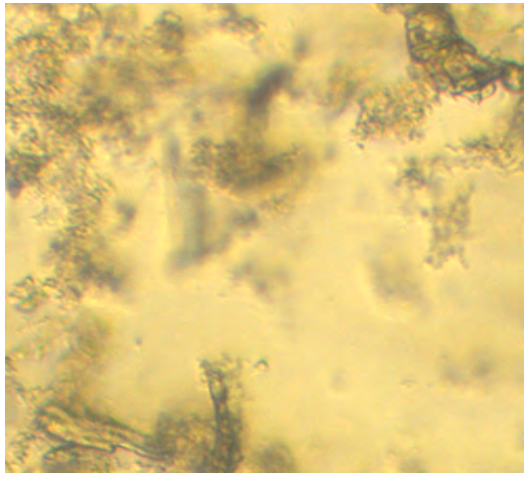
Biofilm Disruption Procedure – (Stick whirling)



Stick in a Tissue Tearor

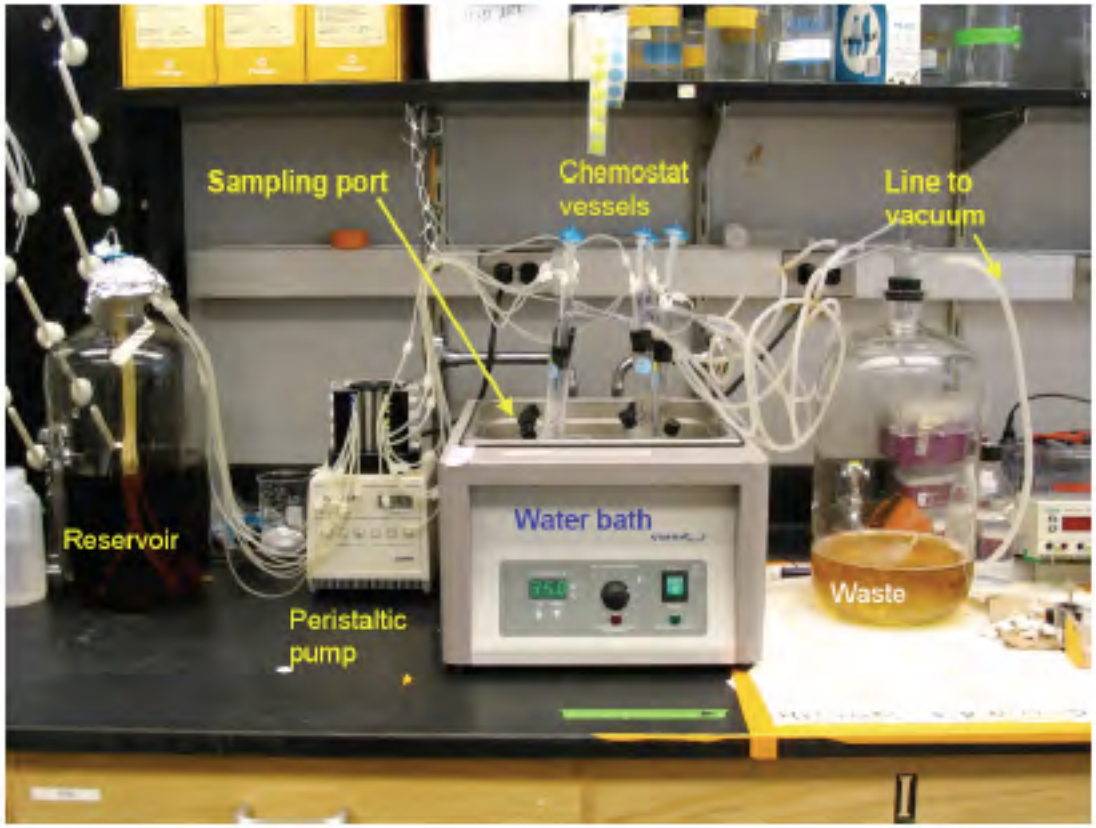


Undisrupted *clsA* biofilm



