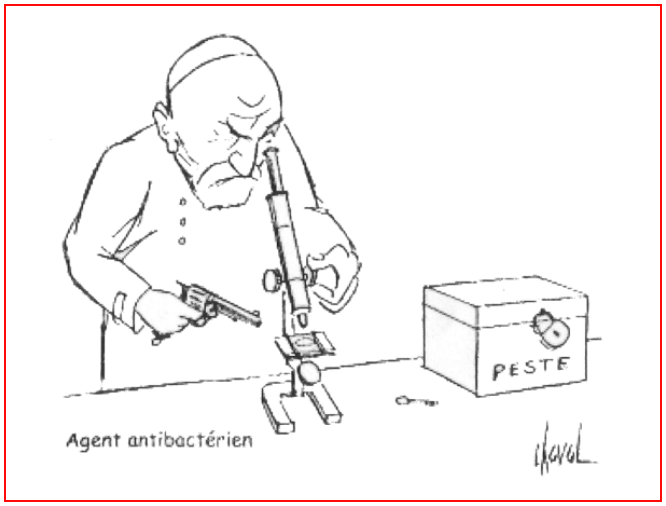
Hunting intracellular bacteria with antibiotics



Françoise Van Bambeke

Pharmacologie cellulaire et moléculaire
Louvain Drug Research Institute
Université catholique de Louvain, Brussels, Belgium

<www.facm.ucl.ac.be>

Traveling from one « Notre Dame » to the other …

A quite nice common experience ….

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 283, NO. 19, pp. 12769–12776, May 9, 2008

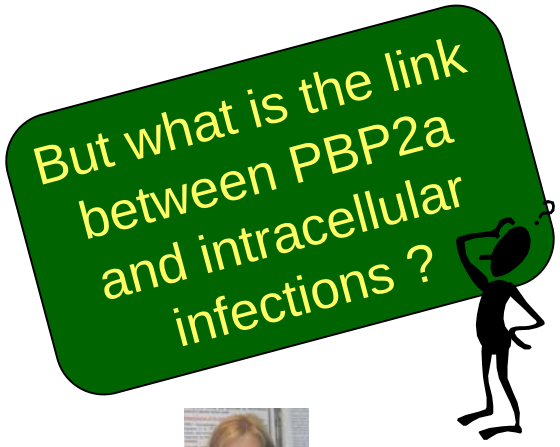
Restoration of Susceptibility of Methicillin-resistant *Staphylococcus aureus* to β-Lactam Antibiotics by Acidic pH

ROLE OF PENICILLIN-BINDING PROTEIN PBP 2a[*S]

Received for publication, January 4, 2008, and in revised form, March 11, 2008 Published, JBC Papers in Press, March 12, 2008, DOI 10.1074/jbc.M800079200

Sandrine Lemaire[‡], Cosimo Fuda[§], Françoise Van Bambeke[‡], Paul M. Tulkens[‡], and Shahriar Mobashery[§]

From the [‡]Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, B-1200 Brussels, Belgium and the [§]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556



Author's Choice

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 285, NO. 31, pp. 24055–24065, July 30, 2010

Insertion of Epicatechin Gallate into the Cytoplasmic Membrane of Methicillin-resistant *Staphylococcus aureus* Disrupts Penicillin-binding Protein (PBP) 2a-mediated β-Lactam Resistance by Delocalizing PBP2[*S]

Received for publication, February 16, 2010, and in revised form, May 5, 2010 Published, JBC Papers in Press, June 1, 2010, DOI 10.1074/jbc.M110.114793

Patricia Bernal[‡], Sandrine Lemaire[§], Mariana G. Pinho[¶2], Shahriar Mobashery[‖], Jason Hinds[**], and Peter W. Taylor[‡3]

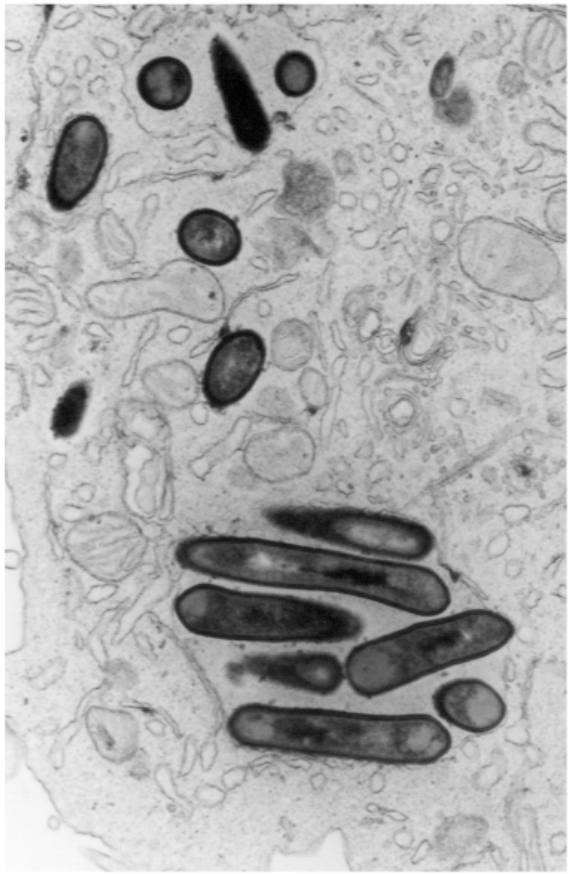
From the [‡]School of Pharmacy, University of London, London WC1N 1AX, United Kingdom, the [§]Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, B-1200 Brussels, Belgium, the [¶]Bacterial Cell Biology Laboratory, Instituto de Tecnologia Quimica e Biológica, Universidade Nova de Lisboa, 2781-901 Oeiras, Portugal, the [‖]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556, and the [**]Department of Cellular and Molecular Medicine, St. George's, University of London, London SW17 0RE, United Kingdom

A quite nice common experience ….

