Intracellular bacteria and antibiotics: *S. aureus, L. monocytogenes et P. aeruginosa*

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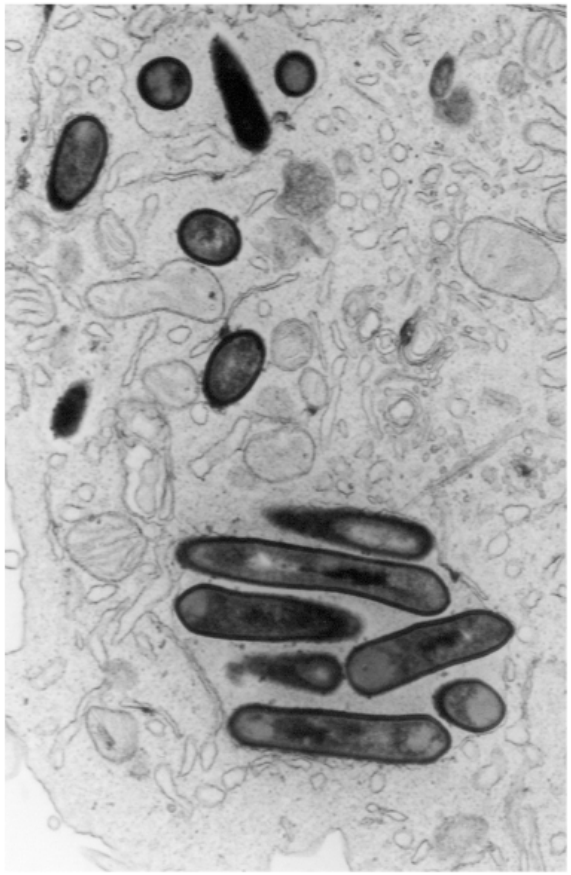
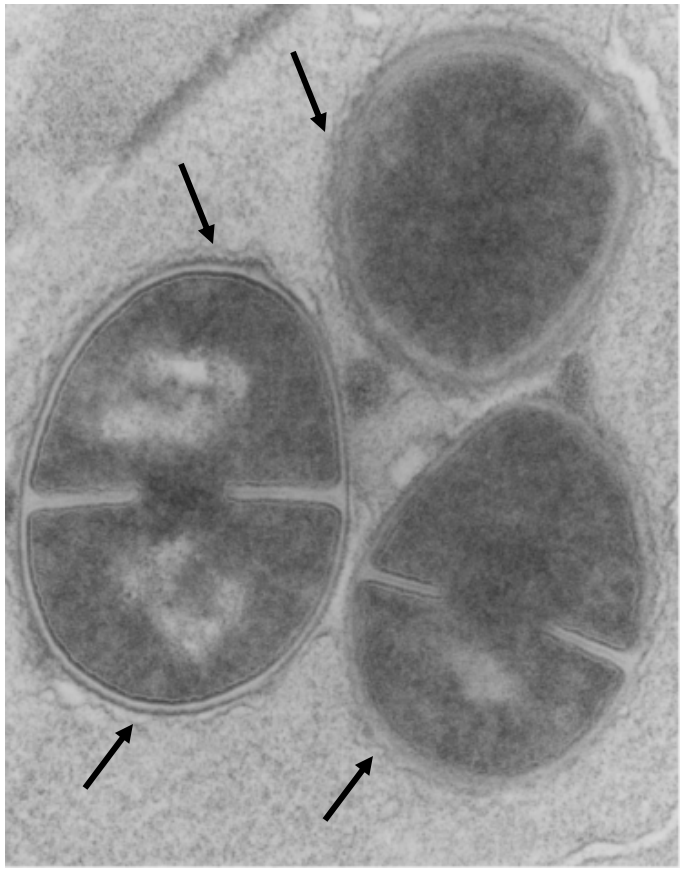
The infected cell: a guided tour …