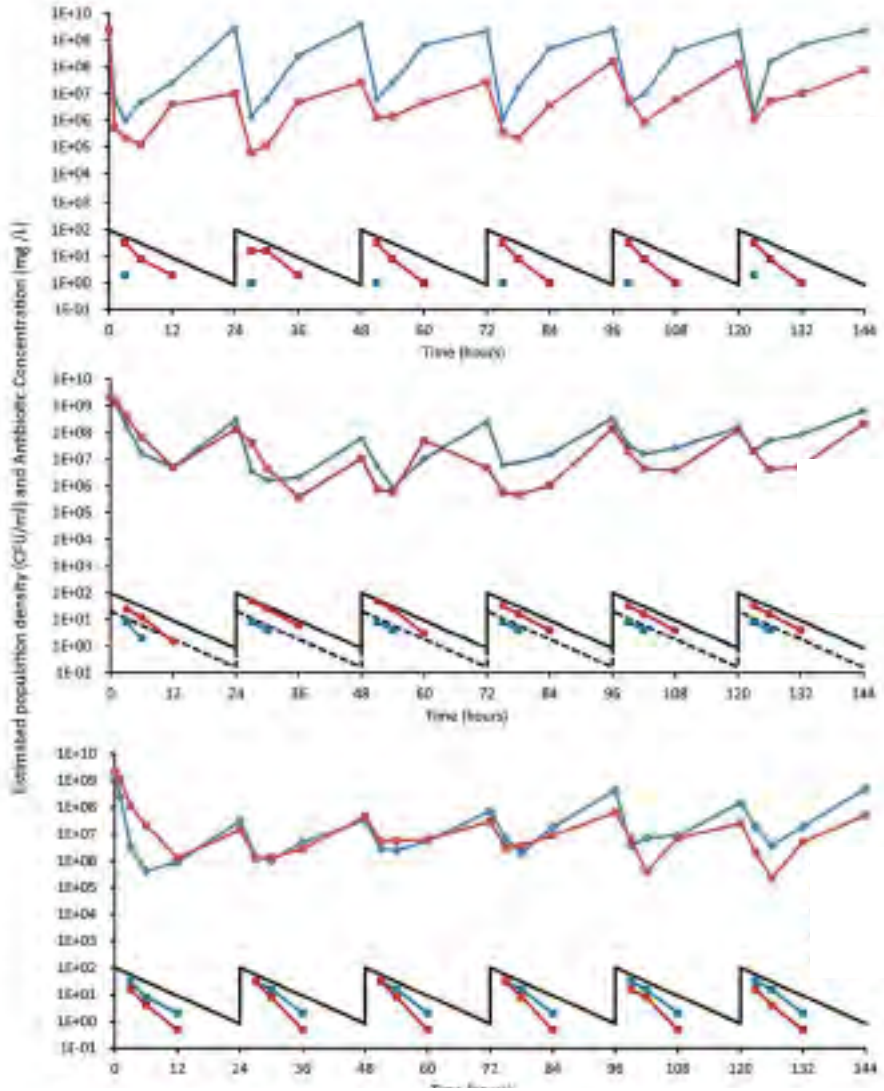
Stick disrupted *clsA* biofilm

S. aureus Newman in Continuous Culture



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

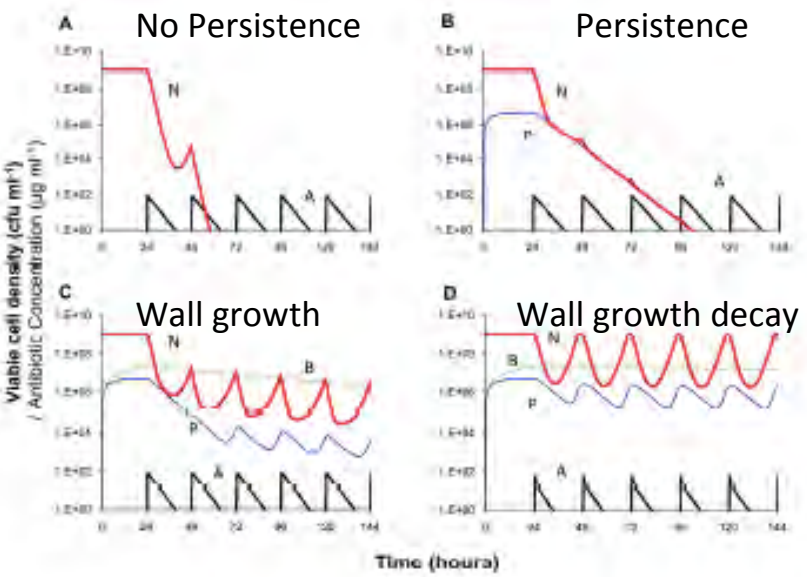