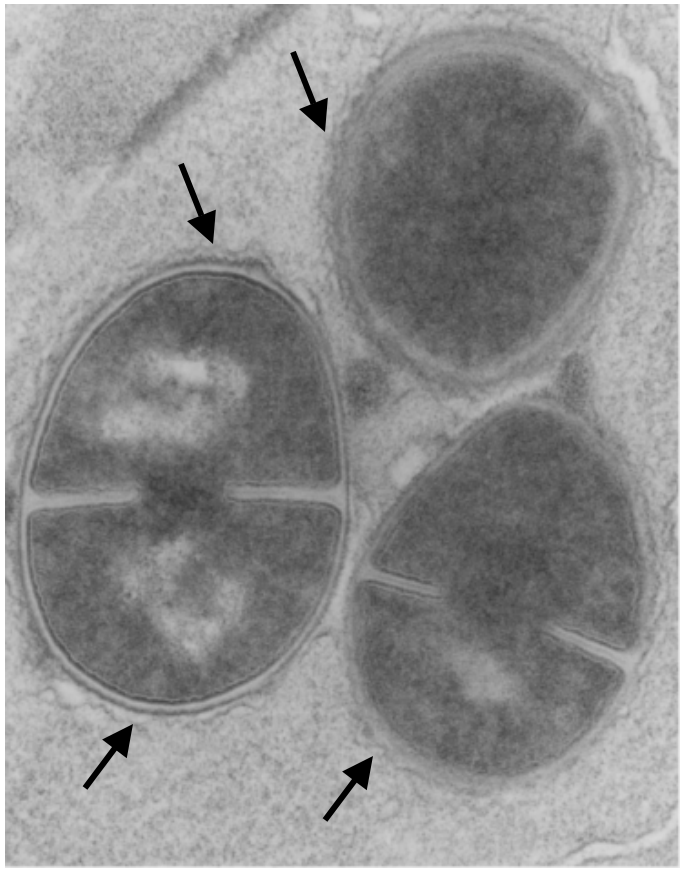
But what is the link between PBP2a and intracellular infections ?

MRSA is everywhere … including inside our cells!