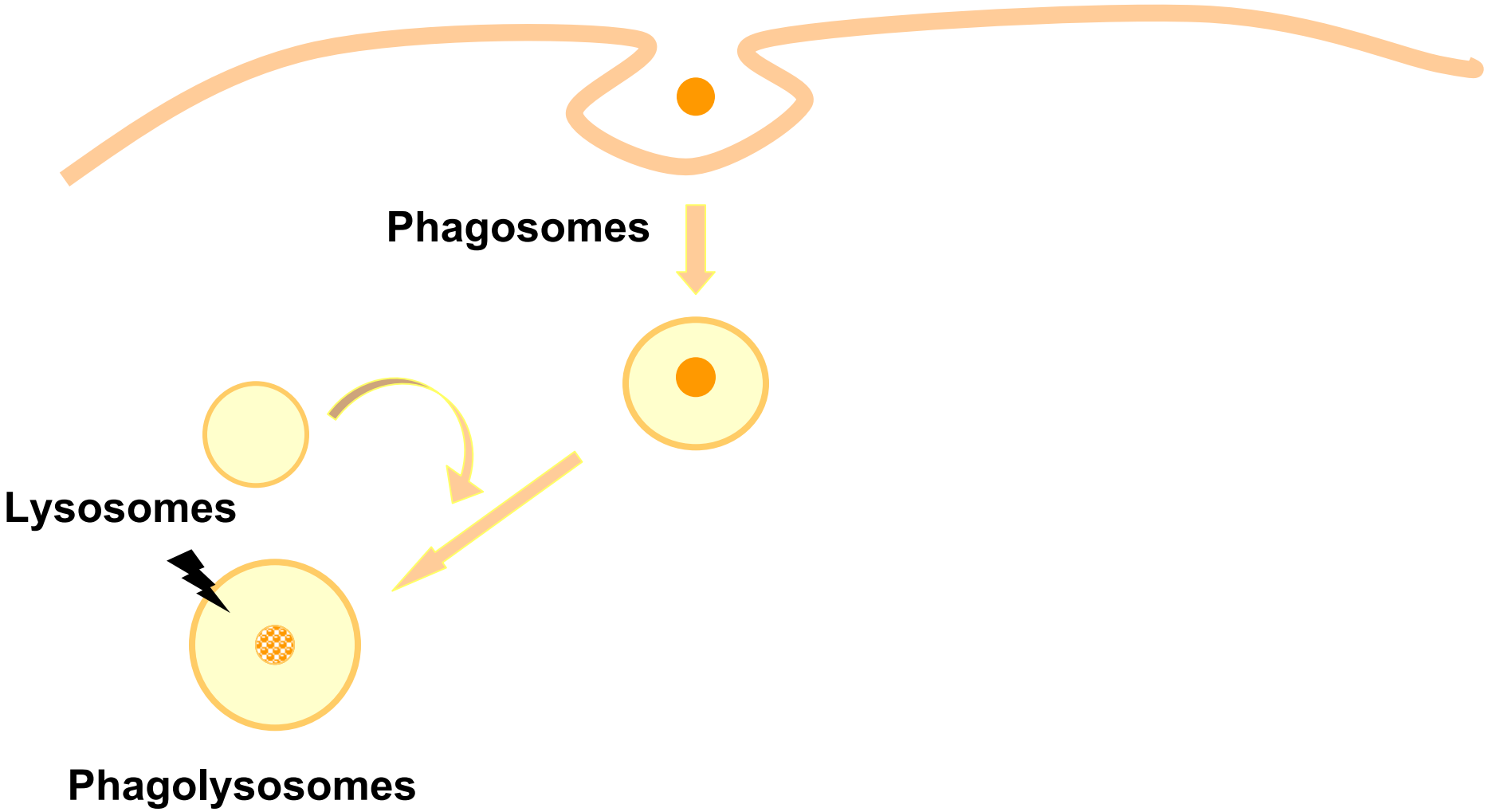


Listeria; cytosol

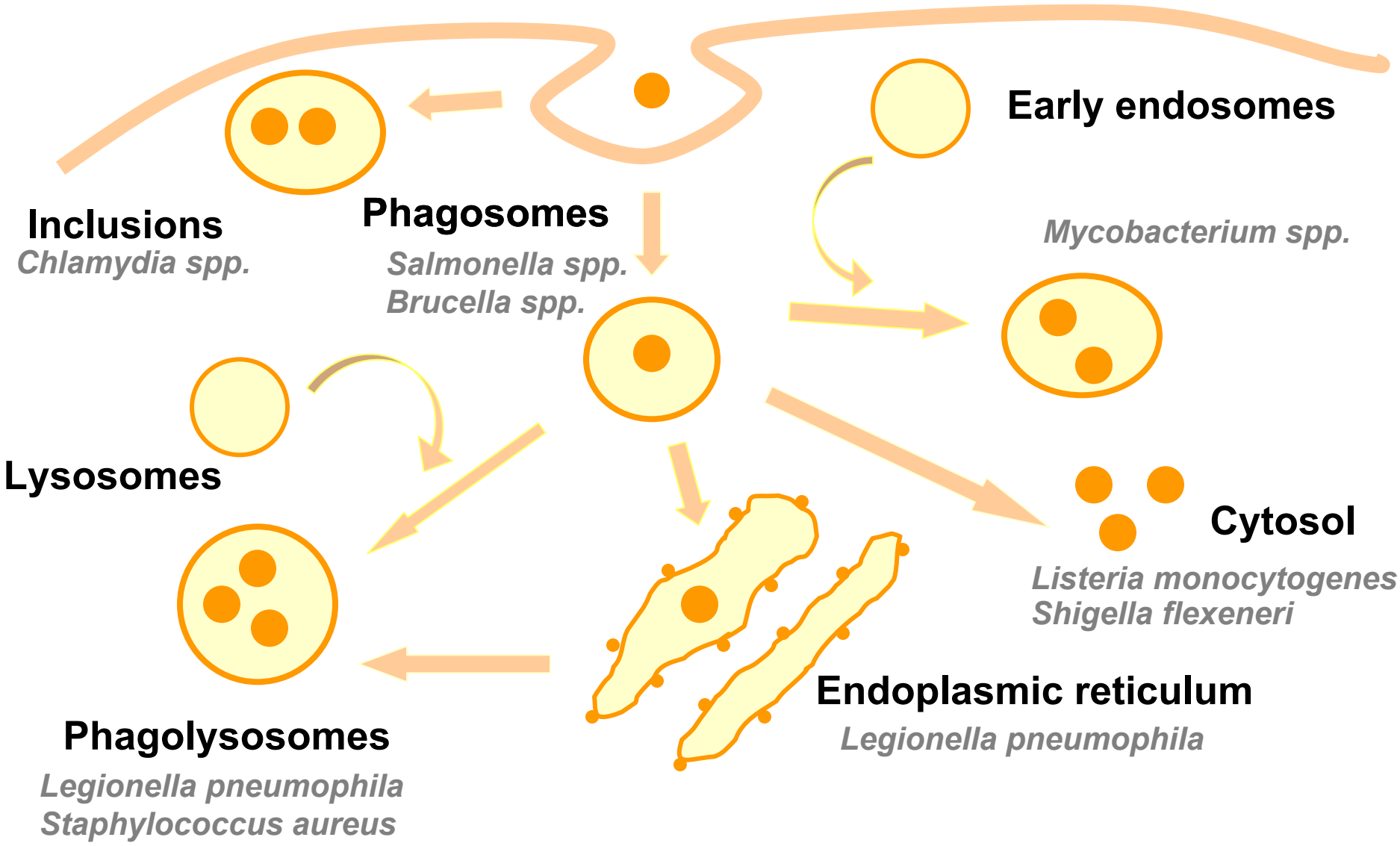


S. aureus; phagolysosomes

Intracellular killing of bacteria by host cell defence mechanisms

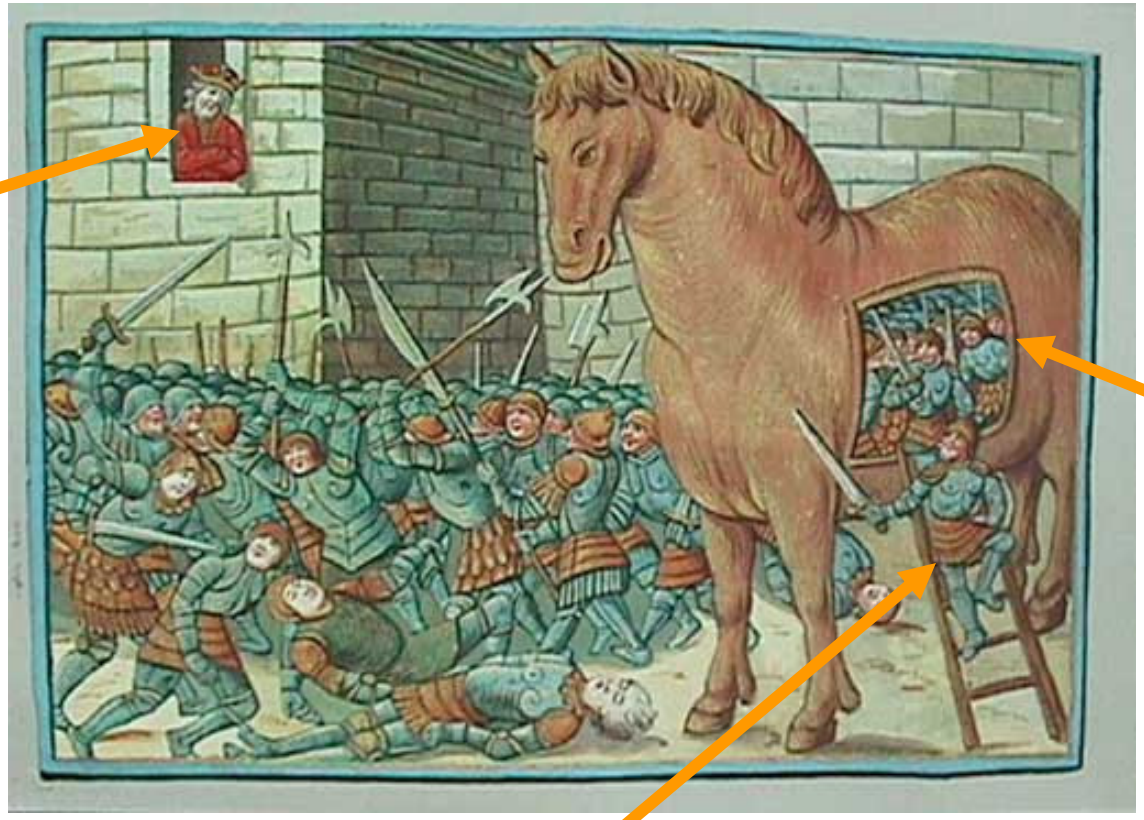


Some bacteria can escape host cell defence mechanisms …



Carryn et al., Infect Dis Clin North Am. (2003) 17:615-34

Benefits of intracellular life

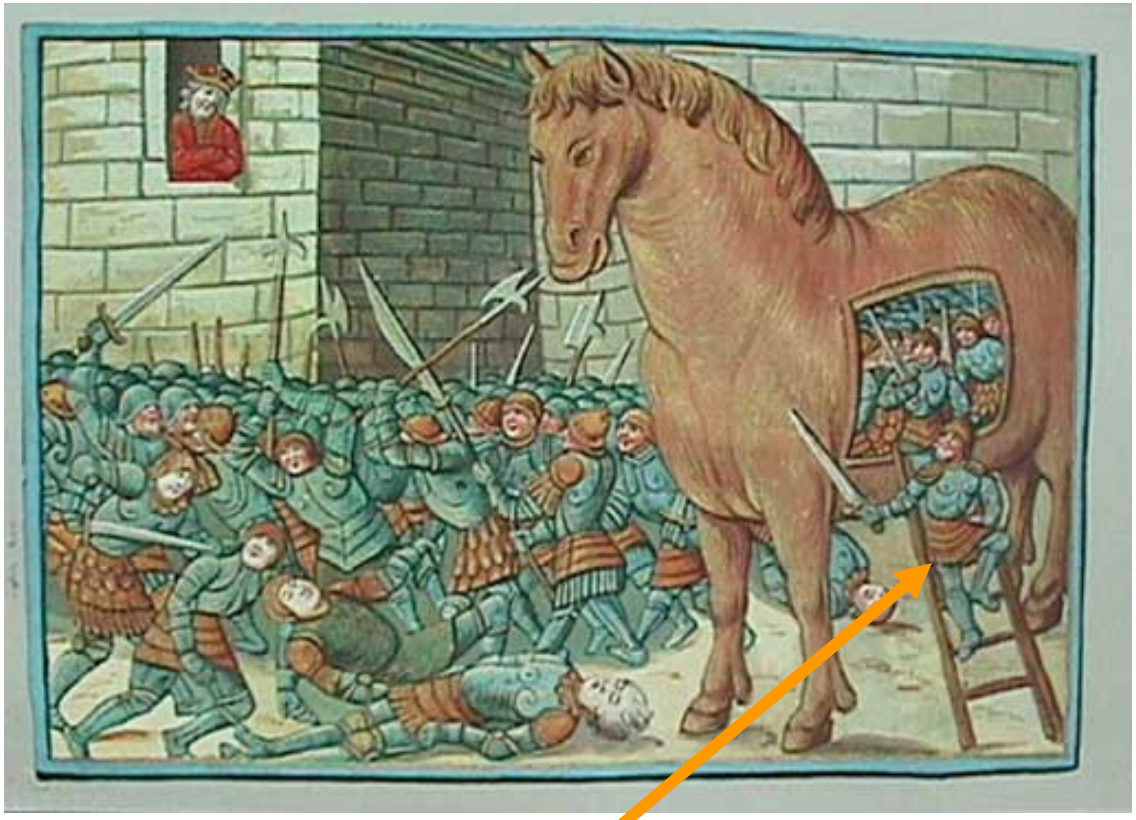


protection

persistence

invasion

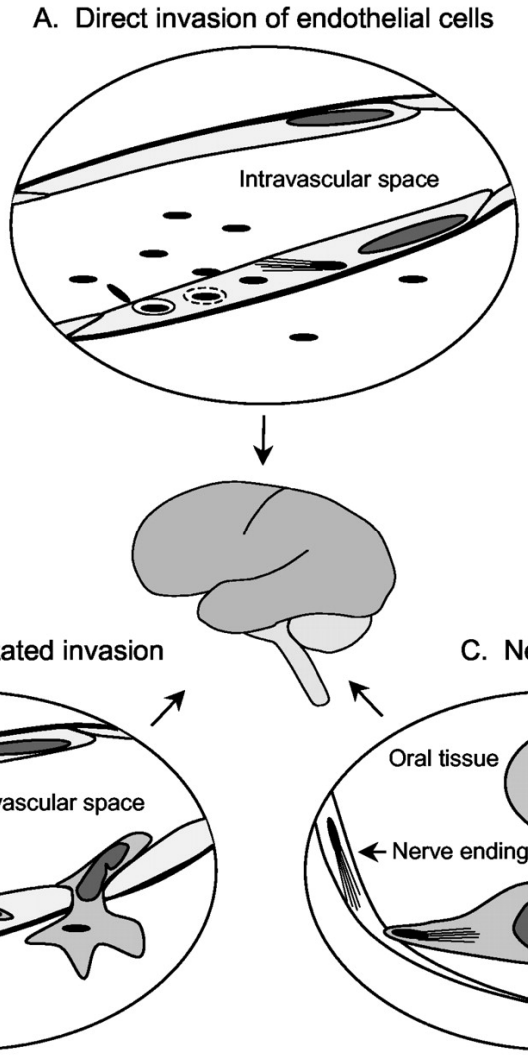
Benefits of intracellular life

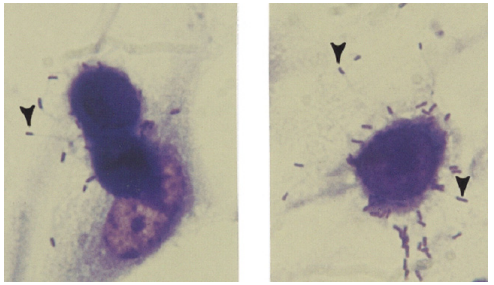


invasion

Migration to the CNS

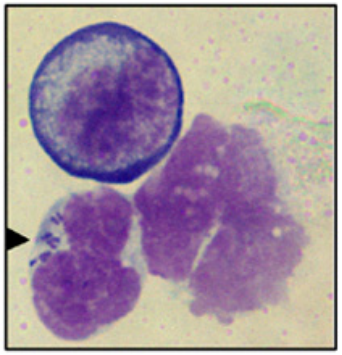
Listeria:
from the gut to the CNS

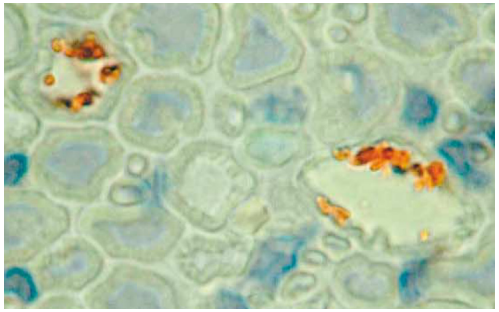




adherence and transfert
from monocytes to endothelial cells

bone-marrow monocyte

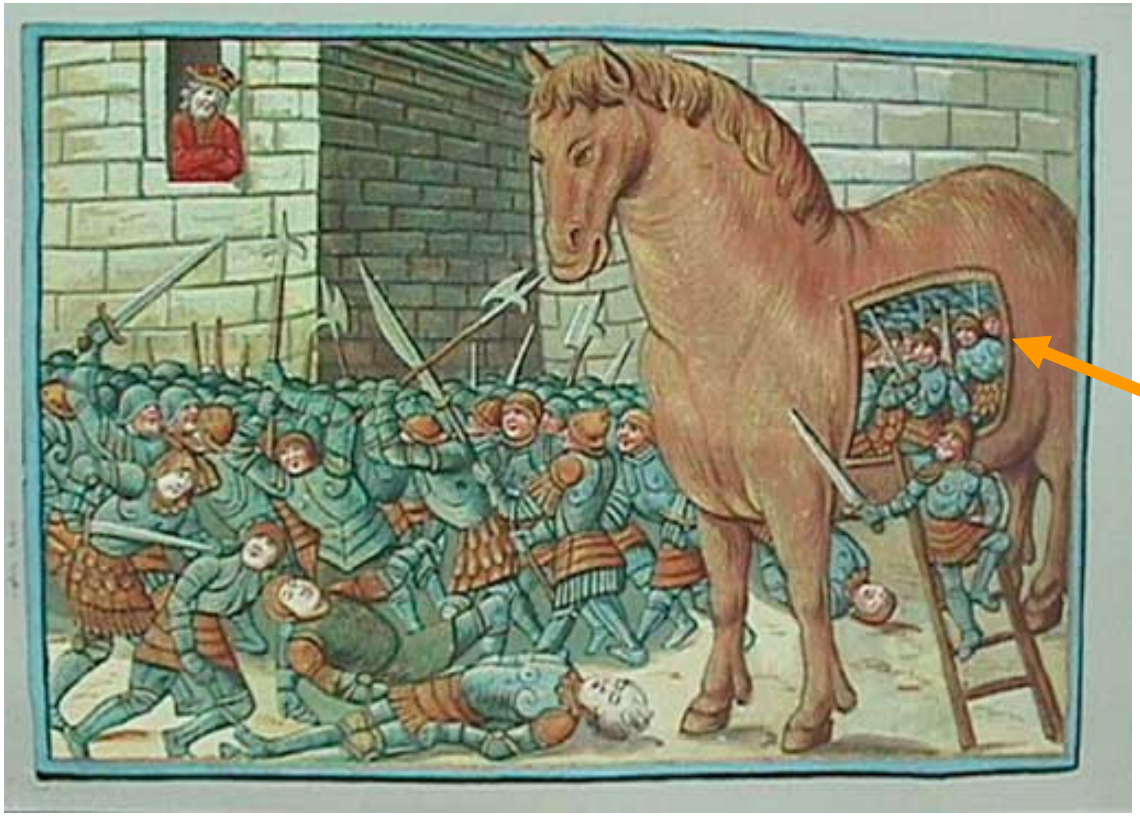




intra-axonal labeling
by anti-listeria antibodies

Antal et al., Brain Pathol. (2001) 11:432-8; Drevets & Bronze, FEMS Immunol Med Microbiol. (2008) 53:151-65
Drevets & Leenen, Microbes Infect. (2000) 2:1609-18; Drevets et al., Clin. Microb. Rev. (2004) 17:323-47

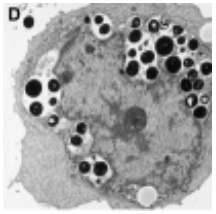
Benefits of intracellular life



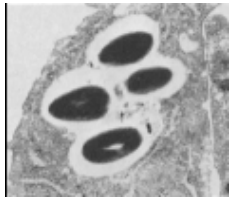
persistence

S. aureus can survive and multiply in several cell types

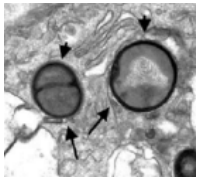
Mechanisms of *Staphylococcus aureus* invasion of cultured osteoblasts.
Ellington et al. Microb Pathog. (1999) 26:317-23.



Invasion of human keratinocytes by *Staphylococcus aureus* and intracellular bacterial persistence represent haemolysin-independent virulence mechanisms that are followed by features of necrotic and apoptotic keratinocyte cell death.
Mempel et al. Br J Dermatol. (2002) 146:943-51.



In vitro ability of *Staphylococcus aureus* isolates from bacteraemic patients with and without metastatic complications to invade vascular endothelial cells.
Park et al. J Med Microbiol. (2007) 56:1290-5.



Staphylococcus aureus invasion of bovine mammary epithelial cells.
Almeida et al. J Dairy Sci. (1996) 79:1021-6.
Brouillette et al. Microb Pathog. (2003) 35:159-68.

S. aureus can survive and multiply in several cell types

Intracellular *Staphylococcus aureus*. A mechanism for the indolence of osteomyelitis.
Ellington et al. J. Bone Joint Surg Br. (2003) 85:918-21

Intracellular persistence of *Staphylococcus aureus* small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with darier's disease.
Von Eiff et al. Clin Infect Dis. (2001) 32:1643-7

Phagocytosis of *Staphylococcus aureus* by cultured bovine aortic endothelial cells: model for postadherence events in endovascular infections.
Hamill et al. Infect Immun. (1986) 54:833-6.

Demonstration of intracellular *Staphylococcus aureus* in bovine mastitis alveolar cells and macrophages isolated from naturally infected cow milk.
Hebert et al. FEMS Microbiol. Lett. (2000) 193:57-72.

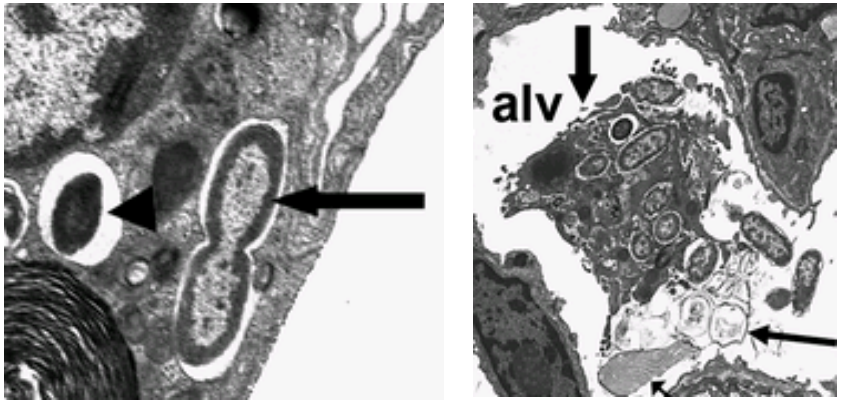
P. aeruginosa as an intracellular pathogen

- penetration inside epithelial cells and phagocytic cells
  → protection from immune defences

- reservoir responsible for persistence
  or recurrence of infections

- intracellular fate not yet fully characterized ….

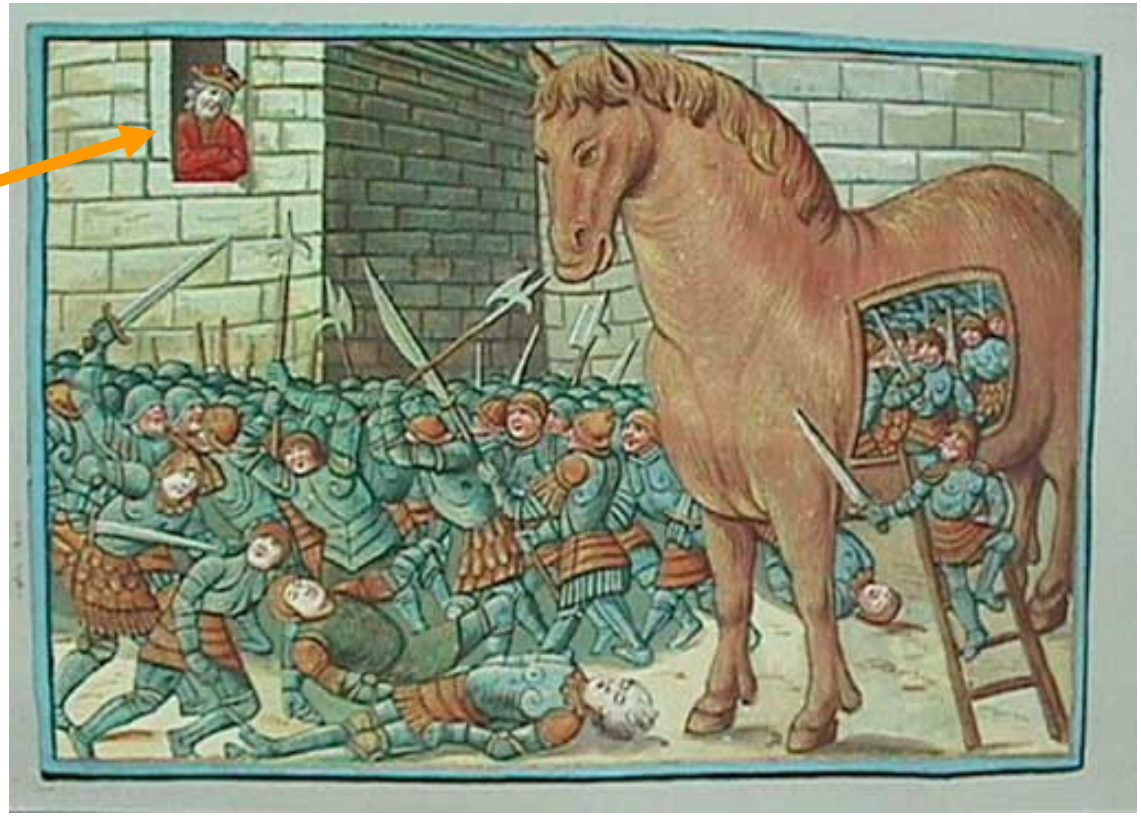
Pneumocytes **Alveolar macrophages**



Schmiedl et al., Cell Tissue Res. (2010) 342:67-73.

Benefits of intracellular life

protection



Failure to eradicate with antibiotics in vitro …

Reduced Ability of Penicillin to Eradicate Ingested Group A Streptococci from Epithelial Cells: Clinical and Pathogenetic Implications

Edward L. Kaplan,[1,a] Gursharan S. Chhatwal,[2] and Manfred Rohde[2]

[1]Department of Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota; and [2]Department of Microbial Pathogenesis; Helmholtz Centre for Infection Research, Braunschweig, Germany

Clinical Infectious Diseases 2006; 43:1398–406

Intracellular Persistence of *Staphylococcus aureus* Small-Colony Variants within Keratinocytes: A Cause for Antibiotic Treatment Failure in a Patient with Darier's Disease

Christof von Eiff,[1] Karsten Becker,[1] Dieter Metze,[2] Gabriele Lubritz,[1] Johannes Hockmann,[2] Thomas Schwarz,[2] and Georg Peters[1]

[1]Institute of Medical Microbiology and [2]Department of Dermatology, Westfälische Wilhelms-Universität Münster, Münster, Germany

Journal of Antimicrobial Chemotherapy (2004) 53, 167–173
DOI: 10.1093/jac/dkh076
Advance Access publication 16 January 2004

JAC

Antibiotic-induced persistence of cytotoxic *Staphylococcus aureus* in non-phagocytic cells

Oleg Krut, Herdis Sommer and Martin Krönke*

OPEN ACCESS Freely available online

PLoS one

Penicillin Induced Persistence in *Chlamydia trachomatis*: High Quality Time Lapse Video Analysis of the Developmental Cycle

Rachel J. Skilton, Lesley T. Cutcliffe, David Barlow, Yibing Wang, Omar Salim, Paul R. Lambden, Ian N. Clarke*

Molecular Microbiology Group, University of Southampton Medical School, Southampton General Hospital, Southampton, United Kingdom

Pediatr Infect Dis J. 2006 Oct;25(10):880-3.

Persistence of erythromycin-resistant group a streptococci in cultured respiratory cells.

Spinaci C, Magi G, Varaldo PE, Facinelli B.

Institute of Microbiology and Biomedical Sciences, Marche Polytechnic University Medical School, Ancona, Italy.

and treatment difficulties …

JEADV (2001) 15, 405–409

ORIGINAL ARTICLE

Electron microscopic evidence of persistent chlamydial infection following treatment

EY Bragina,† MA Gomberg,‡* GA Dmitriev†

†Department of Microbiology, Central Institute of Skin and Venereal Diseases, ‡Laboratory of Viral Urogenital Infections, Central Institute of Skin and Venereal Diseases, Korolenko Str., 3, Moscow, 107076, Russia.

Infection. 1992 Mar-Apr;20(2):99-100.