S. aureus Newman in Continuous Culture



Dap
Gen

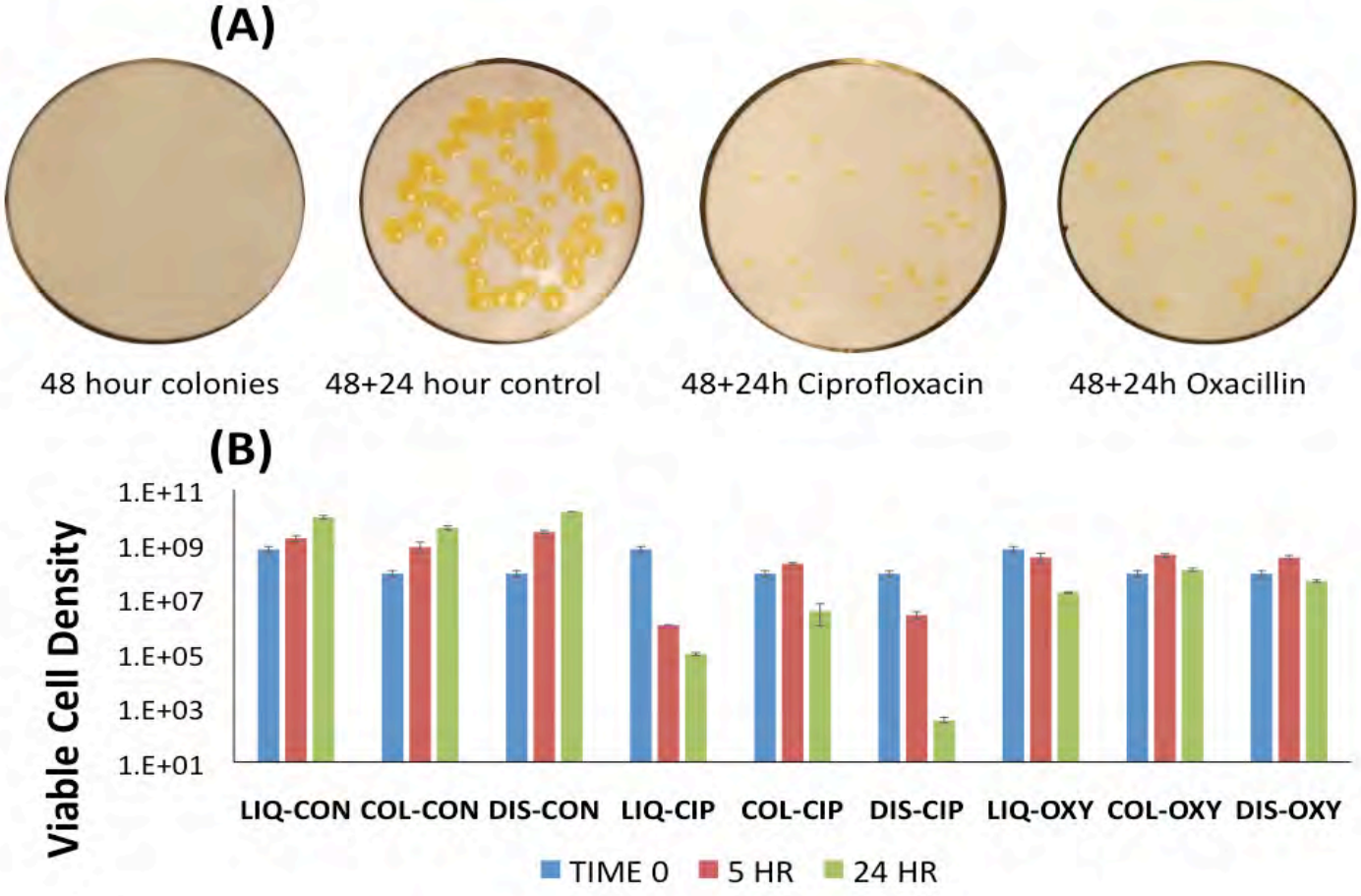
Lin
Van

Cip
Ɔxy



Time (Hours)

Bacterial Growth and Pharmacodynamics and the Pharmacodynamics of bacteria growing as colonies