The infected cell: a guided tour …

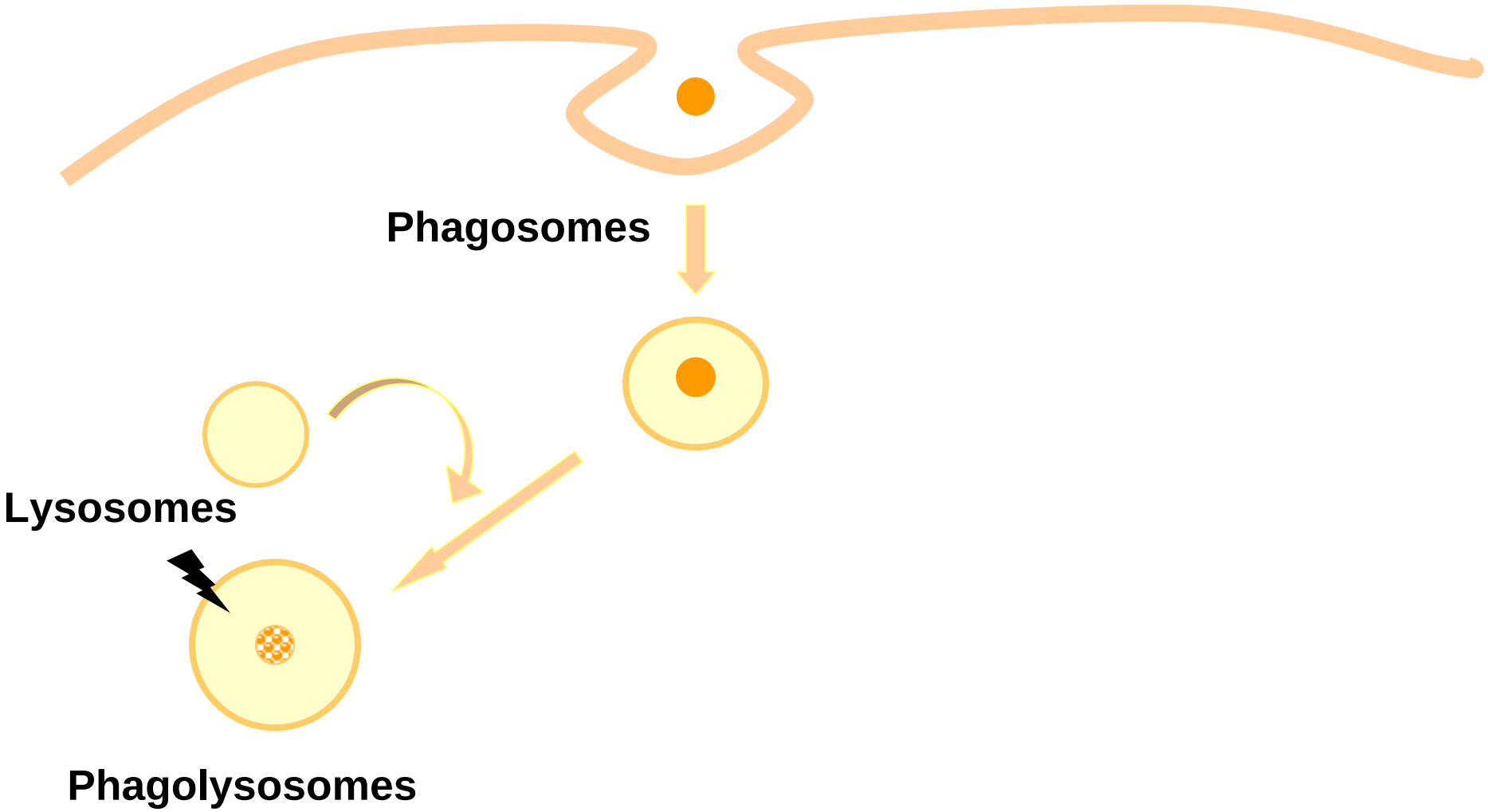


Listeria; cytosol

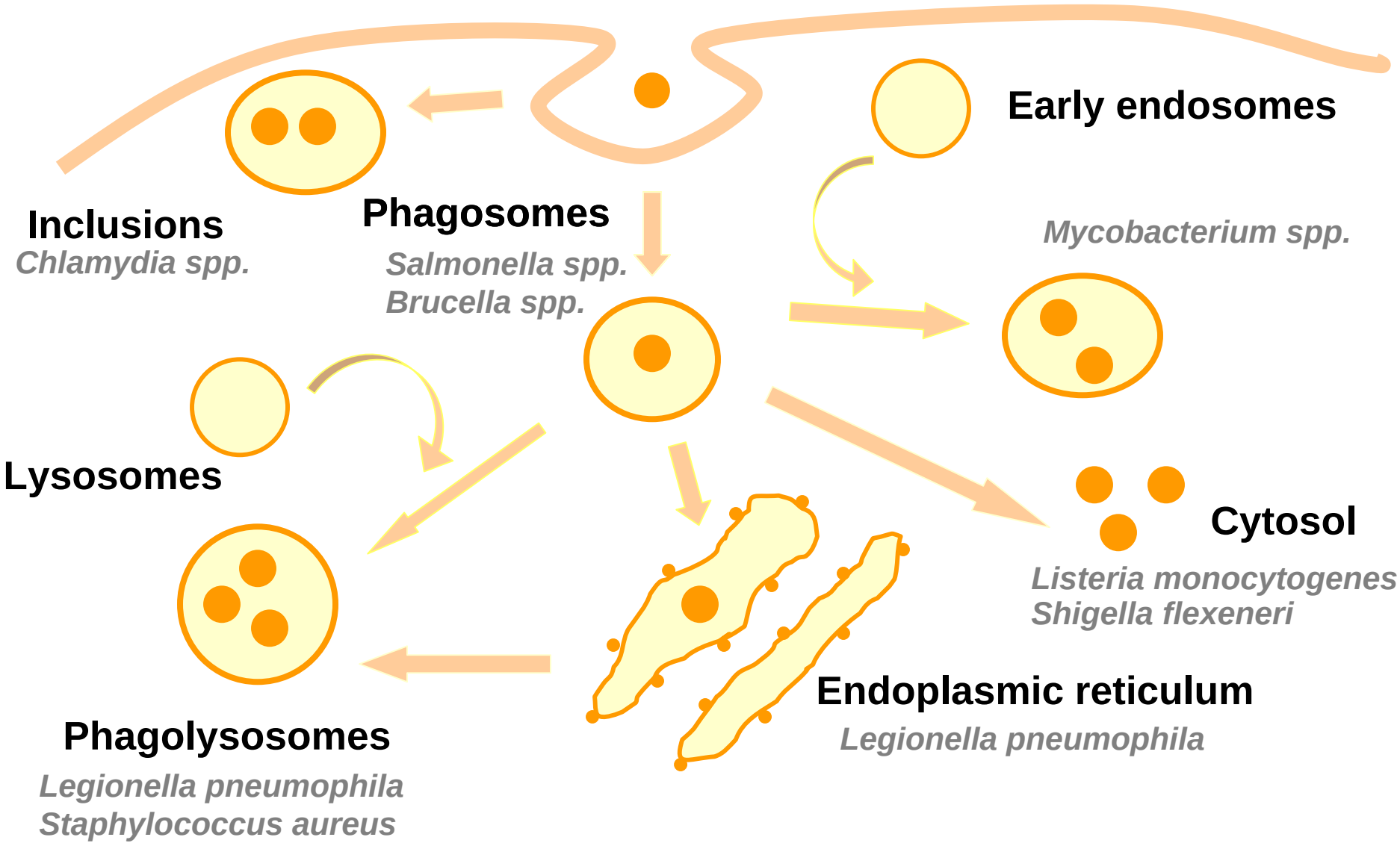


S. aureus; phagolysosomes

Intracellular killing of bacteria by host cell defence mechanisms

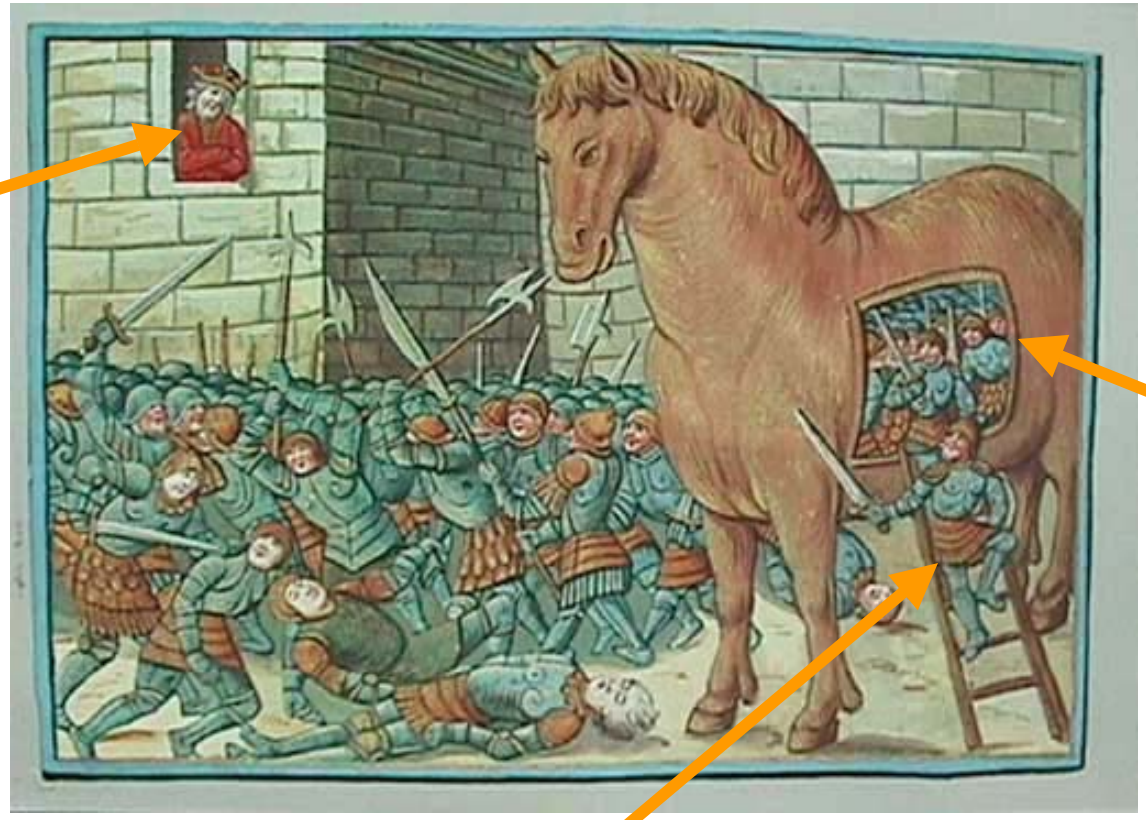


Some bacteria can escape host cell defence mechanisms …



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

Benefits of intracellular life