Fatal Legionella pneumophila pneumonia: treatment failure despite early sequential oral-parenteral amoxicillin-clavulanic acid therapy.

Hohl P, Buser U, Frei R.

Dept. of Internal Medicine, University Hospital, Basel, Switzerland.

Int J Tuberc Lung Dis. 2004 Jan;8(1):31-8.

Development of acquired drug resistance in recurrent tuberculosis patients with various previous treatment outcomes.

Yoshiyama T, Yanai H, Rhiengtong D, Palittapongarnpim P, Nampaisan O, Supawitkul S, Uthaivorawit W, Mori T.

Epidemiology Division, Research Institute of Tuberculosis, Kiyose, Tokyo, Japan.

Pathophysiology of chronic bacterial osteomyelitis. Why do antibiotics fail so often?

J Ciampolini and K G Harding

Postgrad Med J 2000 76: 479-483

Clinical Infectious Diseases 1999; 29:1340–1

Development of Listerial Meningitis during Ciprofloxacin Treatment

Nicholas M. Grumbach, Eleftherios Mylonakis, and Edward J. Wing

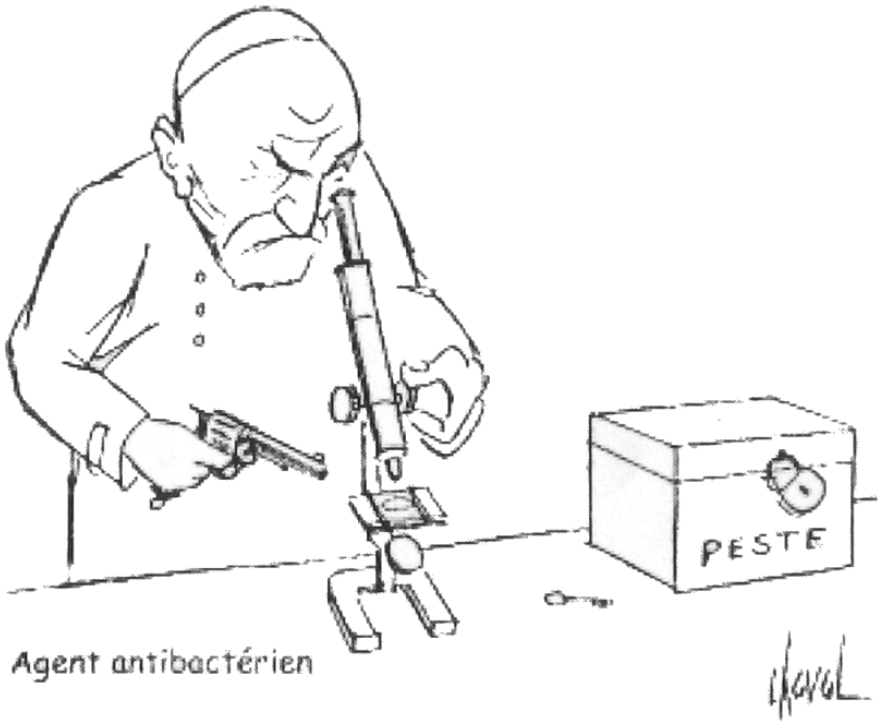
Journal of Antimicrobial Chemotherapy (2005) **55**, 383–386

Intracellular persistence of *Escherichia coli* in urinary bladders from mecillinam-treated mice

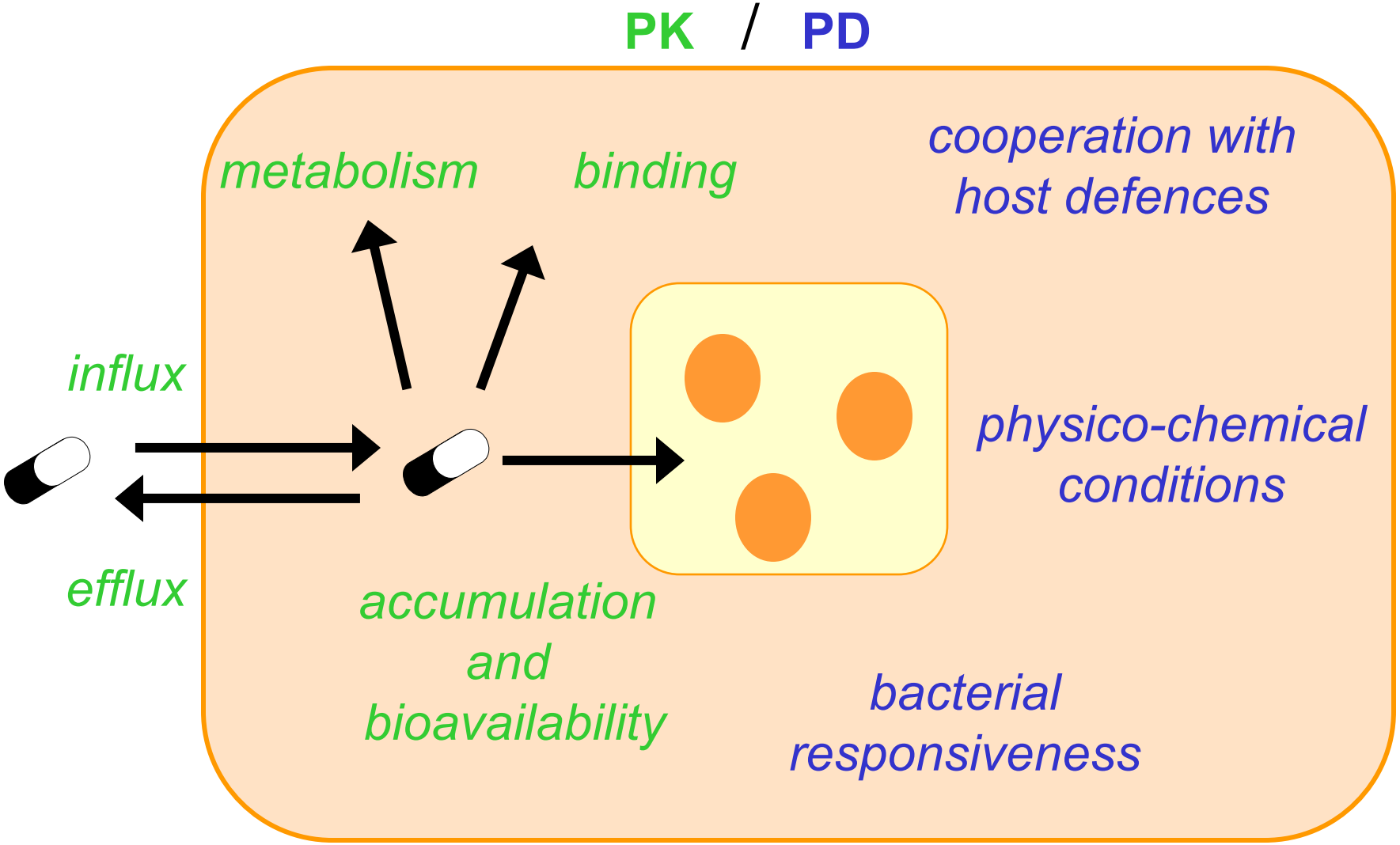
M. B. Kerrn[1,2]*, C. Struve[1], J. Blom[3], N. Frimodt-Møller[2] and K. A. Krogfelt[1]

[1]*Department of Bacteriology, Mycology and Parasitology,* [2]*National Center of Antimicrobials and Infection Control and* [3]*Department of Virology, Statens Serum Institut, Copenhagen, Denmark*

How to hit intracellular bacteria with antibiotics ?



Antibiotic properties for intracellular activity



Carryn et al., Infect Dis Clin North Am. (2003) 17:615-34

Antibiotic accumulation and subcellular distribution

diffusion

endocytosis

β-lactams; fast; ~ 1 x

fluoroquinolones : fast
CIP, LVX : 4-10 x
MXF, GAR, GMF : 10-20 x

?

linezolid: ~ 1 x
lincosamides: 1-4 x
tetracyclines: 2-4 x
rifampin : 2-10 x
synercid: 30-50x

aminoglycosides: slow ; 2-4 x
glycopeptides: slow
VAN ~ 8 x
TLV ~ 50 x
ORI ~ 150-300 x

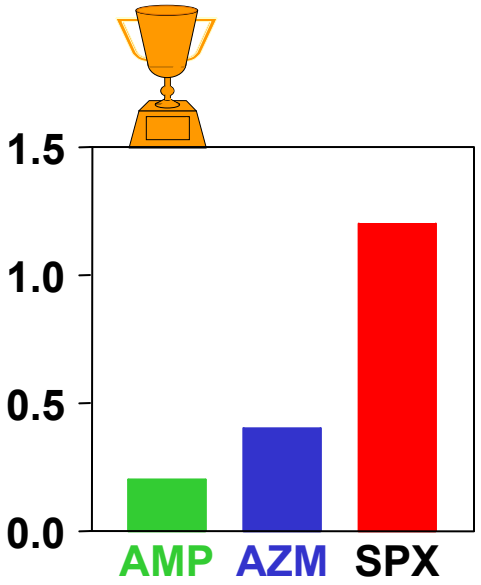
macrolides: fast
ERY: 4-10 x
CLR, ROX, TEL: 10-50x
AZM: > 50 x
SOL: 350 x

oxazolidinones: fast
RDZ : 10 x

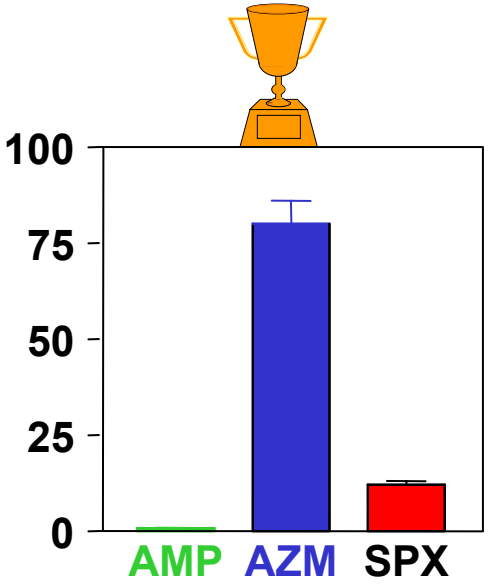
diffusion/
segregation

Can we simply predict intracellular activity based on MIC and antibiotic accumulation?

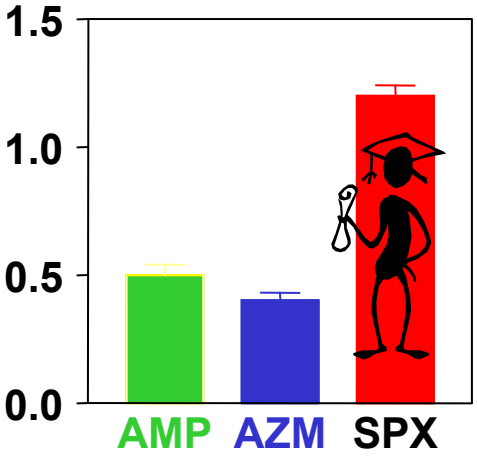
MIC
(L. monocytogenes)



antibiotic accumulation



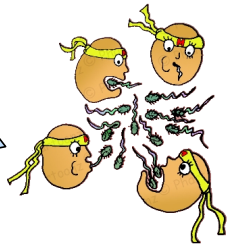
activity on intracellular *Listeria*
(5 h; 10 x MIC)



Ouadrhiri et al (1999) AAC 43:1242-51

In vitro model of intracellular infection

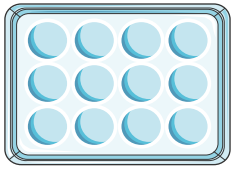
Opsonization
9 mL RPMI +
1 mL human serum



Phagocytosis

Extracellular Wash
Gentamicin 100 X MIC h)

5 -10 x10^5 CFU/mg prot.
Time 0



Incubation (with antibiotics)
For up to 24 h

In vitro model of intracellular infection

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9 mL RPMI +
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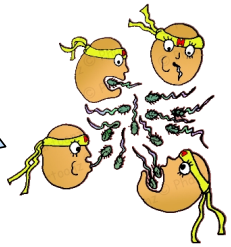

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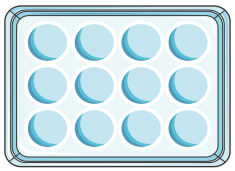
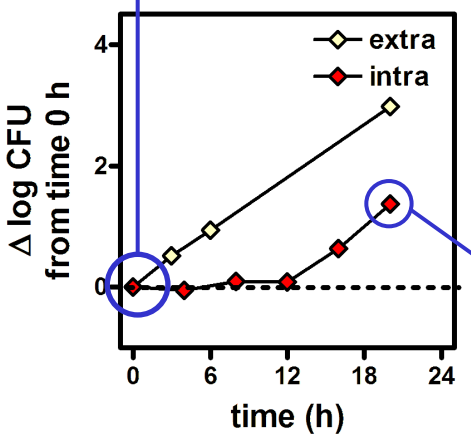
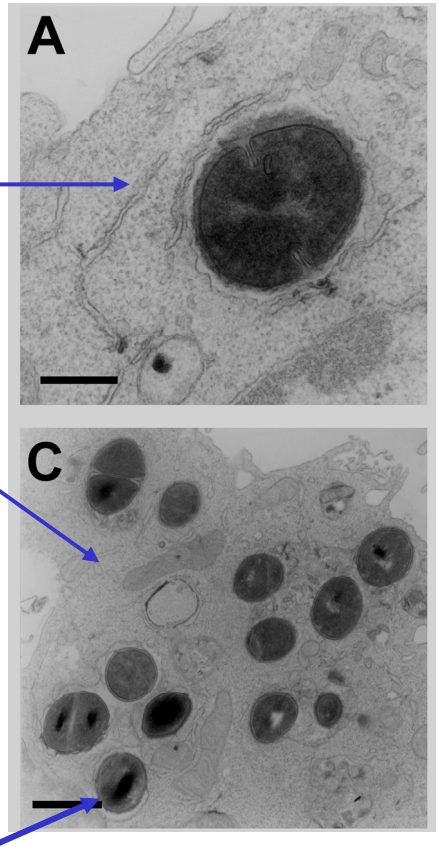