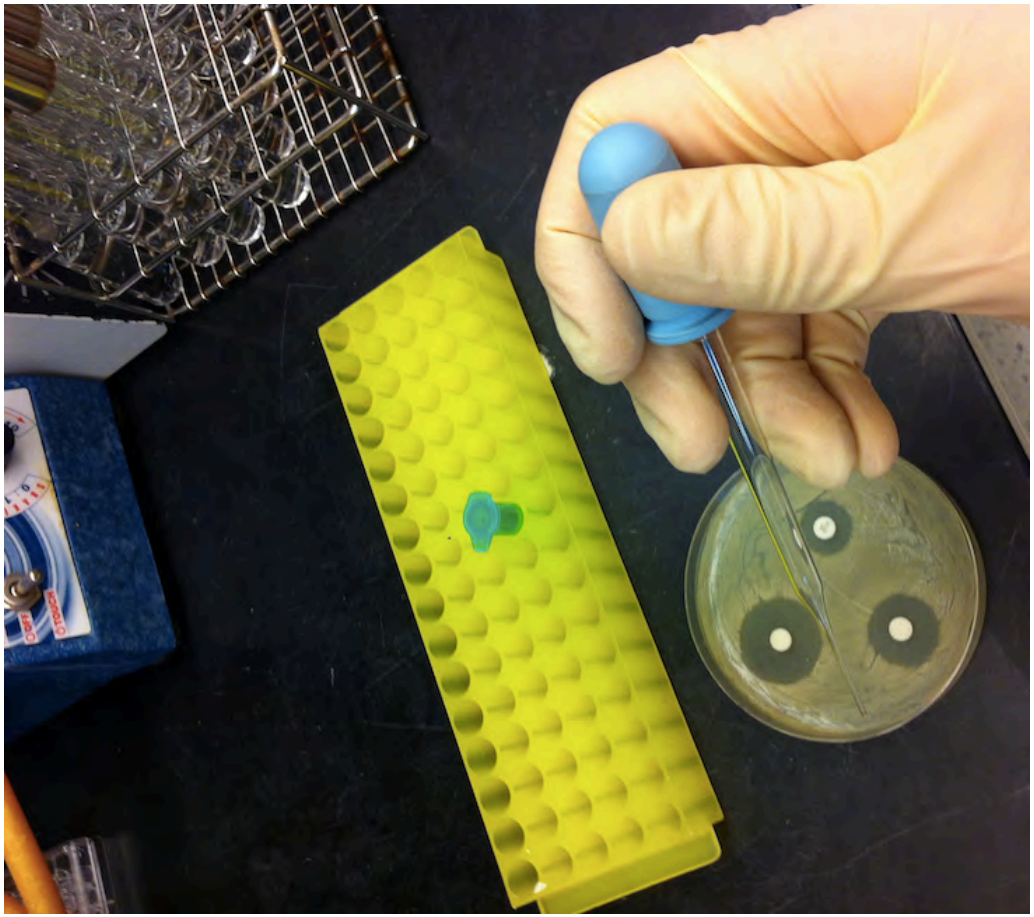


Xinxian Shao

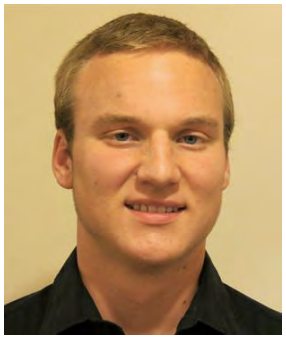
Jason Jeong

Justin Young KIm

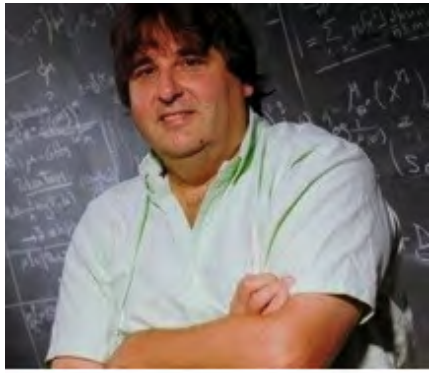
The Pharmacodynamics of Physically Structured Populations of Bacteria: What Disk Diffusion Assays Tell us