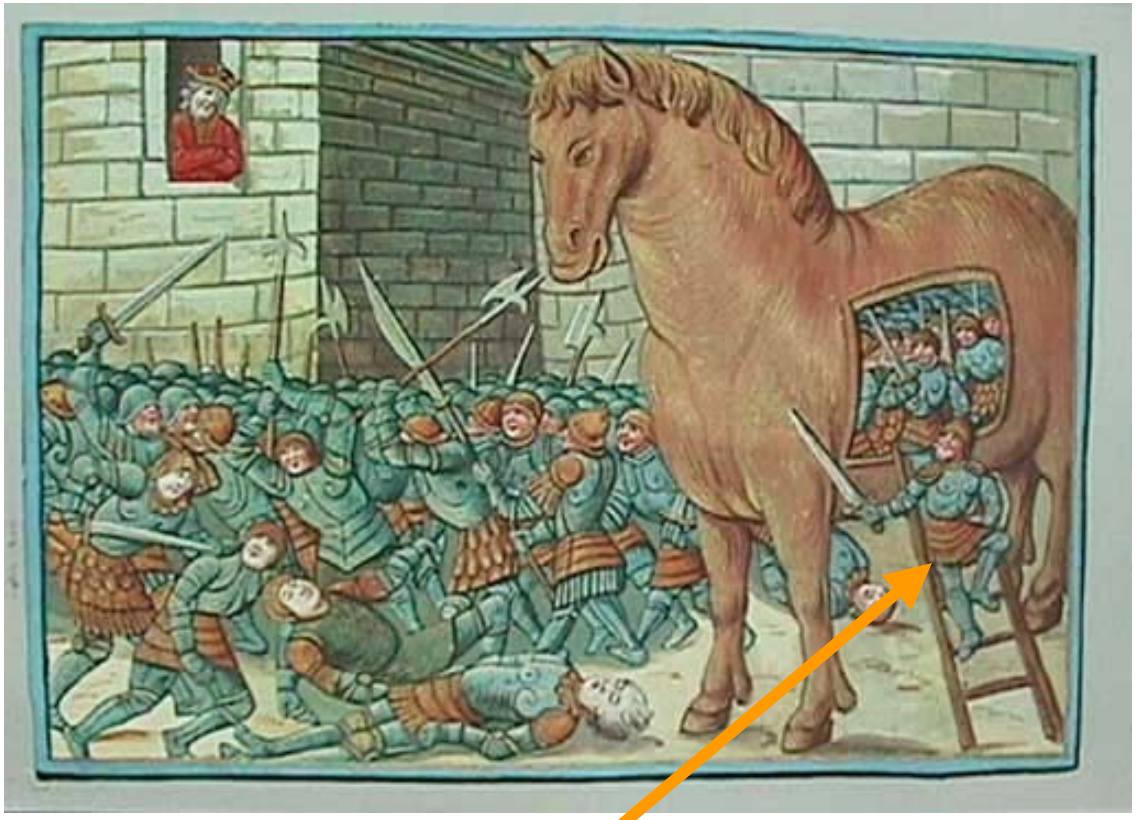


protection

persistence

invasion

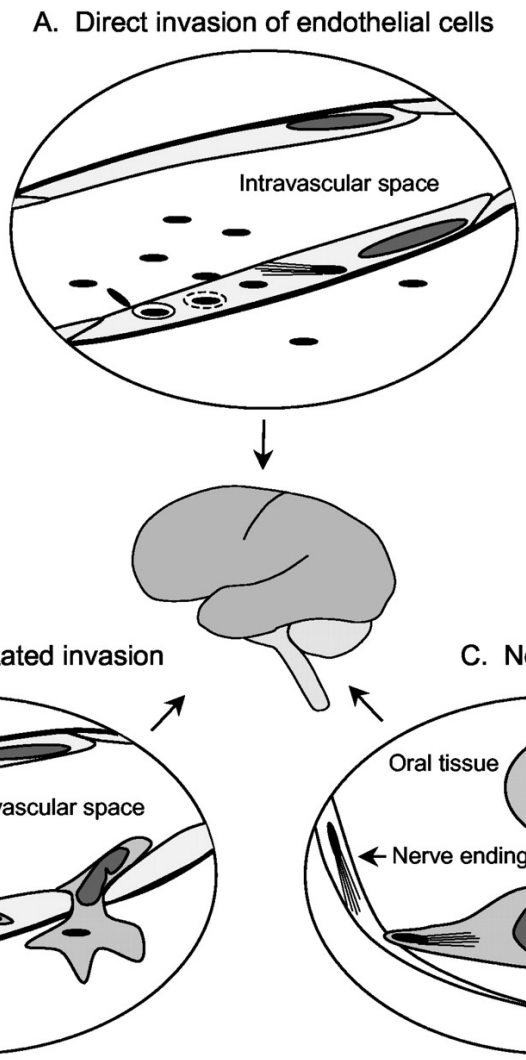
Benefits of intracellular life

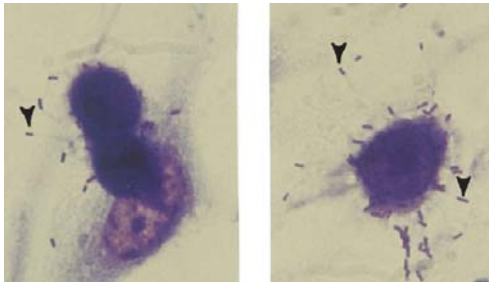


invasion

Migration to the CNS

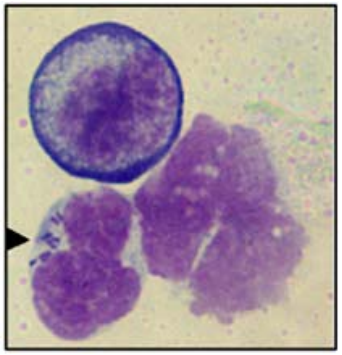
Listeria:
from the gut to the CNS

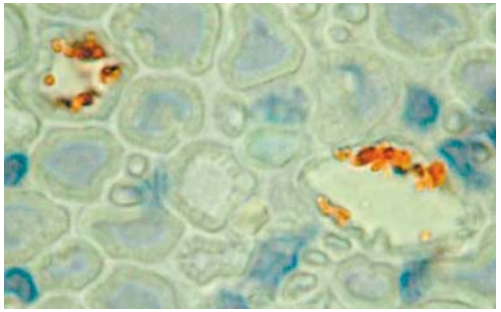




adherence and transfert
from monocytes to endothelial cells

bone-marrow monocyte