Incubation (with antibiotics)
For up to 24 h

S. aureus as an example



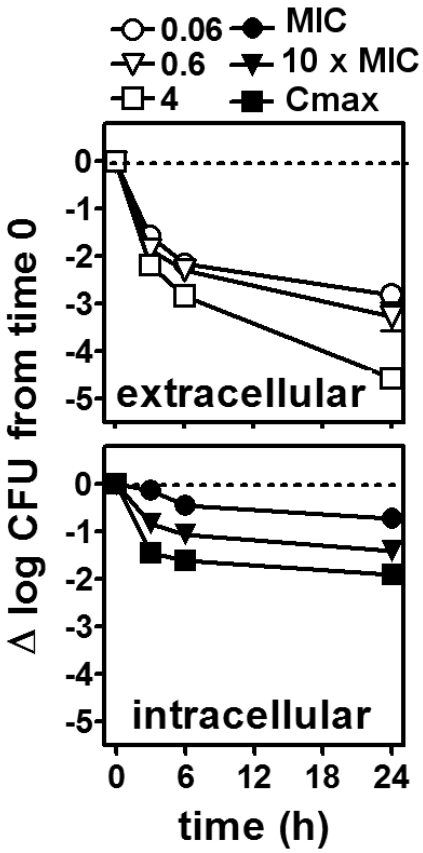


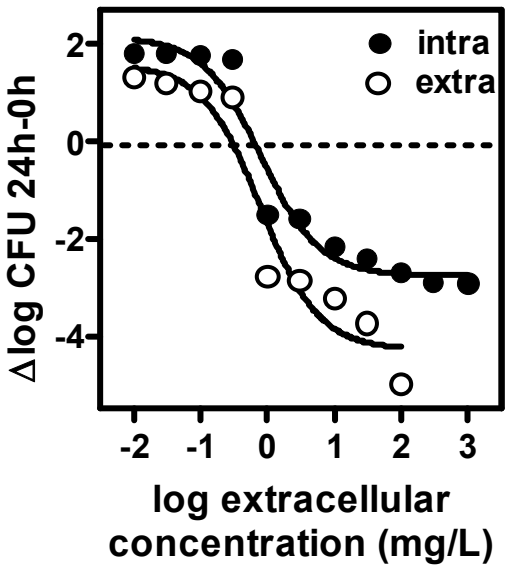
remains in vacuoles

Seral et al., Antimicrob. Agents Chemother. (2003) 47:2283-2292

Setting-up appropriate models for the study of cellular activity of antibiotics

moxifloxacin & *S. aureus*

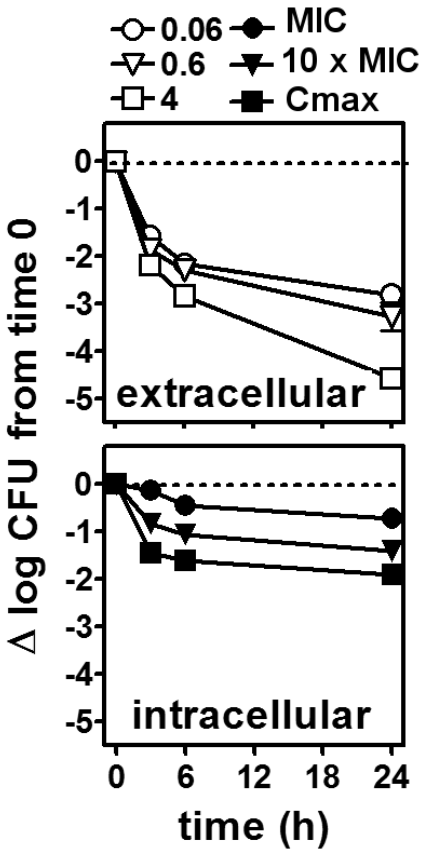


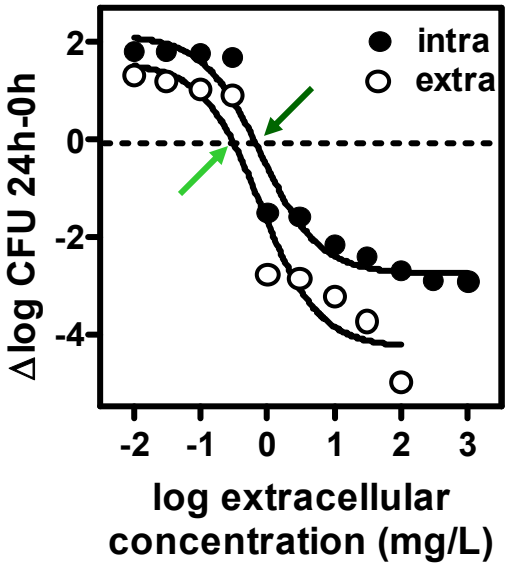


Barcia-Macay et al, AAC (2006) 50:841-51

Setting-up appropriate models for the study of cellular activity of antibiotics

moxifloxacin & *S. aureus*





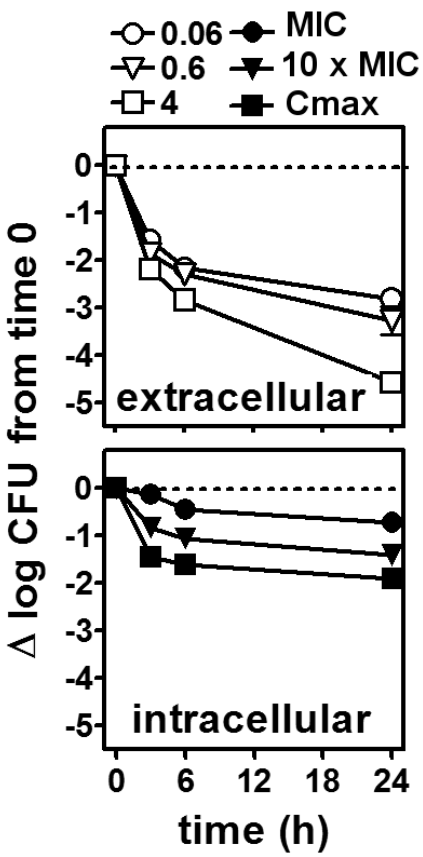
model	C_{stat} (x MIC)
extra	0.27
intra	0.63

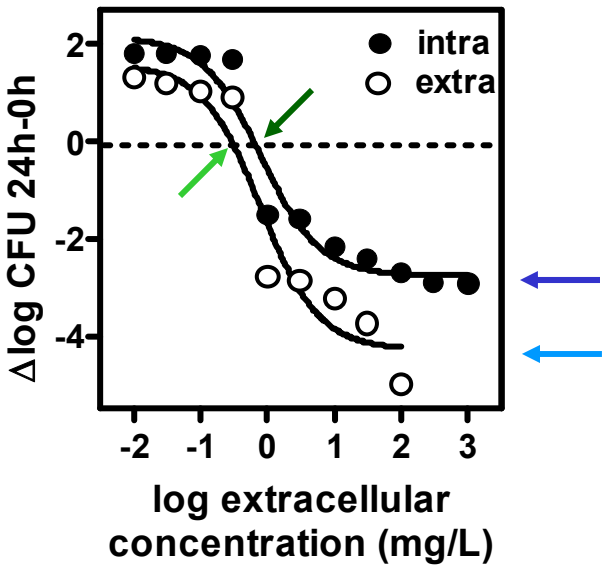
relative potency

Barcia-Macay et al, AAC (2006) 50:841-51

Setting-up appropriate models for the study of cellular activity of antibiotics

moxifloxacin & *S. aureus*





model	C_{stat} (x MIC)	E_{max}
extra	0.27	-3.86 (5.22 to 2.51)
intra	0.63	-2.77 (3.31 to 2.22)

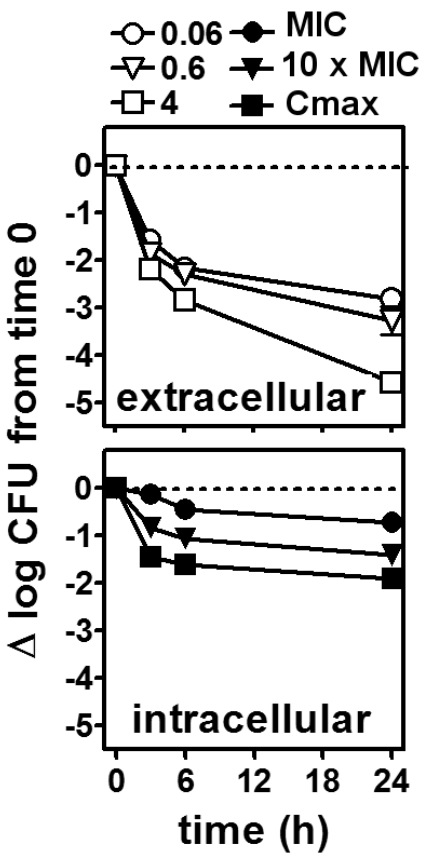
relative potency

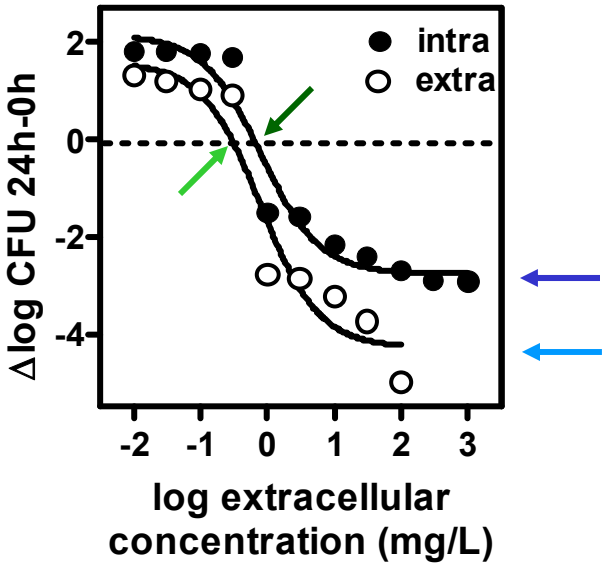
maximal efficacy

Barcia-Macay et al, AAC (2006) 50:841-51

Setting-up appropriate models for the study of cellular activity of antibiotics

moxifloxacin & *S. aureus*





model	C_{stat} (x MIC)	E_{max}
extra	0.27	-3.86 (5.22 to 2.51)
intra	0.63	-2.77 (3.31 to 2.22)

relative potency

maximal efficacy

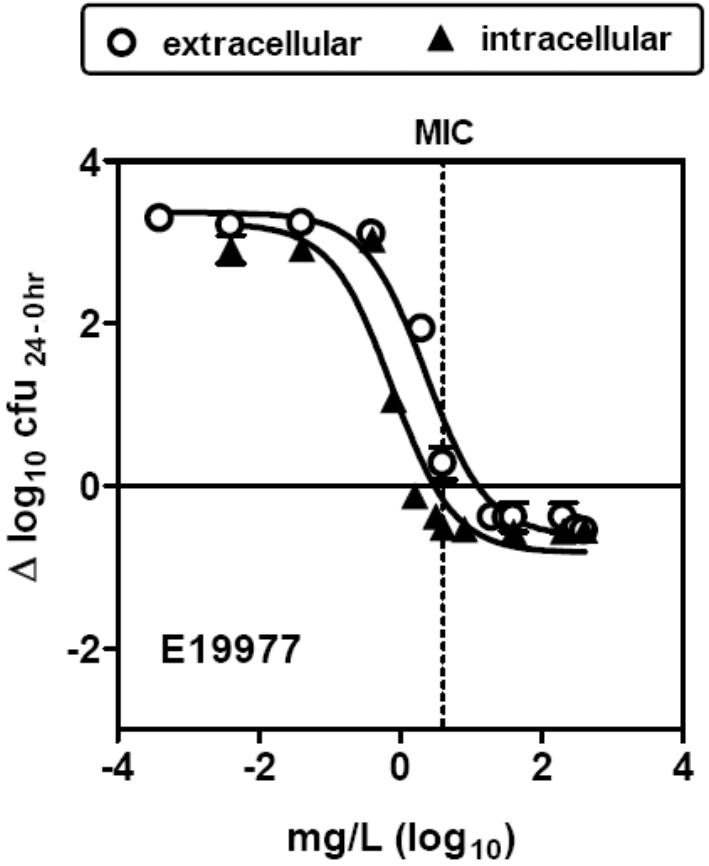
Quantitative comparison
~ models
~ drugs

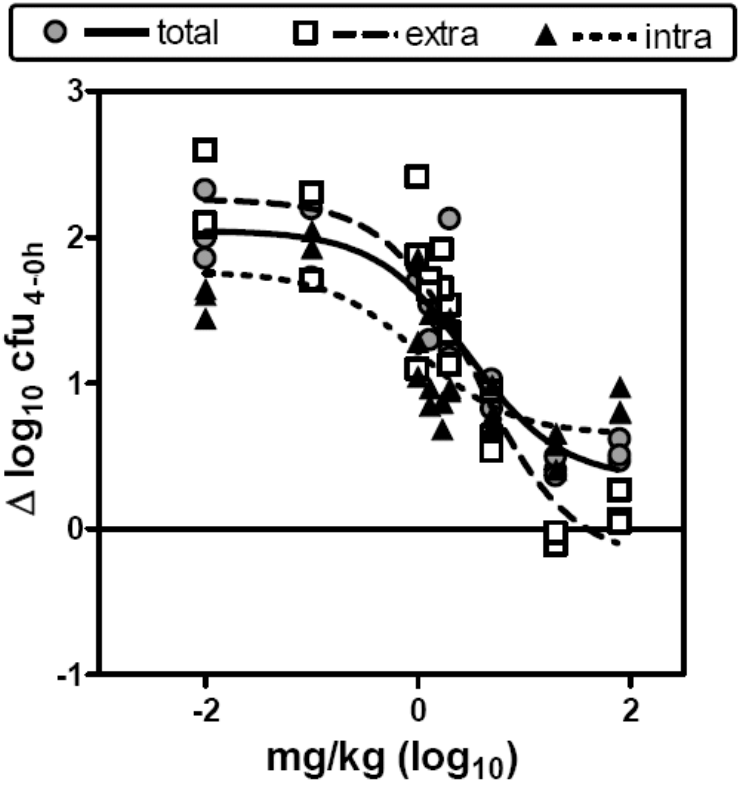
in vitro vs *in vivo*

Linezolid & *S. aureus*

in vitro
(macrophages)

in vivo
(peritonitis)

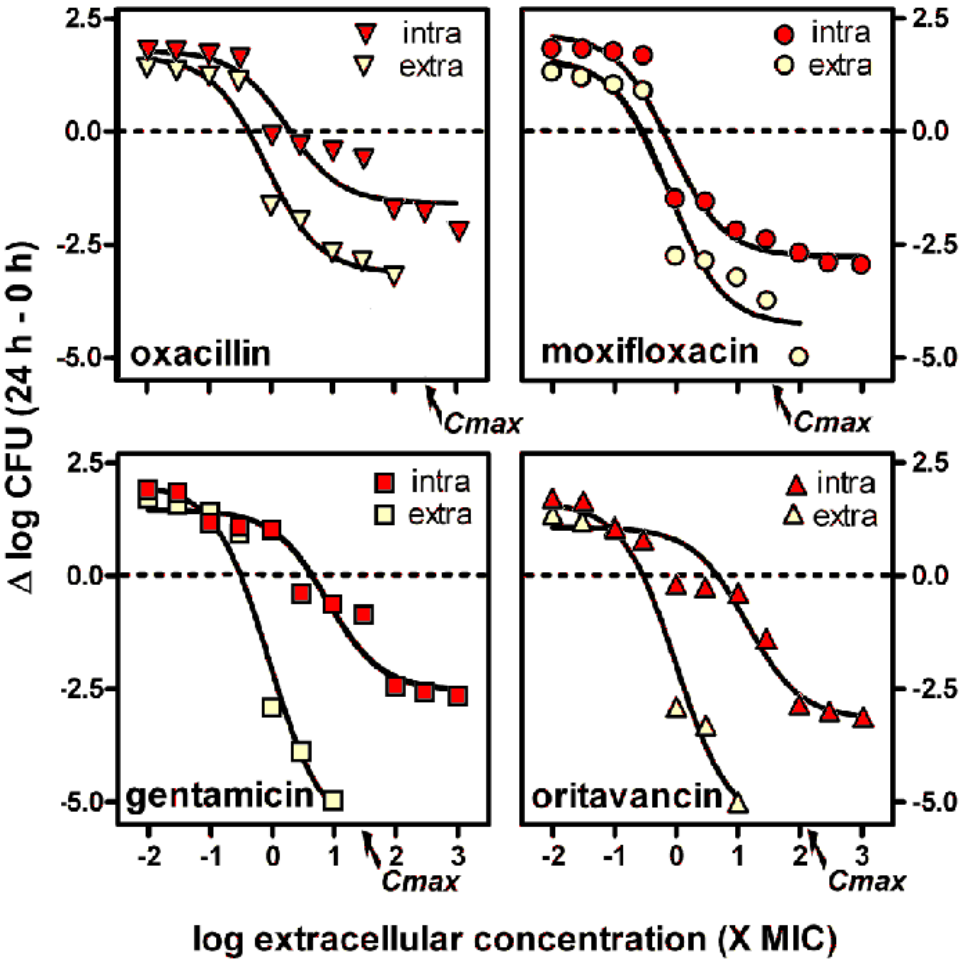




Sandberg et al. JAC (2010) 65:962-973

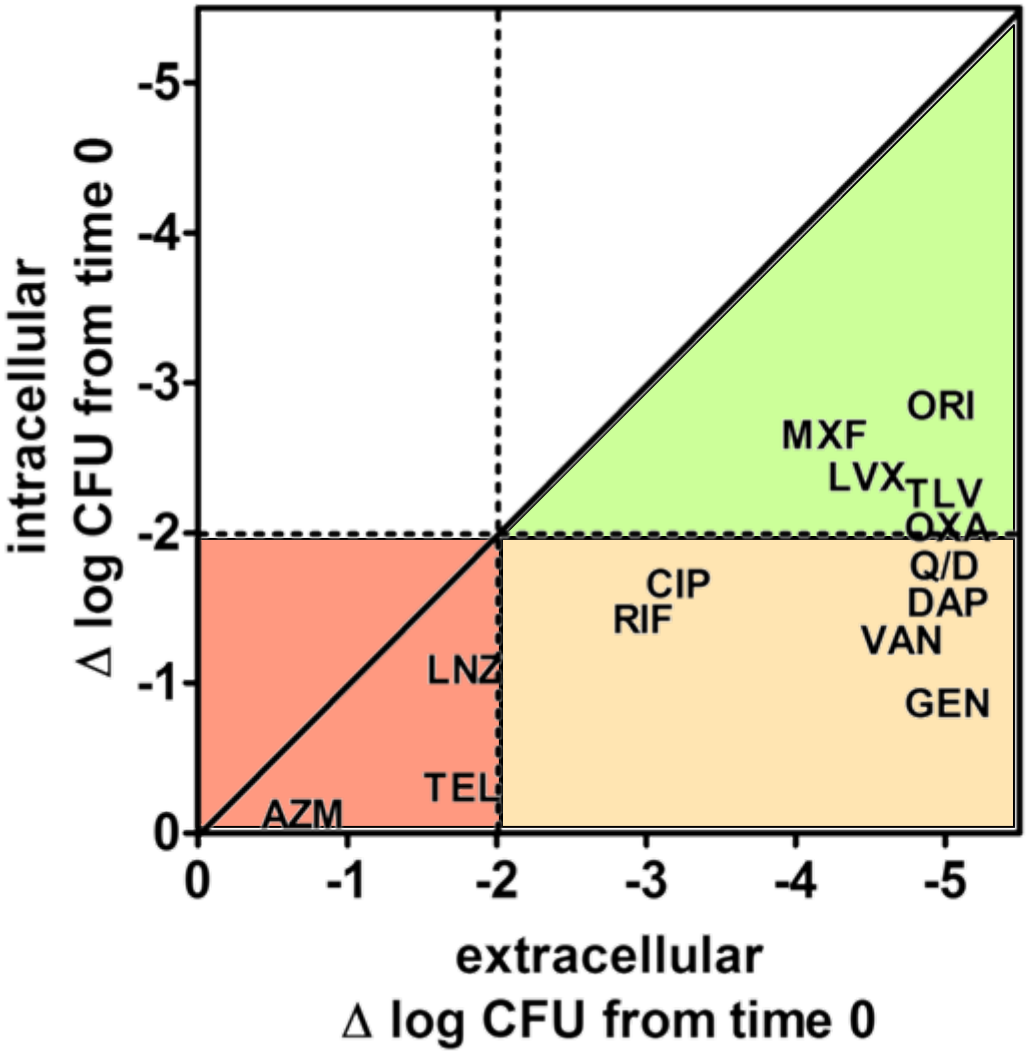
Comparing different drugs against a single species