Veronique Perrot

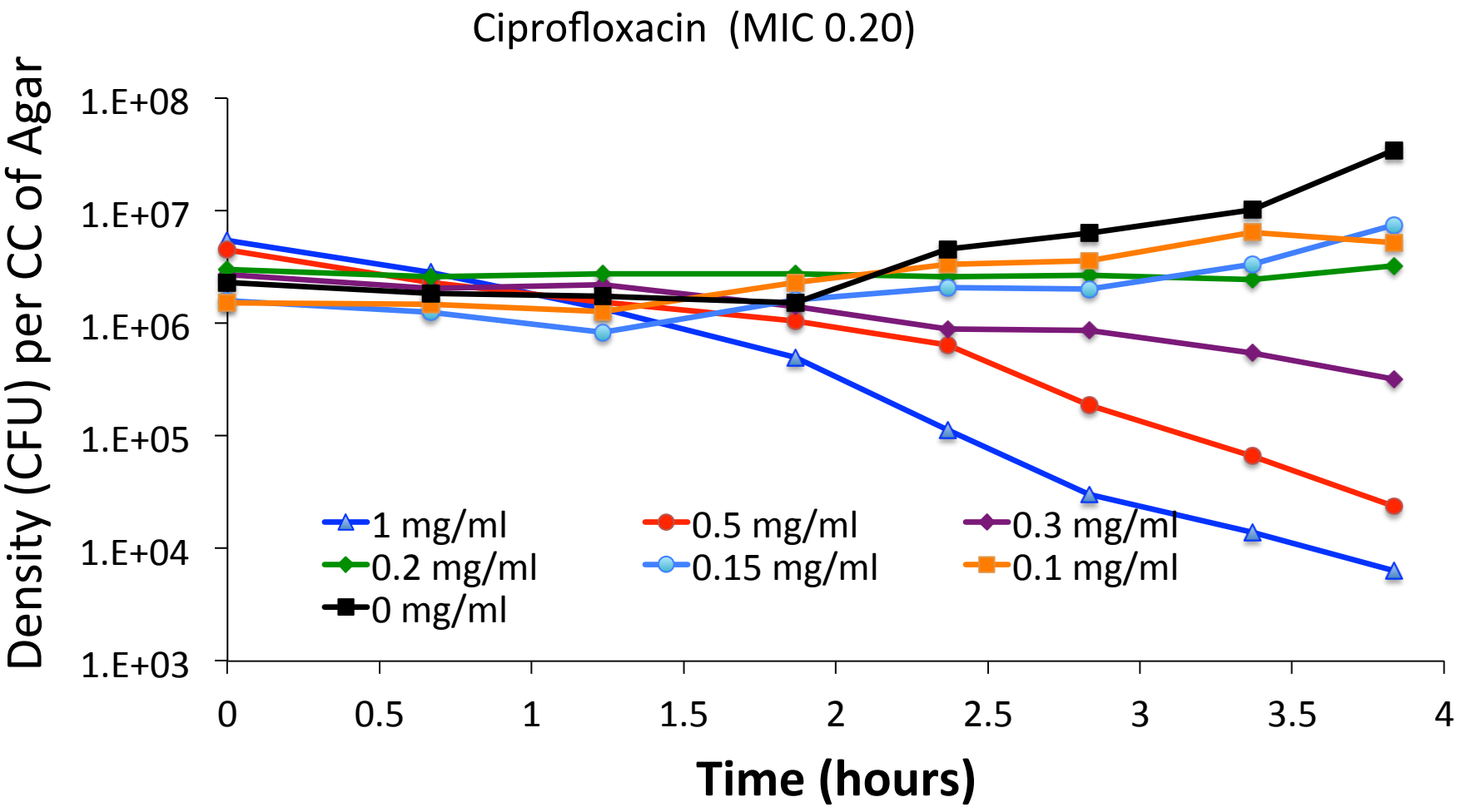


James Dickey

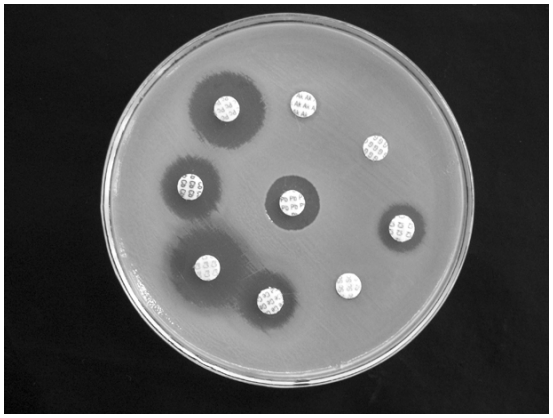


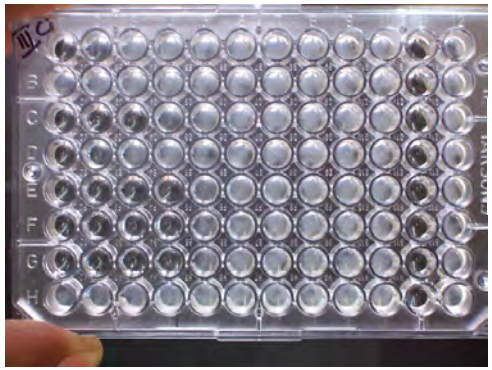
Howie Weiss

Time Kill Experiment on Plates

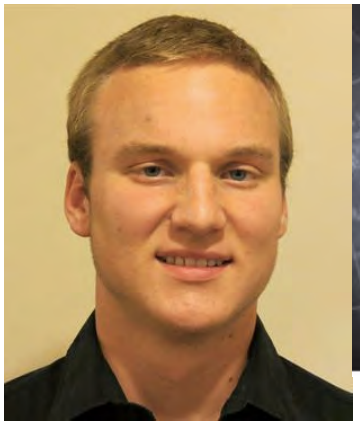


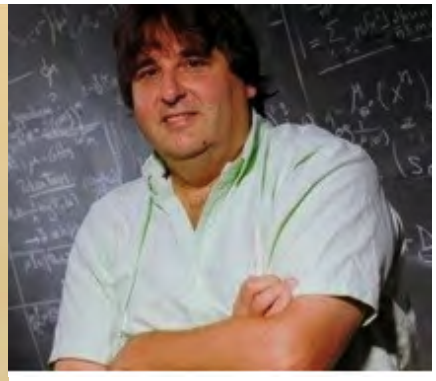
Estimating MICs from Zones of Inhibition Data





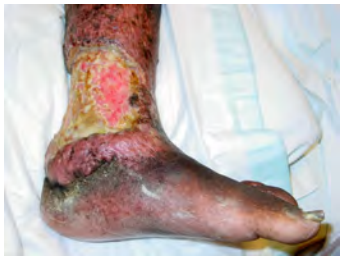
EUCAST
EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

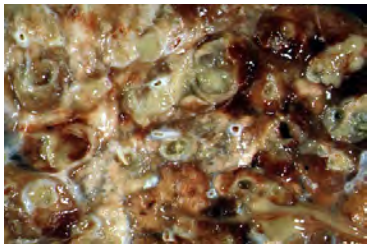




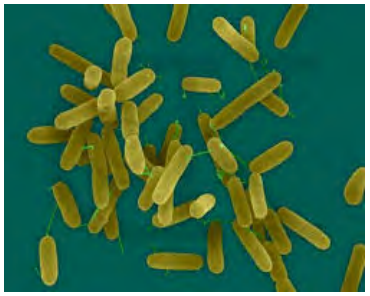
Veronique Perrot Roland Regoes Nathanaël Hozé James Dickey Howie Weiss