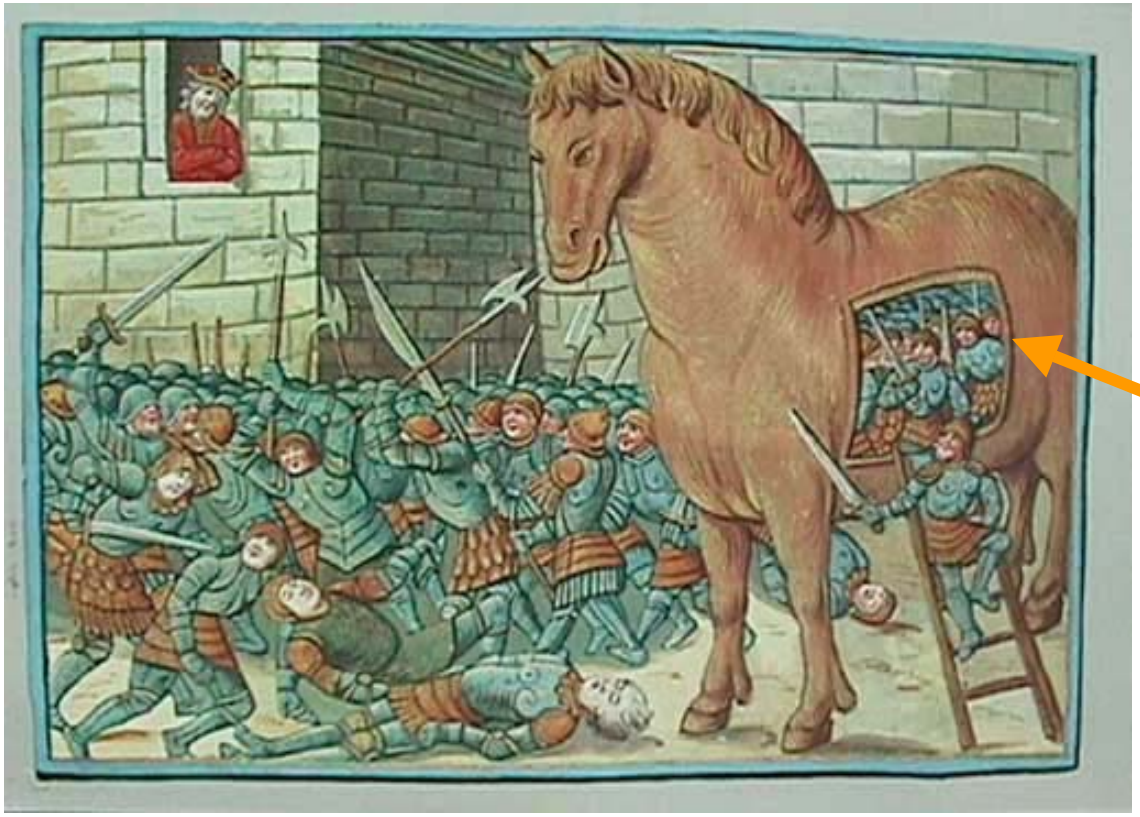




intra-axonal labeling
by anti-listeria antibodies

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

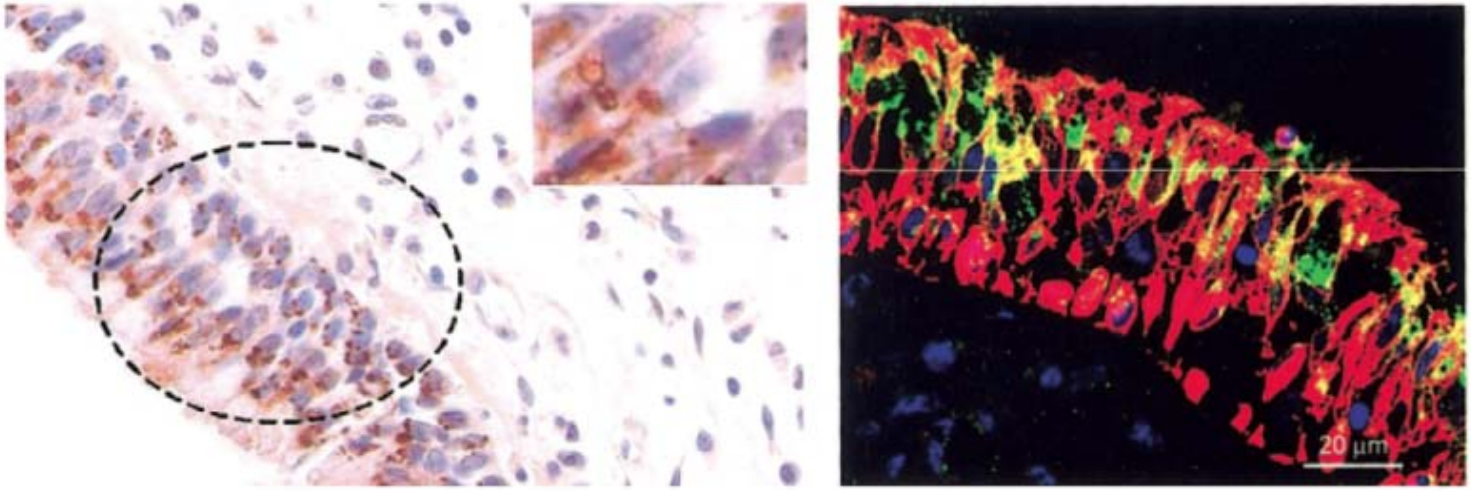
Benefits of intracellular life



persistence

S. aureus persistent infections

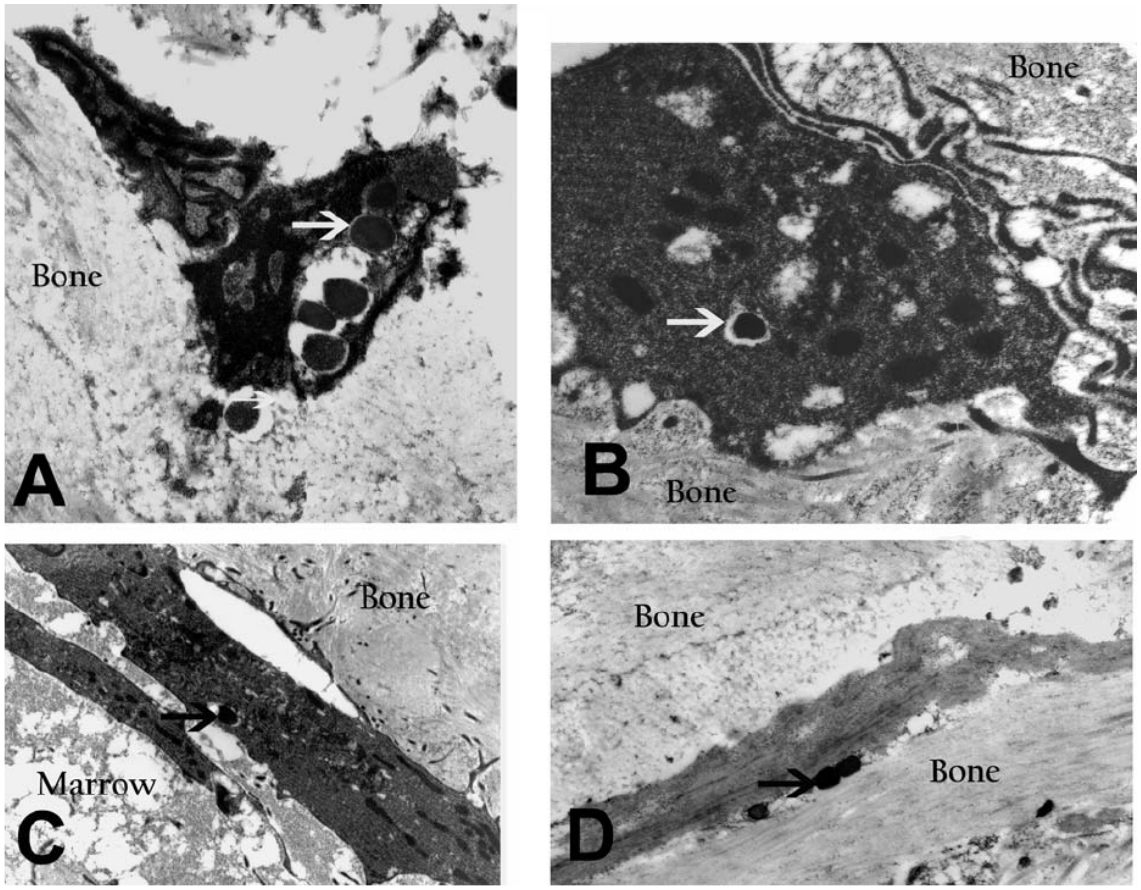
Evidence of an intracellular reservoir in the nasal mucosa of patients with recurrent *Staphylococcus aureus* rhinosinusitis