Activity at 24 h against *S. aureus* – fully susceptible strain



Barcia-Macay et al., Antimicrob Agents Chemother. (2006) 50:841-51

Balance of extra-and intracellular activity of antibiotics against *S. aureus*



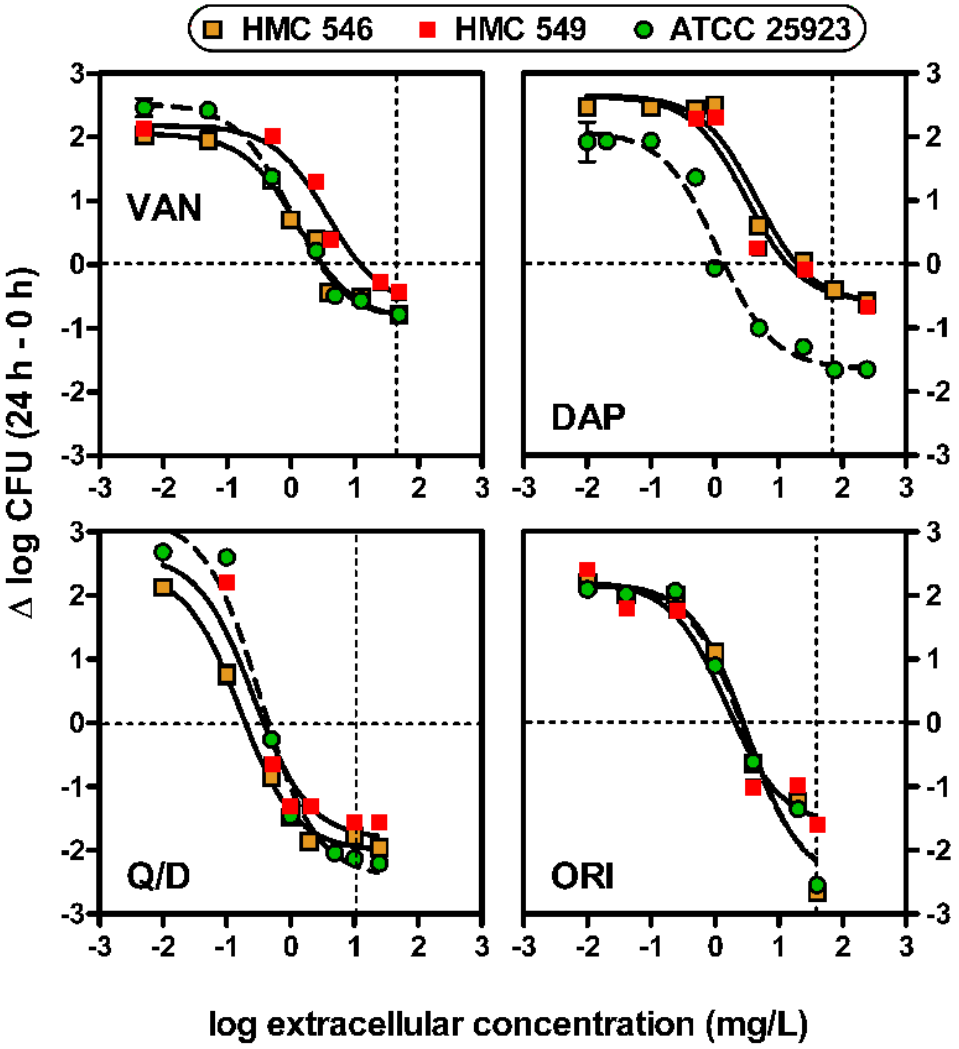
Adapted from Van Bambeke et al., Curr Opin Drug Discov Devel. (2006) 9:218-30

What about resistant strains ?

VISA and DAP-resistant strains isolated from a patient with endocarditis

higher intracellular EC_{50}

no effect of resistance phenotype



higher intracellular EC_{50}

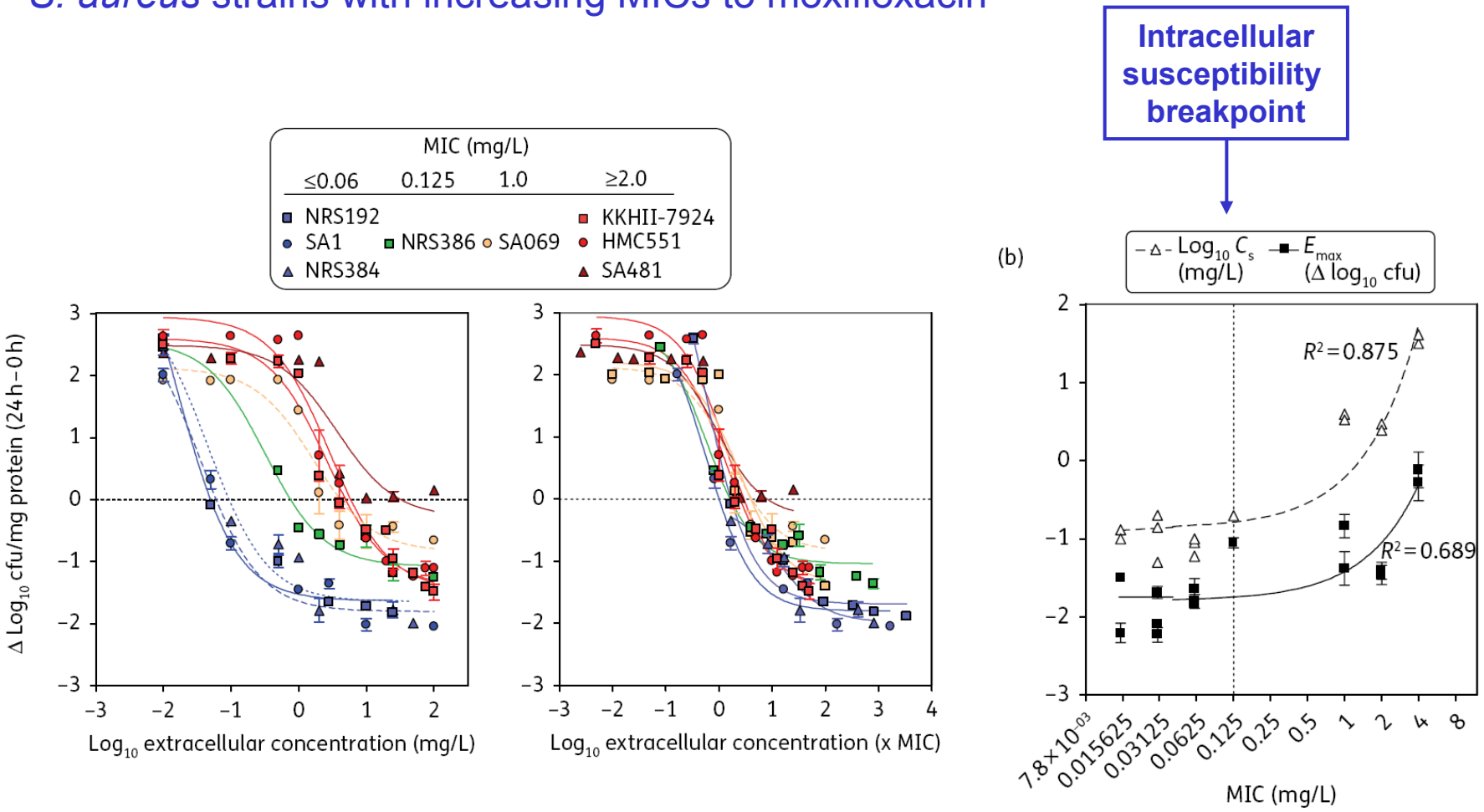
lower intracellular Emax

no effect of resistance phenotype

Lemaire et al., Clin. Microbiol. Infect. (2008) 14:766-777

What about resistant strains ?

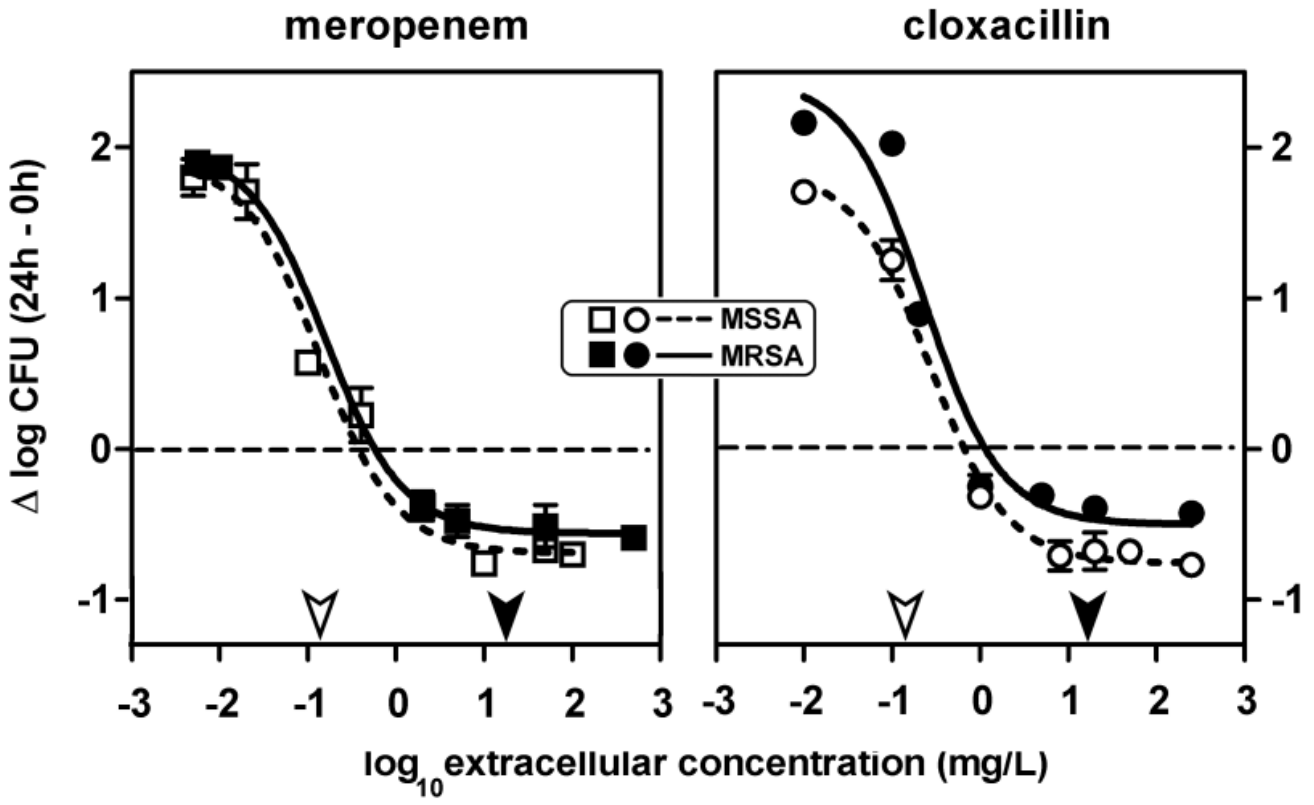
S. aureus strains with increasing MICs to moxifloxacin



Lemaire et al., J. Antimicrob. Chemother. (2011) 66:596-607

Beta-lactams ~ MSSA and MRSA

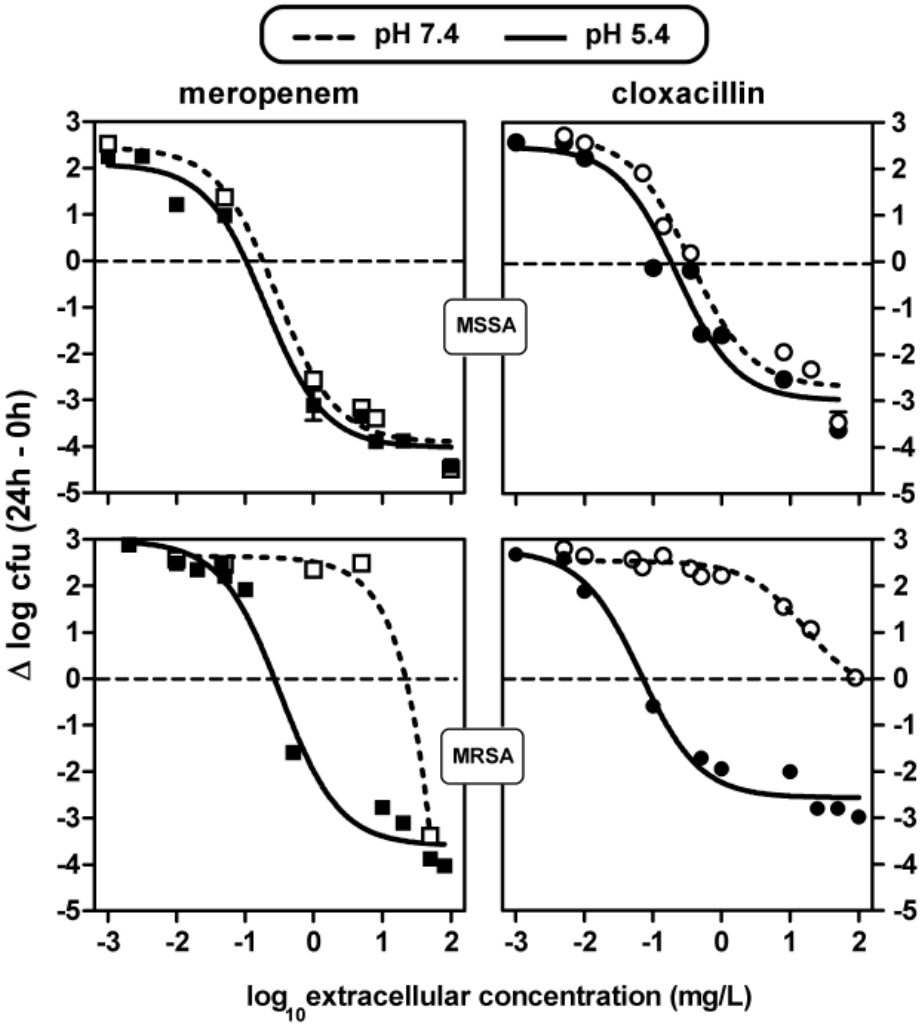
MRSA are as susceptible as MSSA to β-lactams when intracellular !