Combined Phage and Antibiotic Treatment





Diabetic skin ulcers Cystic Fibrosis Lung Biofilms

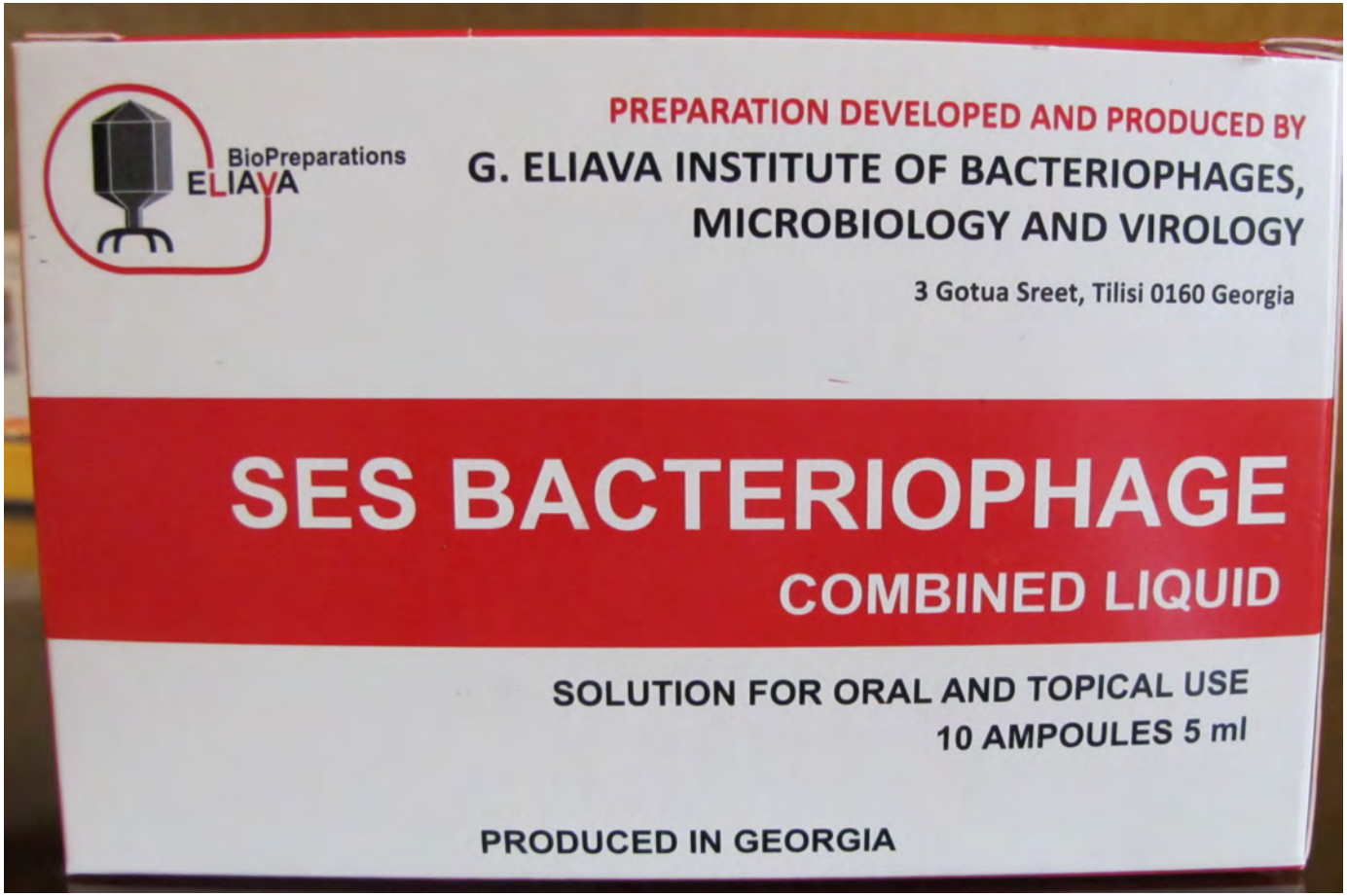


Pseudomonas aeruginosa

Waqas Chaudhry

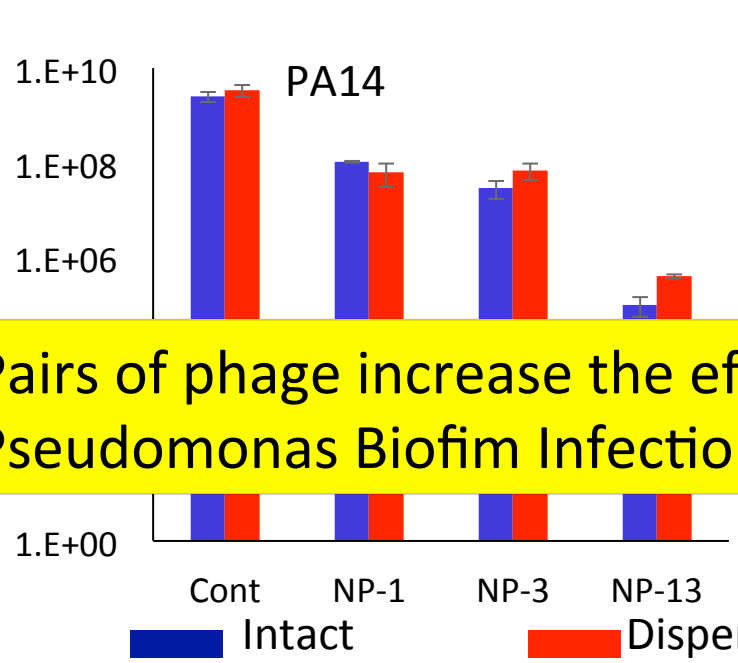
Jeny Concepcion-Acevedo

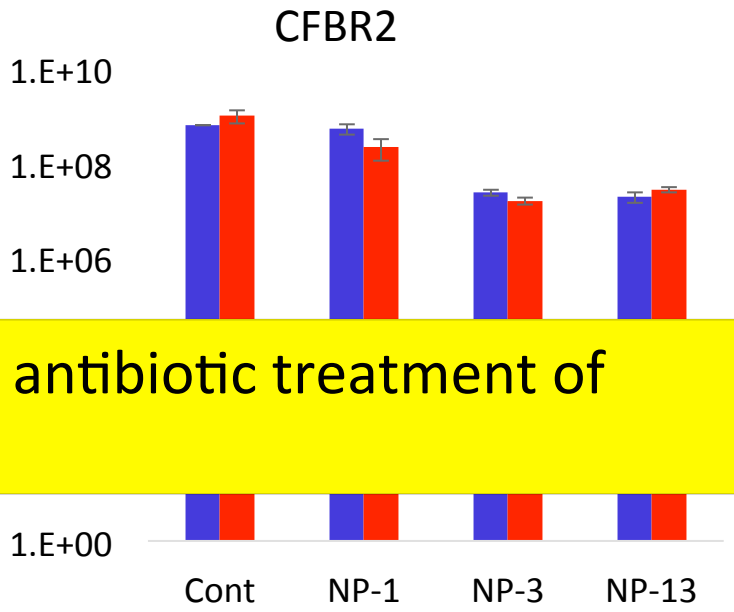
Therapeutic Phage from the Eliava Institute in Tbilisi (2013)