Clement et al., J Infect Dis. (2005) 192:1023-8

S. aureus persistent infections

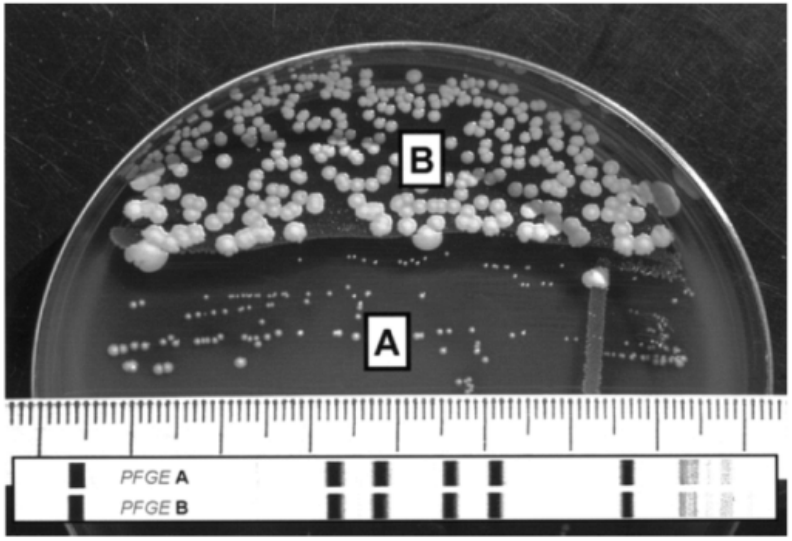
Evidence of an intracellular reservoir in osteocytes (A,B), osteoblasts (C) and bone matrix of a patient with recurrent osteomyelitis



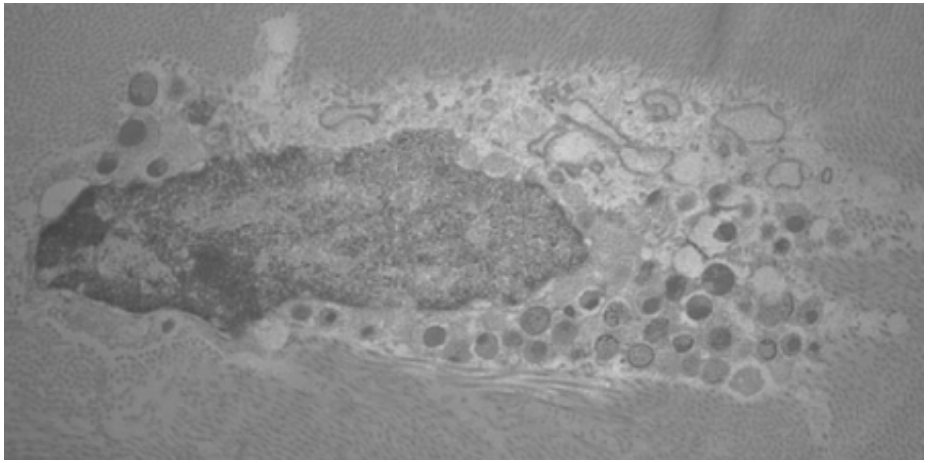
Bosse et al., J Bone Joint Surg Am. (2005) 87:1343-7

S. aureus persistent infections

Evidence of Small Colony Variants and of intracellular *S. aureus* after treatment failure * in patients with prosthetic joint infections



Small colony variant *(A)* and normal-phenotype *Staphylococcus aureus (B)* isolated from patient 1 on Columbia blood agar.