Lemaire et al., Antimicrob. Agents Chemother. (2007) 51:1627-1632

MRSA *vs.* MSSA: extracellular activity of β-lactams

MRSA are as susceptible as MSSA in broth at acidic pH



Lemaire et al., AAC (2007) 51:1627-32

PBP2a conformation is modified by acidic pH

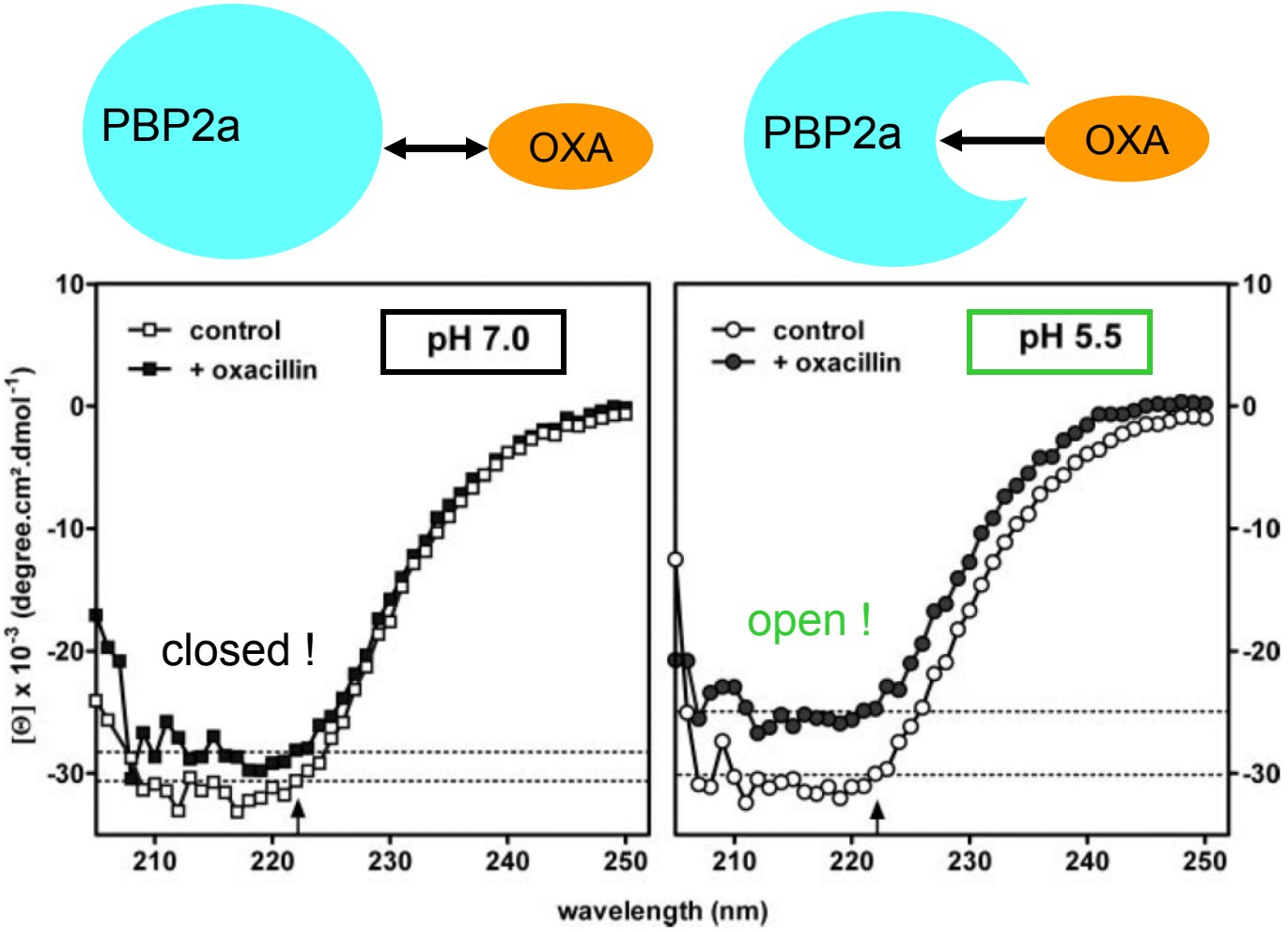
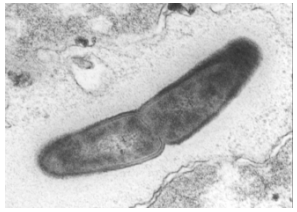


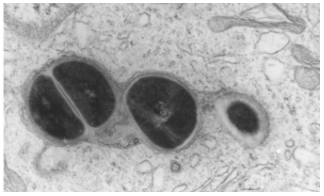
FIGURE 4. **Circular dichroic spectra of PBP 2a at pH 7. 0 (*left panel*) and pH 5.5 (*right panel*) in the absence (*open symbols*) and in the presence (*closed symbols*) of oxacillin (30 μM) for 30 min at 25 °C.** The *thin dotted lines* in each graph represent minima of PBP 2a molar ellipticity at 222 nm (*vertical arrow* on the *abscissa*) for each condition. The spectrum of oxacillin has been subtracted from all data points.

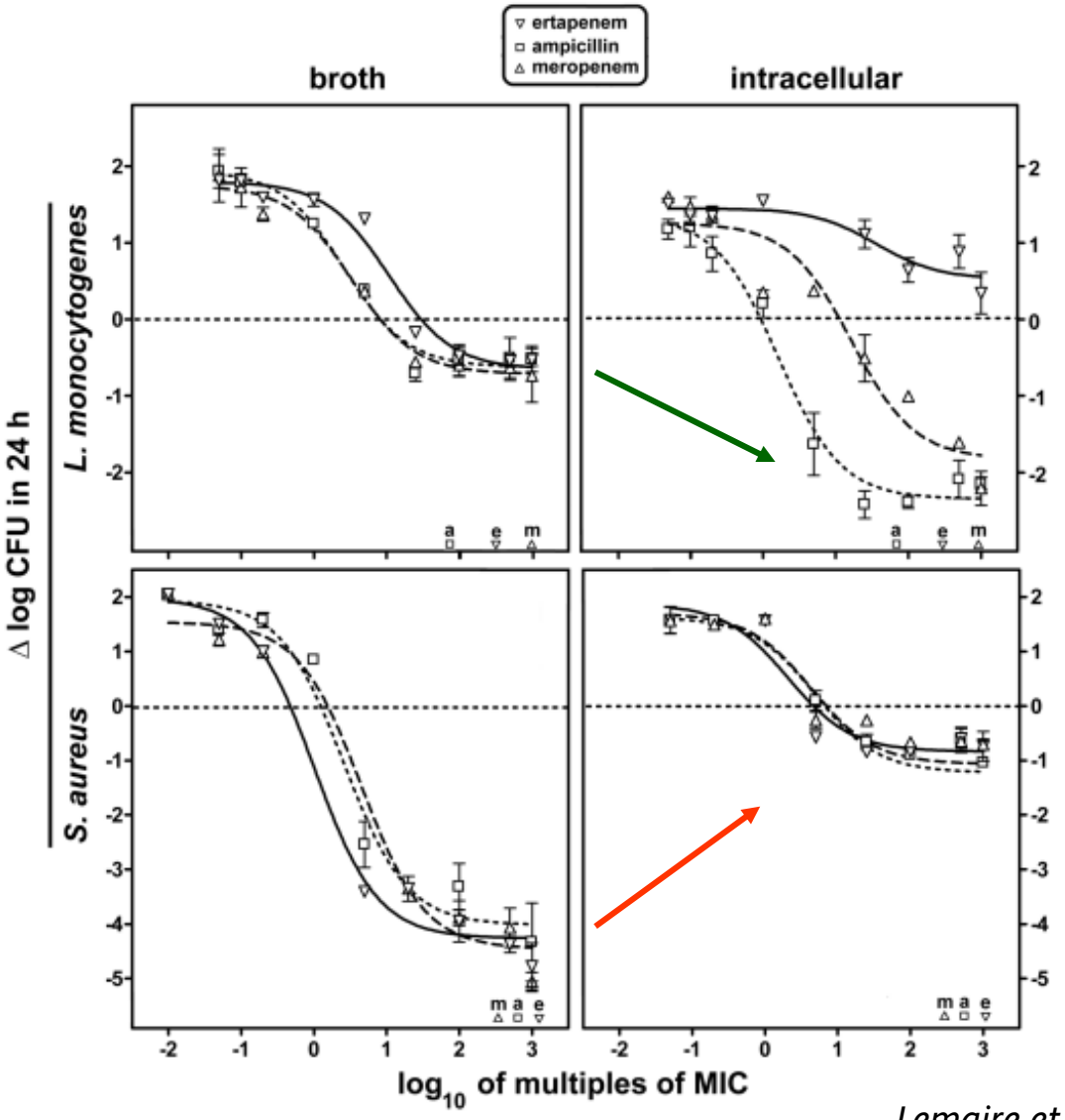
Lemaire et al., JBC (2008) 283:12769-76

Comparing a class of drugs against different bacteria

Beta-lactams vs. *L. moncytogenes* and *S. aureus*





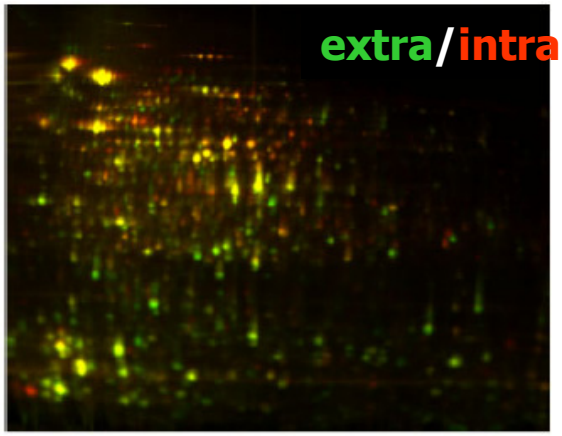


more active against intracellular bacteria ?

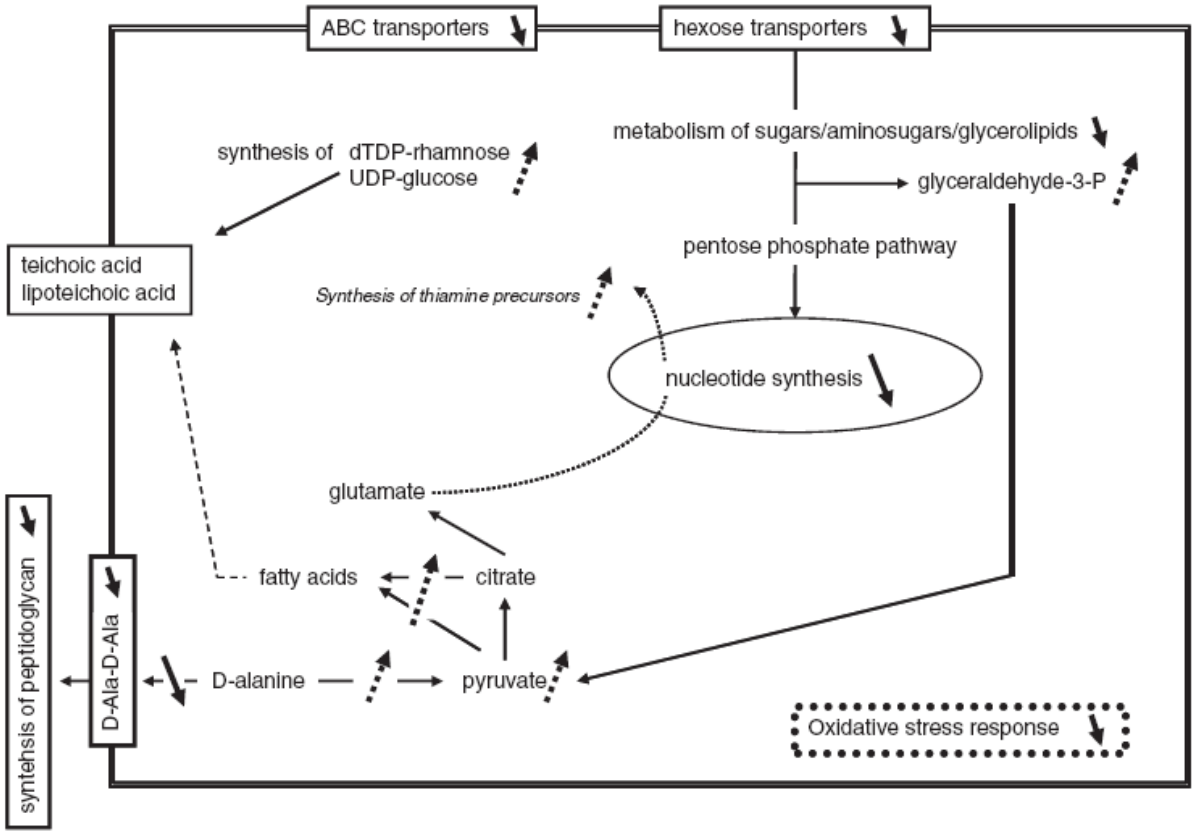
Lemaire et al. JAC (2005) 55:897–904

Why are beta-lactams so active against intracellular *L. monocytogenes ?*

Proteome analysis of intra- vs extra- *Listeria*



Reduced cell wall synthesis

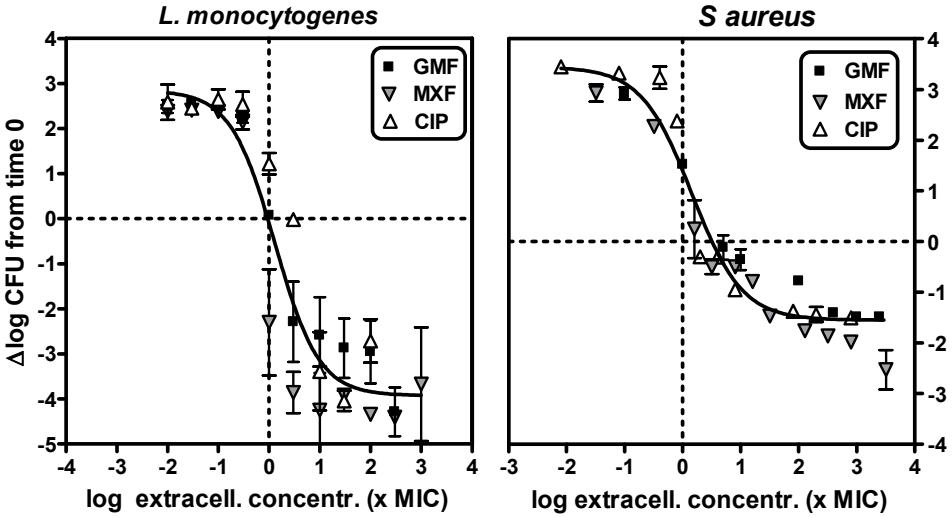


Van de Velde et al. Proteomics (2009) 9:5484-5496

Comparing a class of drugs against different bacteria

Fluoroquinolones against *L. monocytogenes* vs. *S.aureus*

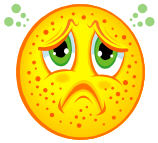
They all look the same …



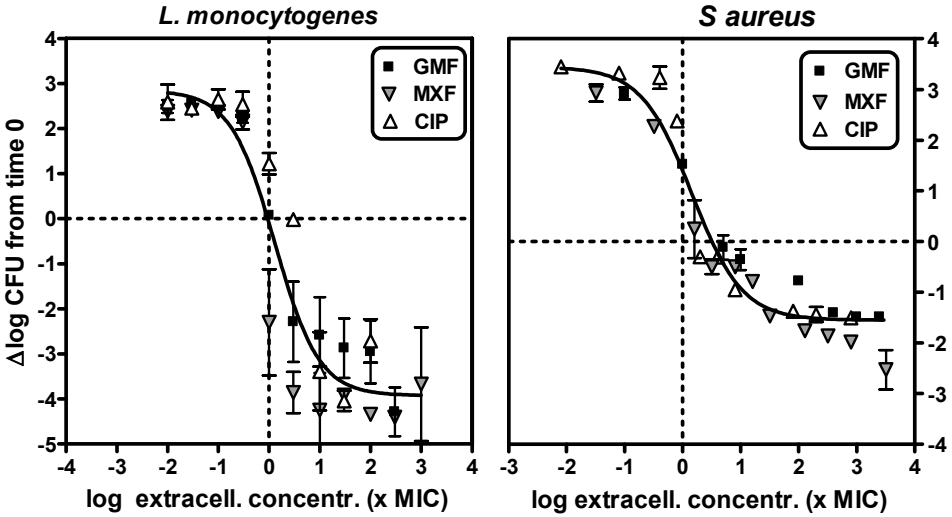
Vallet et al. Int. J. Antimicrob. Ag. (2011) 38:249-256.