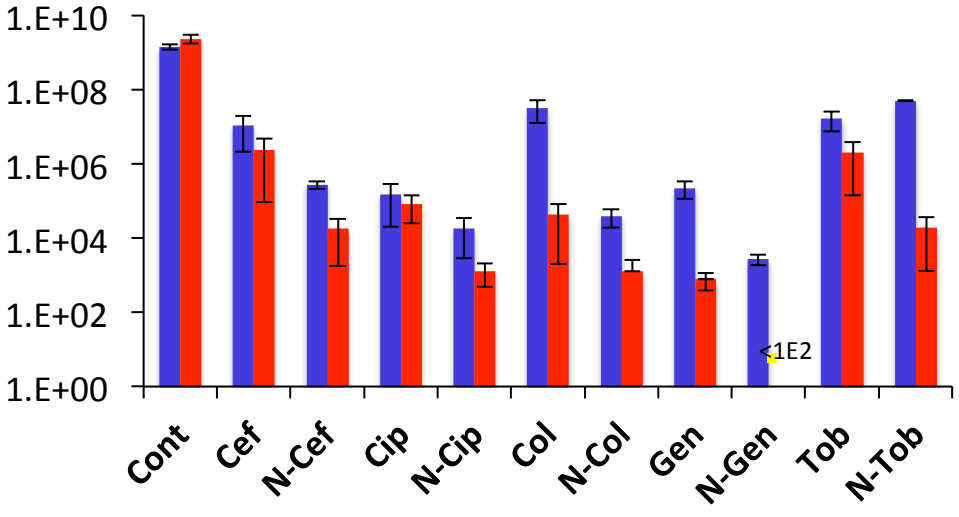
Phage and antibiotic Treatment

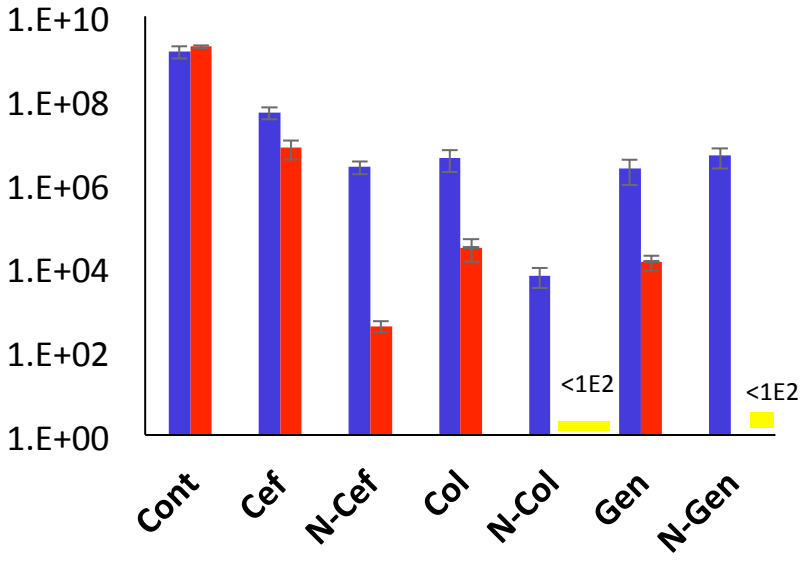
Pseudomonas aeruginosa



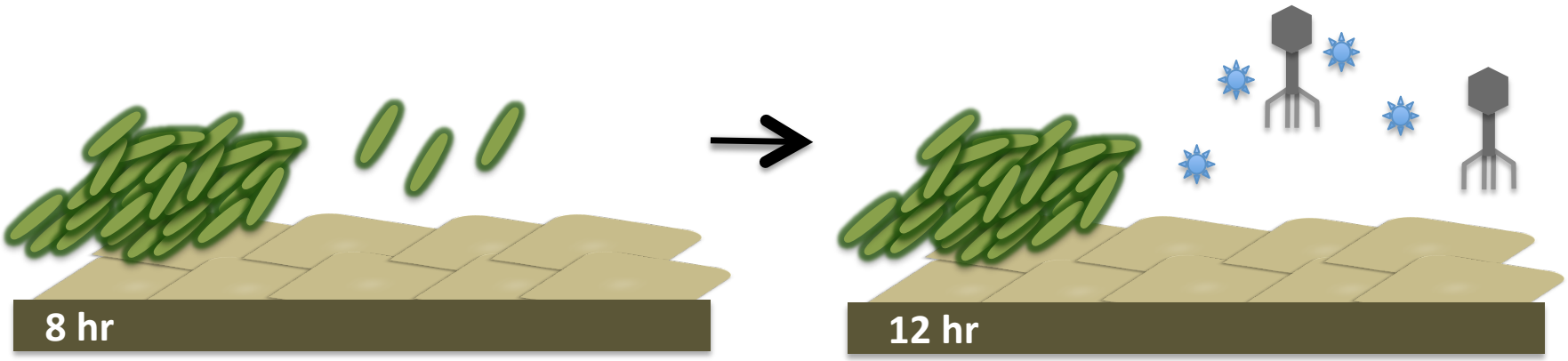


Pairs of phage increase the effiacy of antibiotic treatment of Pseudomonas Biofim Infections