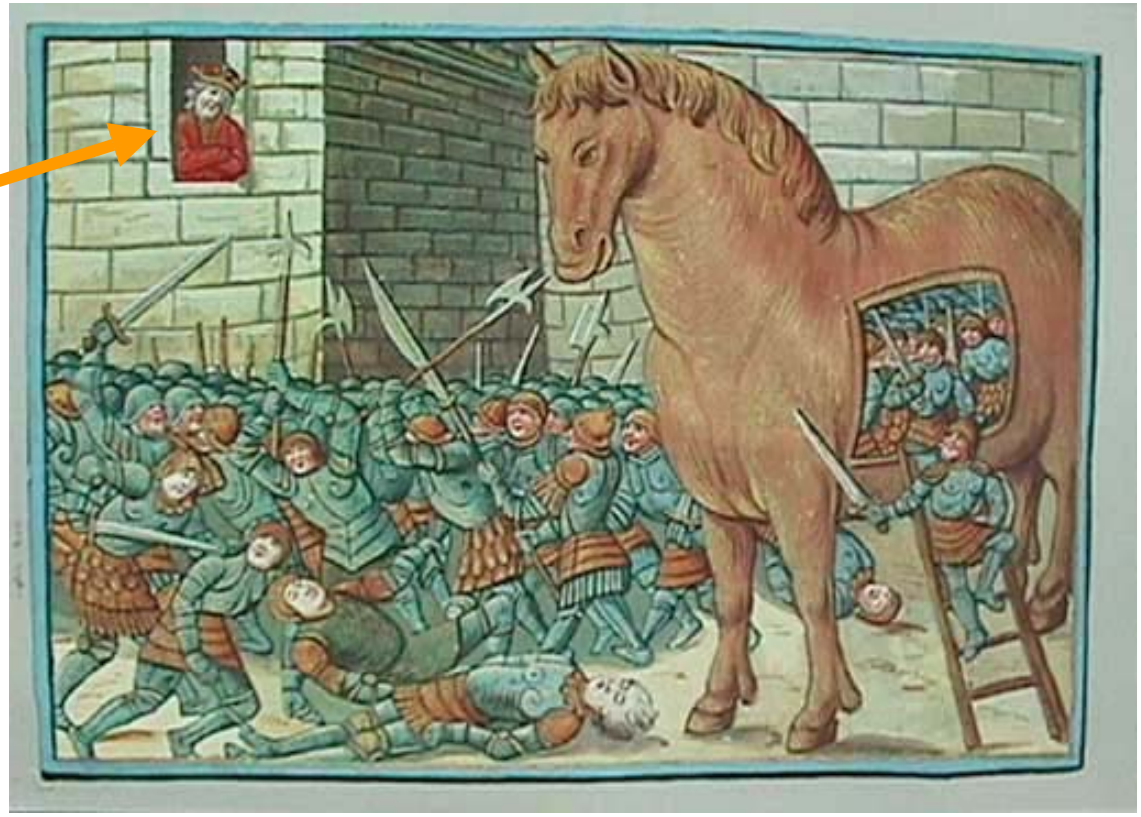


* Fluclox, CIP+ RIF, VAN + FEP

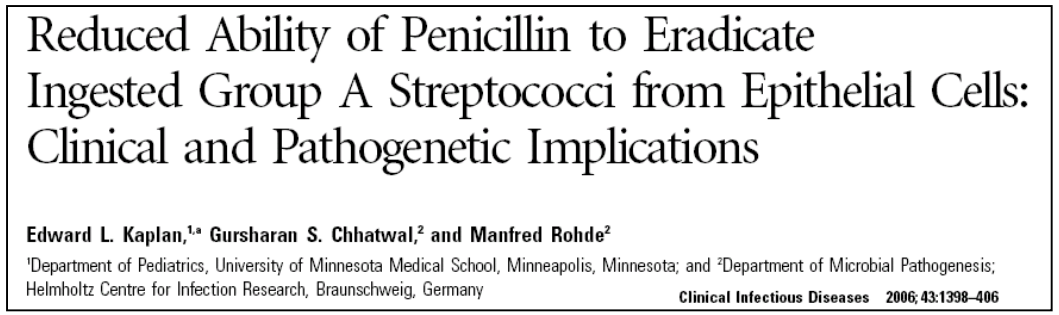
Sendi et al., Clin Infect Dis. (2006) 43:961-7

Benefits of intracellular life

protection



Failure to eradite with antibiotics in vitro …

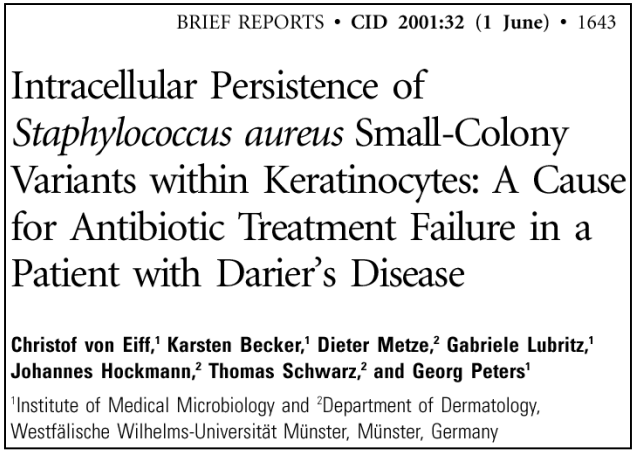


Reduced Ability of Penicillin to Eradicate Ingested Group A Streptococci from Epithelial Cells: Clinical and Pathogenetic Implications

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

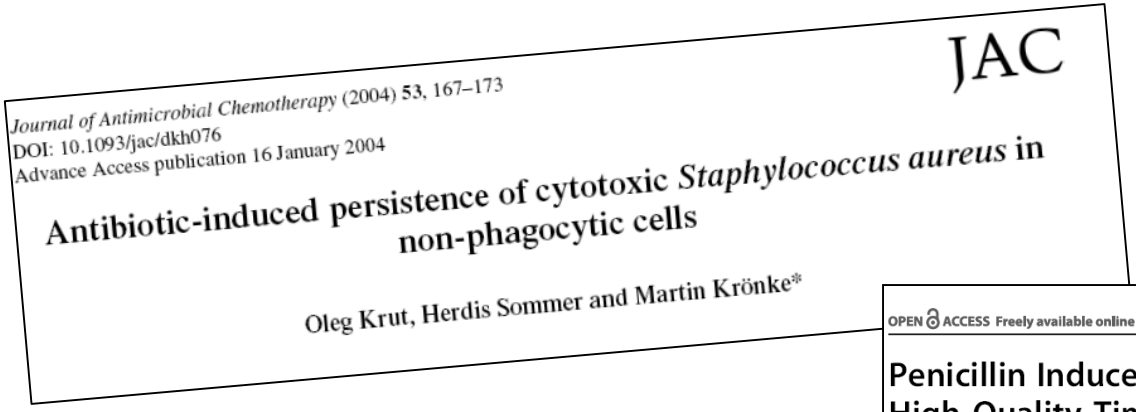
Clinical Infectious Diseases 2006; 43:1398–406