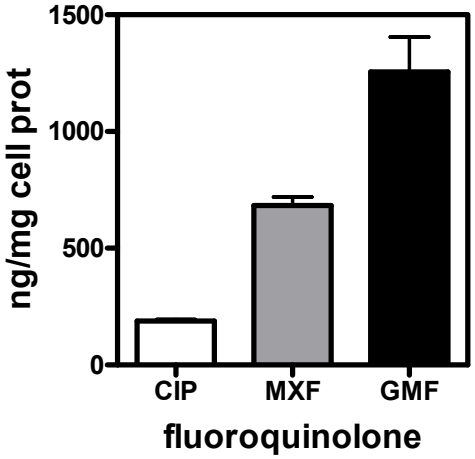
Comparing a class of drugs against different bacteria

Fluoroquinolones against *L. monocytogenes* vs. *S.aureus*



…eventhough they accumulate to variable levels



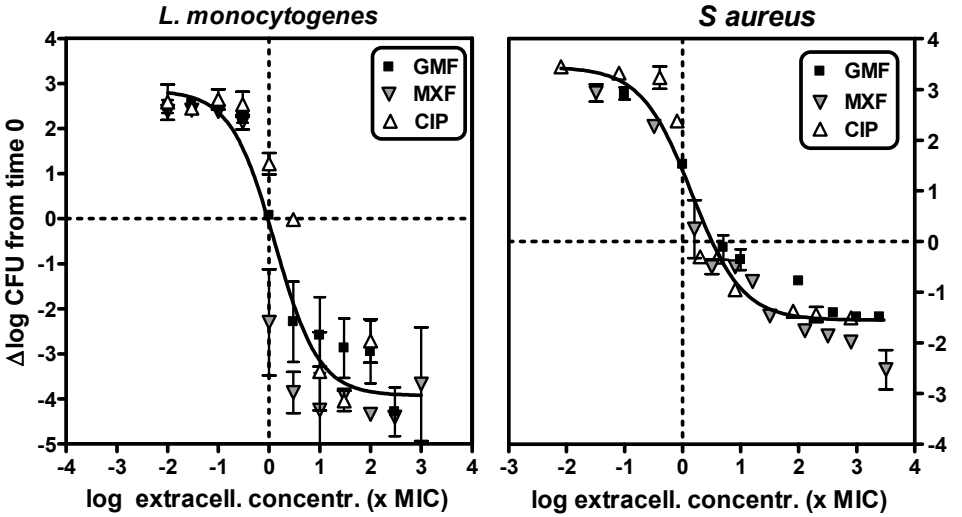


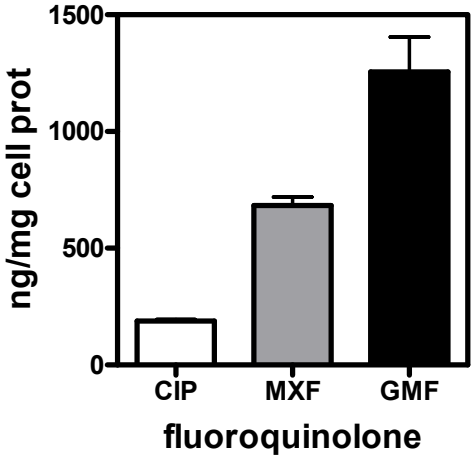
Vallet et al. Int. J. Antimicrob. Ag. (2011) 38:249-256.

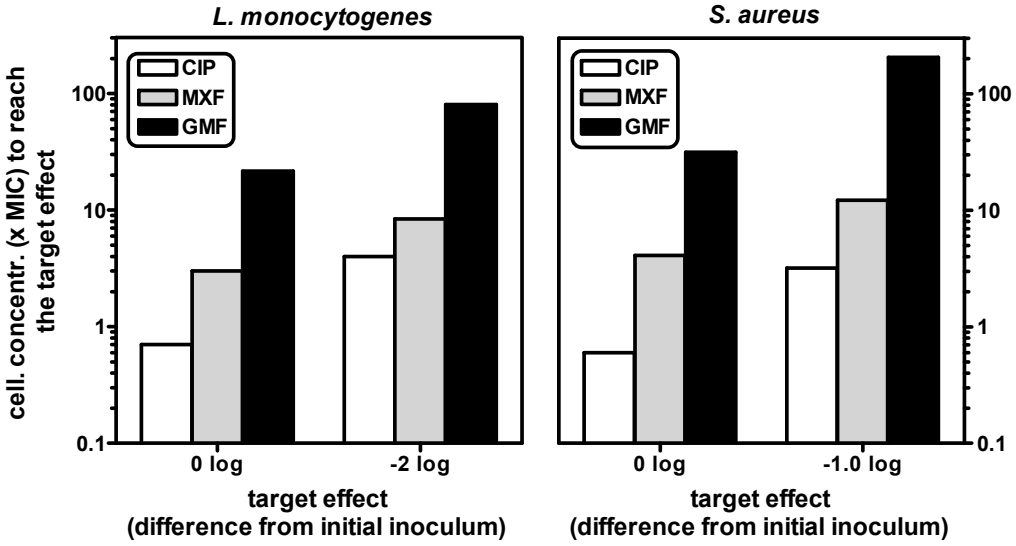
Comparing a class of drugs against different bacteria

Fluoroquinolones against *L. monocytogenes* vs. *S.aureus*

intracellular bioavailability ?





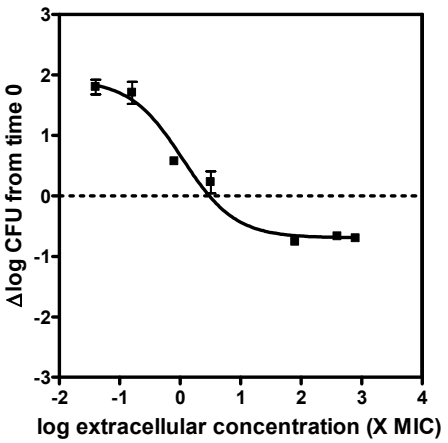


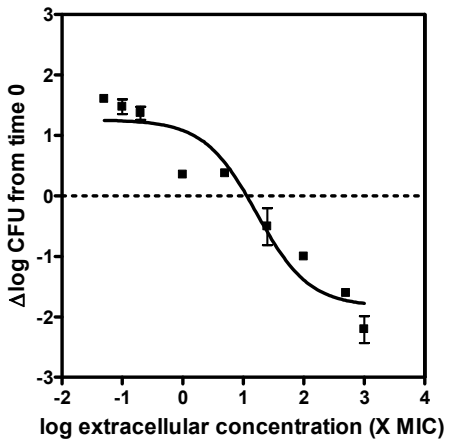
Vallet et al. Int. J. Antimicrob. Ag. (2011) 38:249-256.

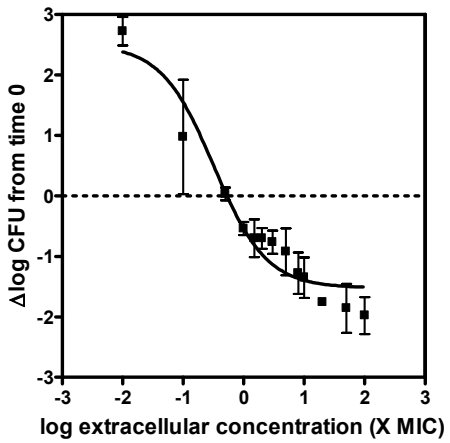
What about *P. aeruginosa* ?

These two look alike …

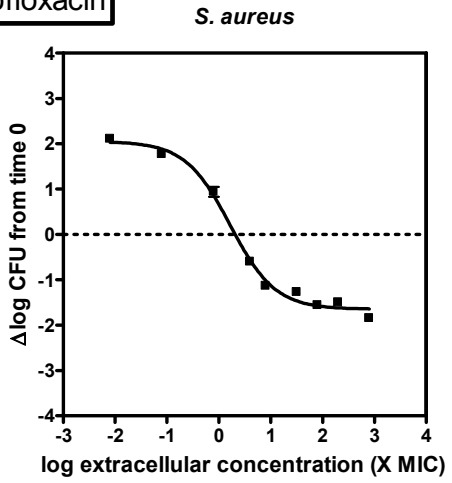
meropenem

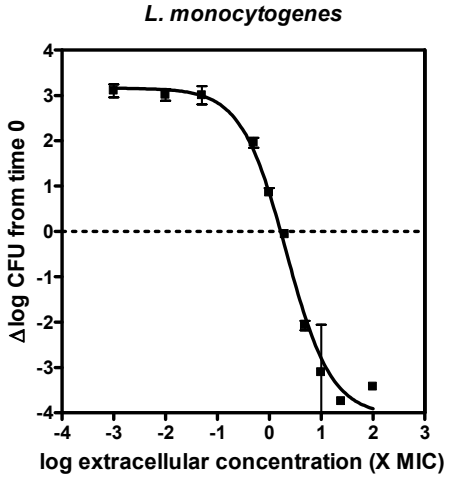


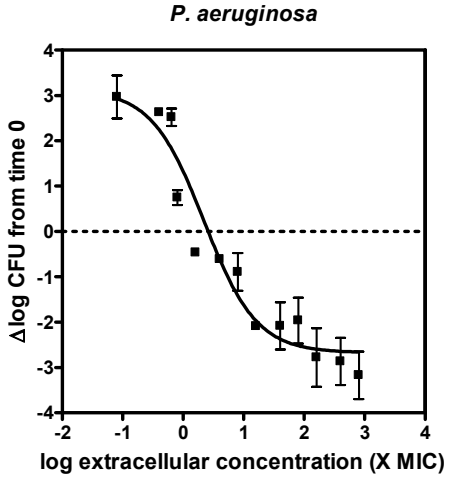




ciprofloxacin

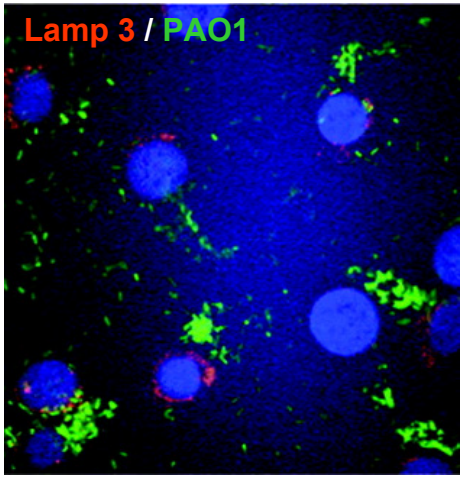




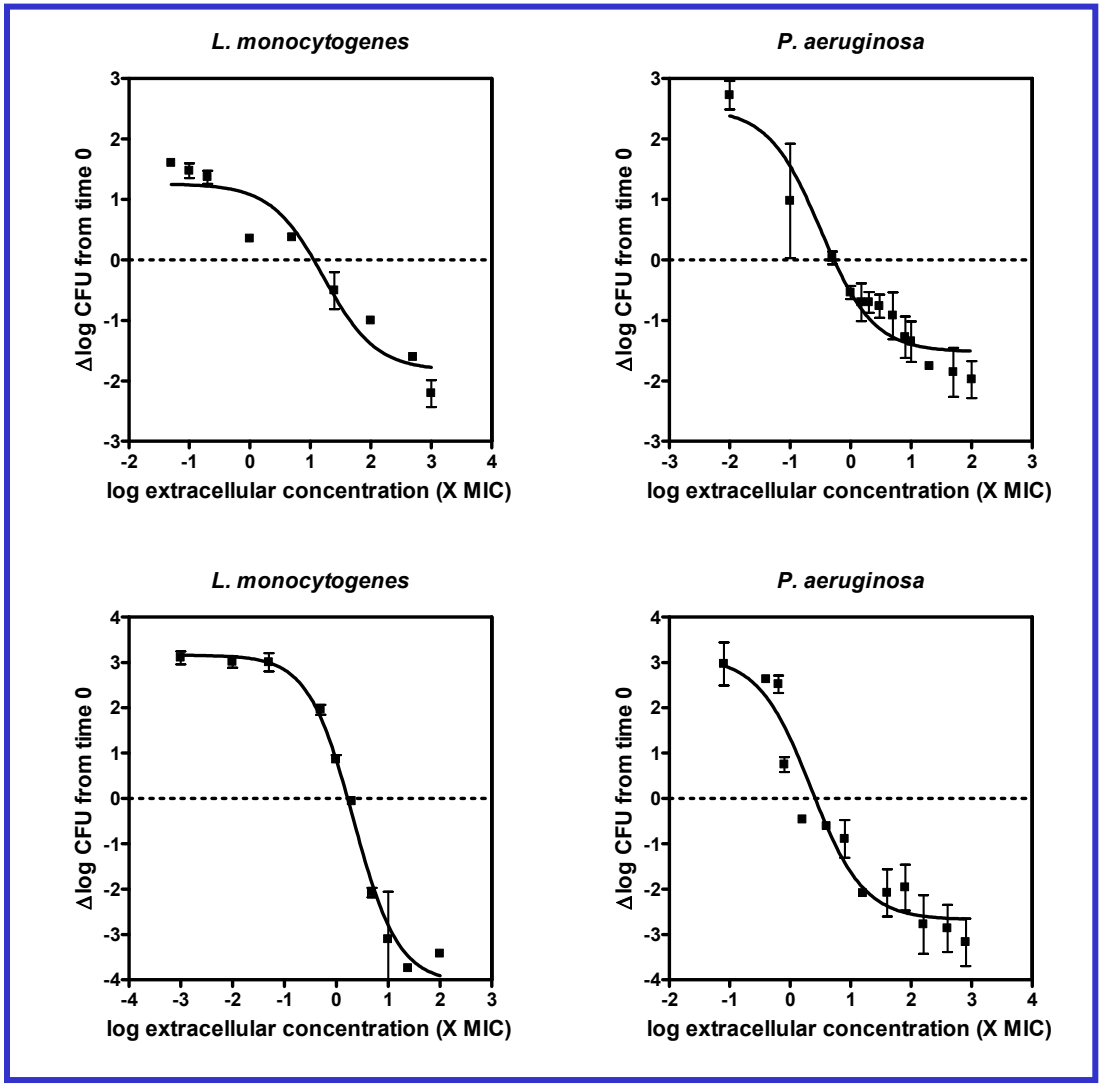


What about *P. aeruginosa* ?

These two look alike …



not associated with lysosomes after 8 h (in epithelial cells)

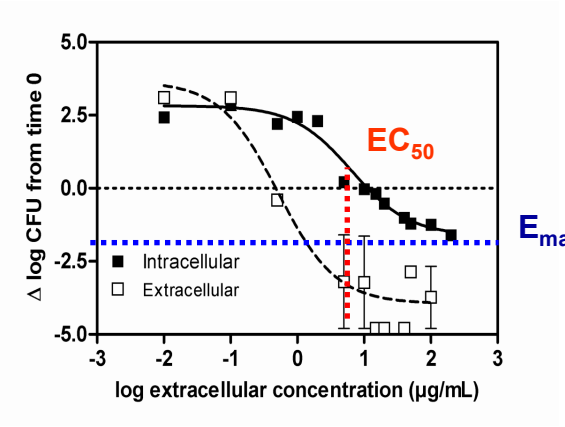


Can we do better ?

"You are completely free to carry out whatever research you want, so long as you come to these conclusions."

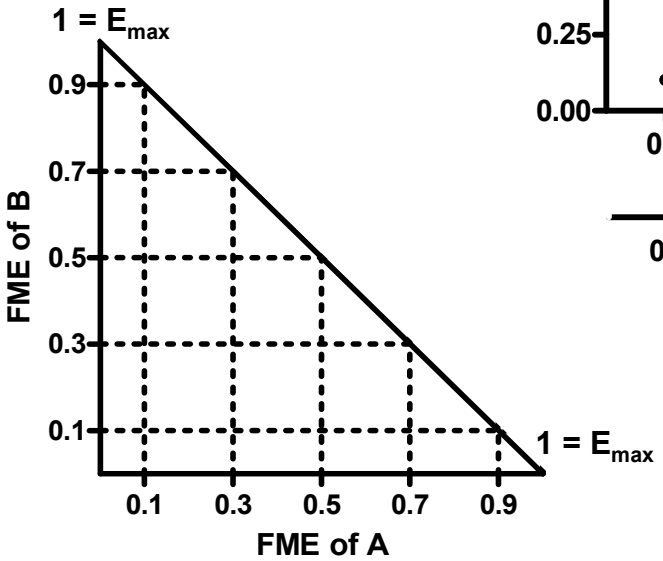
Can we do better ?