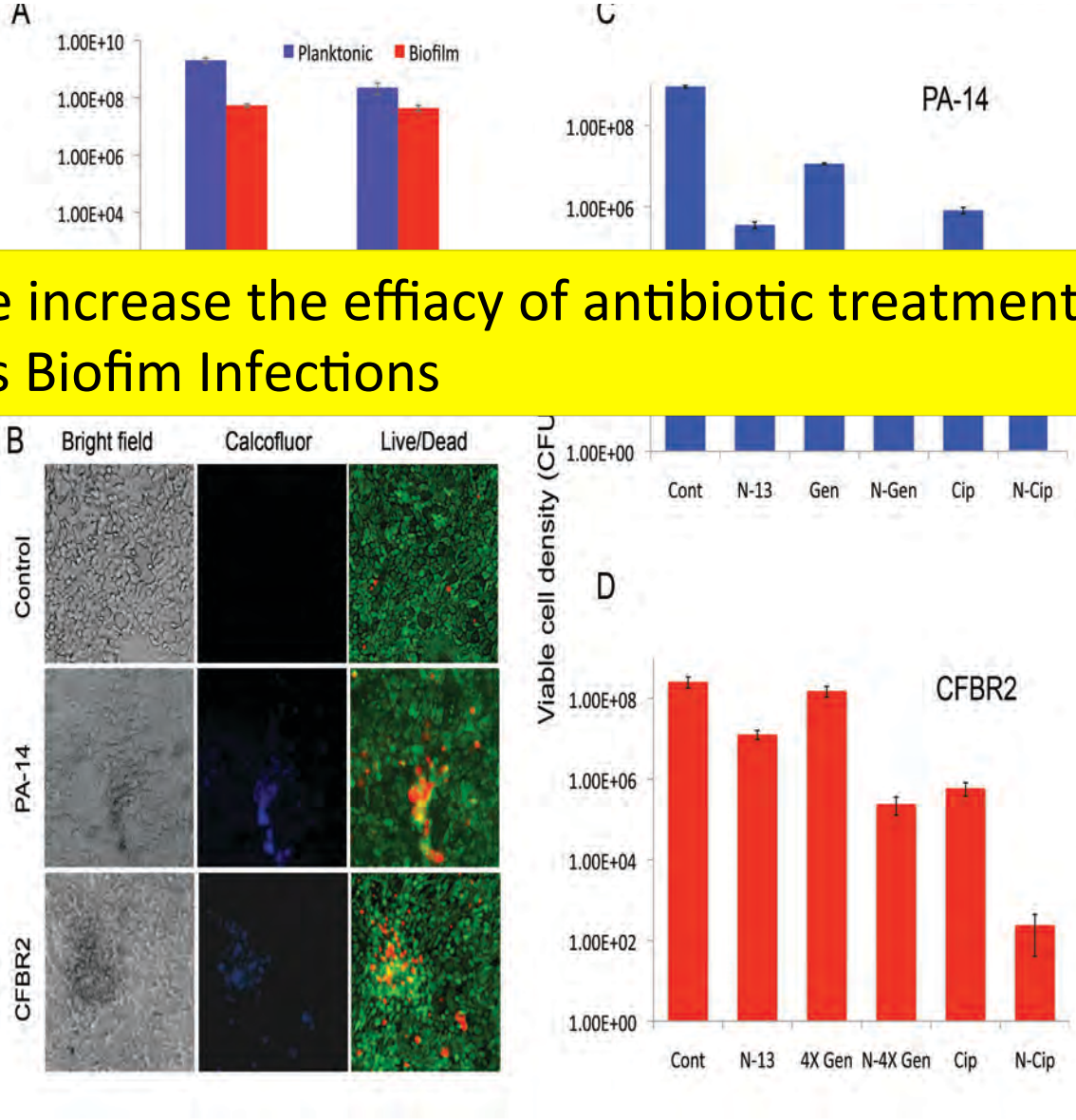




Biofilm Cultures on Epithelial cells



Antibiotic Pharmacodynamics of P. aeruginosa biofilms on epithelial cells



Pairs of phage increase the effiacy of antibiotic treatment of Pseudomonas Biofim Infections

Thank you and Happy Independence Day