Antibiotic combinations

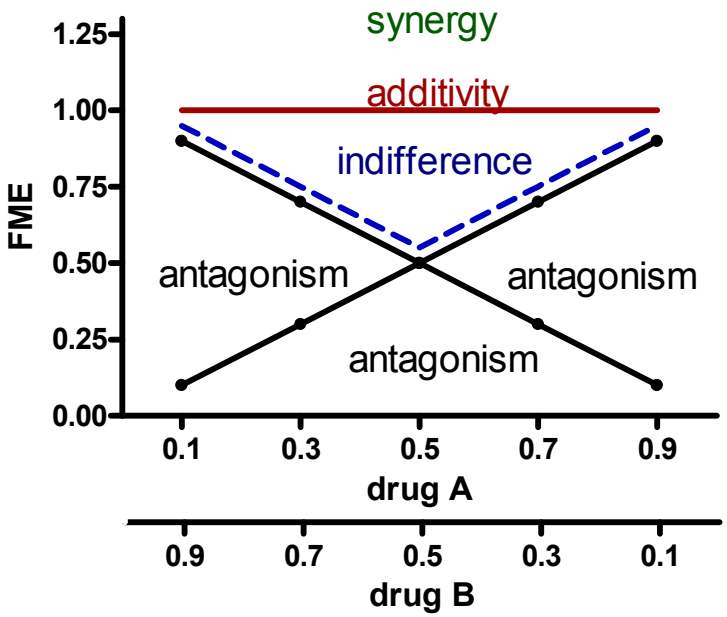


$$C_A = \frac{FME_A.EC_{50A}}{1-FME_A}$$

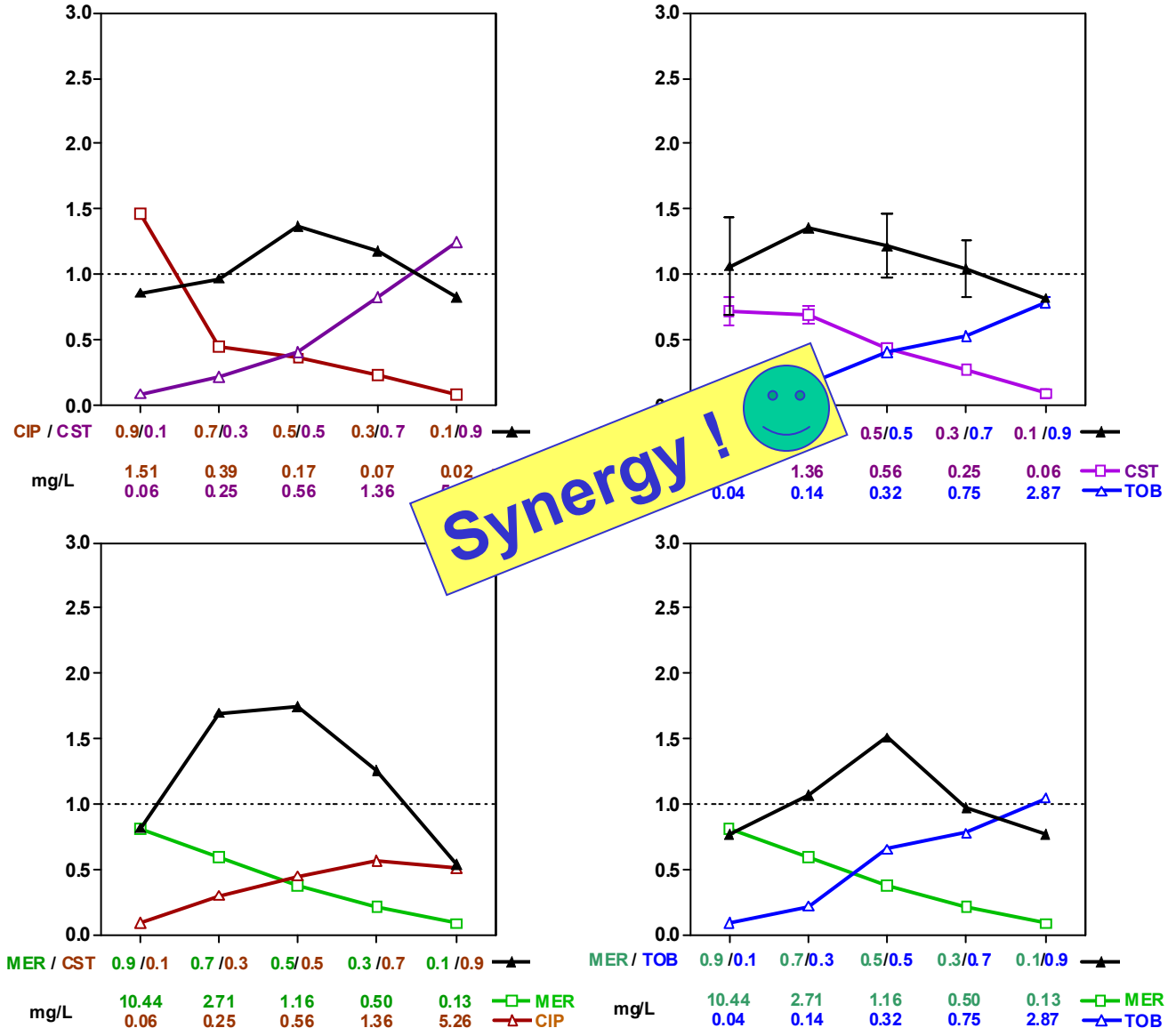
$$C_B = \frac{FME_B.EC_{50B}}{1-FME_B}$$



$FME_{comb.} = FME_A + FME_B = 1$

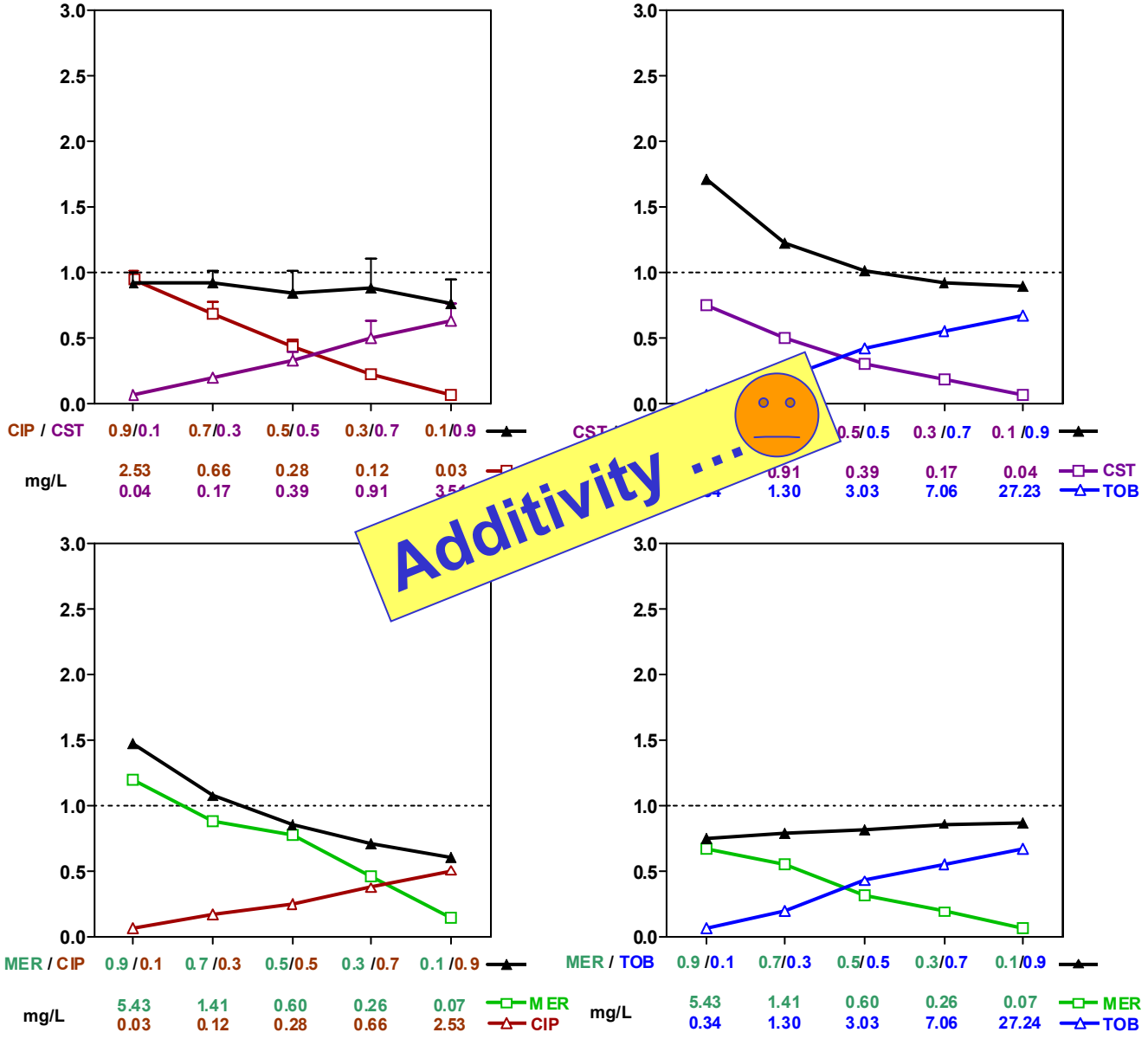


Combinations against extracellular *P. aeruginosa*



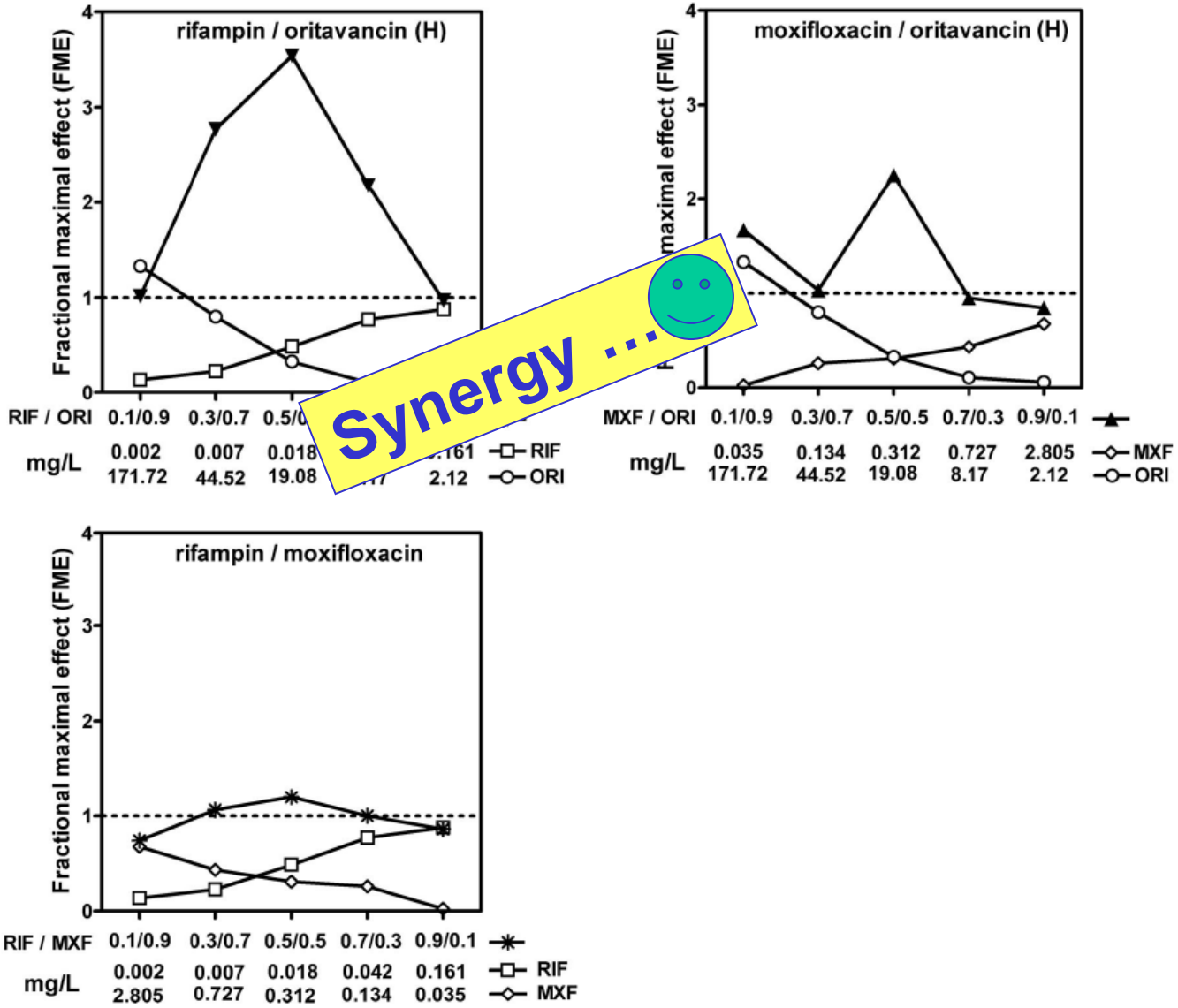
Buyck et al, ICAAC 2011

Combinations against intracellular *P. aeruginosa*



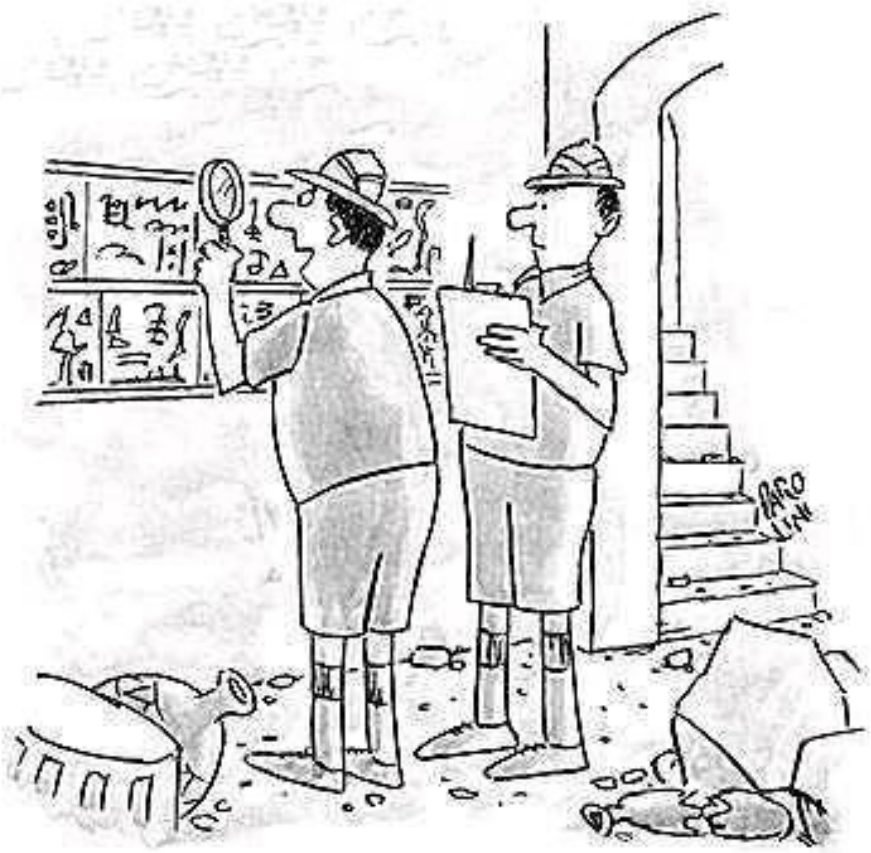
Buyck et al, ICAAC 2011

Combinations against intracellular *S. aureus*



Nguyen et al. Antimicrob. Ag. Chemother. (2009) 53:1443-1449

What do these models tell us ?

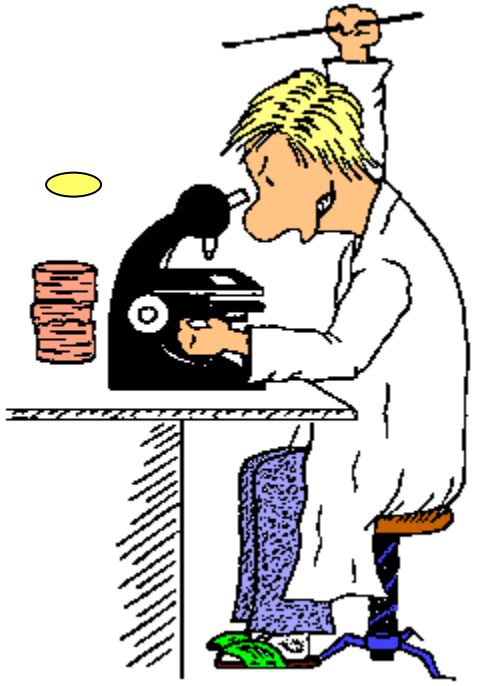


"THIS STUFF IS A SNAP FOR ME. I USED TO BE A PHARMACIST."

What do these models tell us ?

Now, I know how to catch them !

- high intracellular biodisponibility
- capacity to rejoin the infected compartment
- not substrate for efflux pumps
- low MIC at both neutral and acidic pH
- highly bactericidal, including against slow growing bacteria
- no cell toxicity
- cooperation with cell defence mechanisms



Use of these models to position new molecules

Guidance for Industry

Microbiological Data for Systemic Antibacterial Drug Products — Development, Analysis, and Presentation

"That must be the new miracle drug."

DRAFT GUIDANCE

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

September 2009
Clinical Antimicrobial

C. Intracellular Antimicrobial Concentration Assessment

The ability of an antibacterial drug product to achieve significant intracellular concentrations may have clinical importance when the target organism can reside within the cell (e.g., *Listeria*, *Chlamydophila*, *Legionella*). In situations where the antimicrobial drug product is intended to treat infections caused by microorganisms that reside within the cell, sponsors should provide data on the drug product's ability to penetrate into host cells and demonstrate the drug product's activity inside the cell against target microorganisms.

Take home message

1. identify your target
2. choose the right weapon

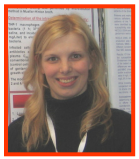
you may avoid serious problems ...

Acknowledgments

Our intracellular PK/PD team over the years …


C. Seral


M. Barcia-Macay


S. Lemaire


H.A. Nguyen


A. Olivier

S. aureus

P. Baudoux


L. Garcia


Y. Ouadhriri

L. monocytogenes


S. Carryn


S. Vandevelde


A. Lismond

P. aeruginosa


J. Buyck


G. de Laminne


H. Jacqmin


A. Anantharajah