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

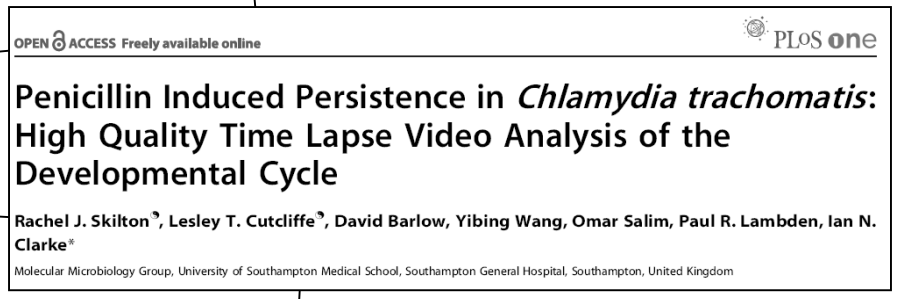


JAC

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

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

Oleg Krut, Herdis Sommer and Martin Krönke*



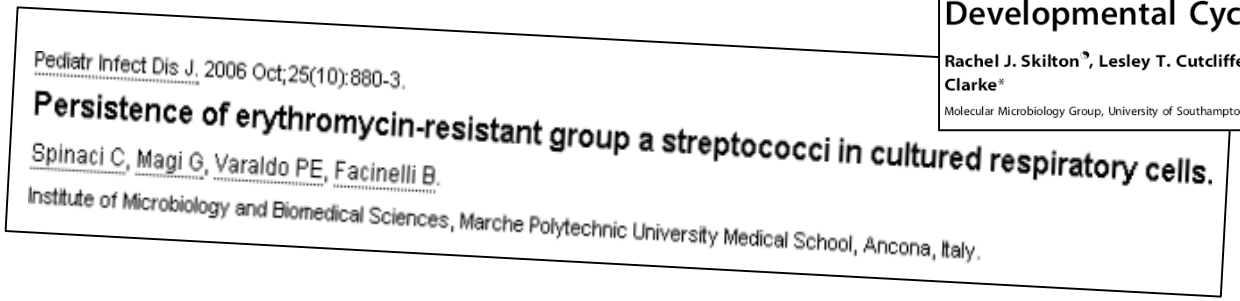
OPEN ACCESS Freely available online

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

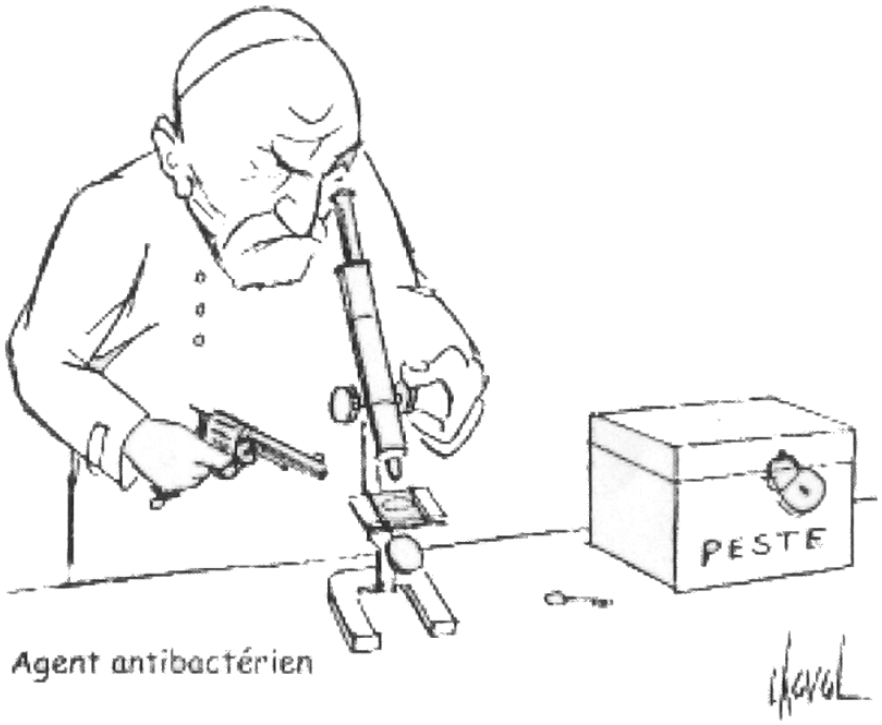
Journal of Antimicrobial Chemotherapy (2005) **55**, 383–386

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

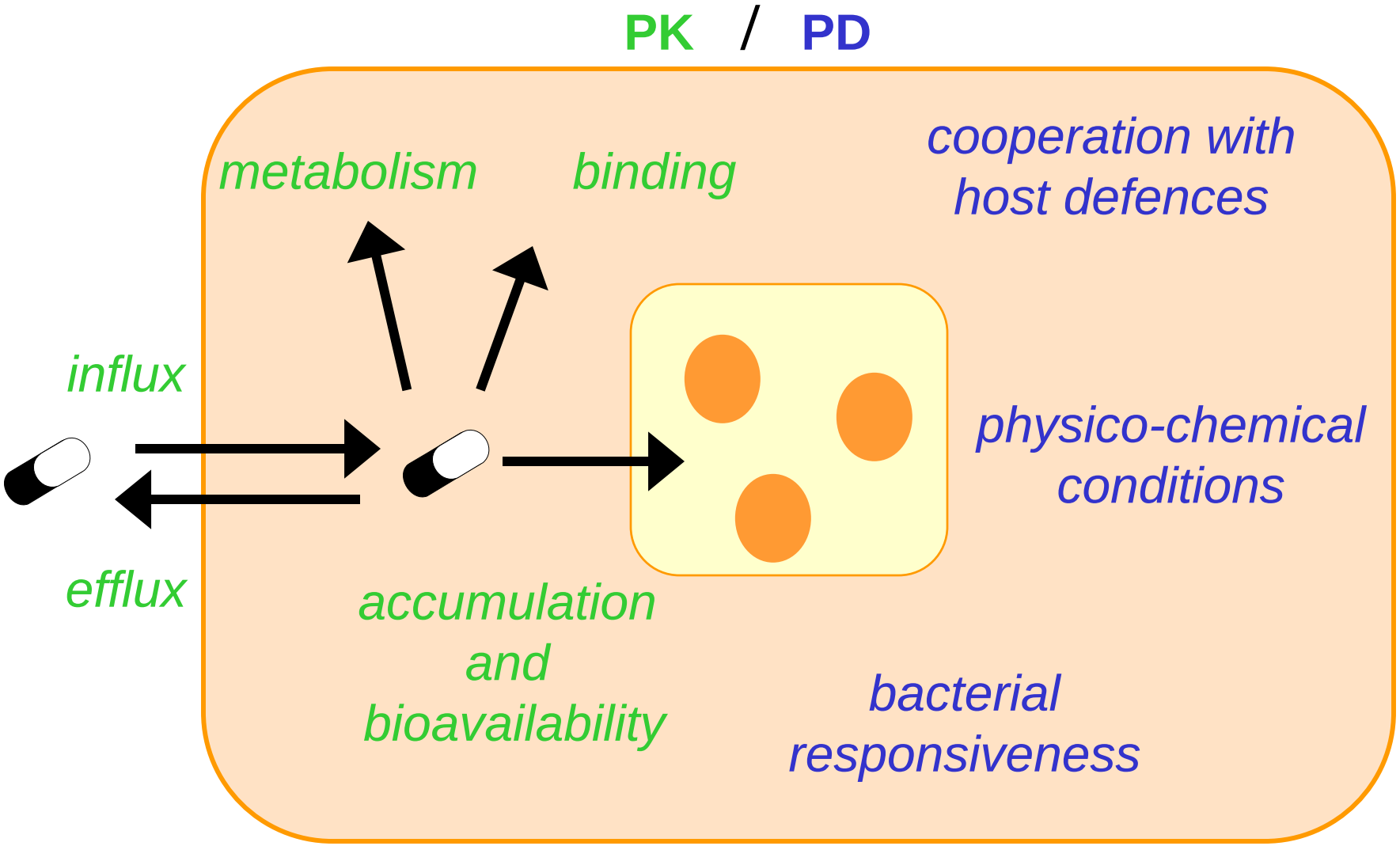
M. B. Kerrn[1,2]*, C. Struve[1], J. Blom[3], N. Frimodt-Møller[2] and K. A. Krogfelt[1]

[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 ?

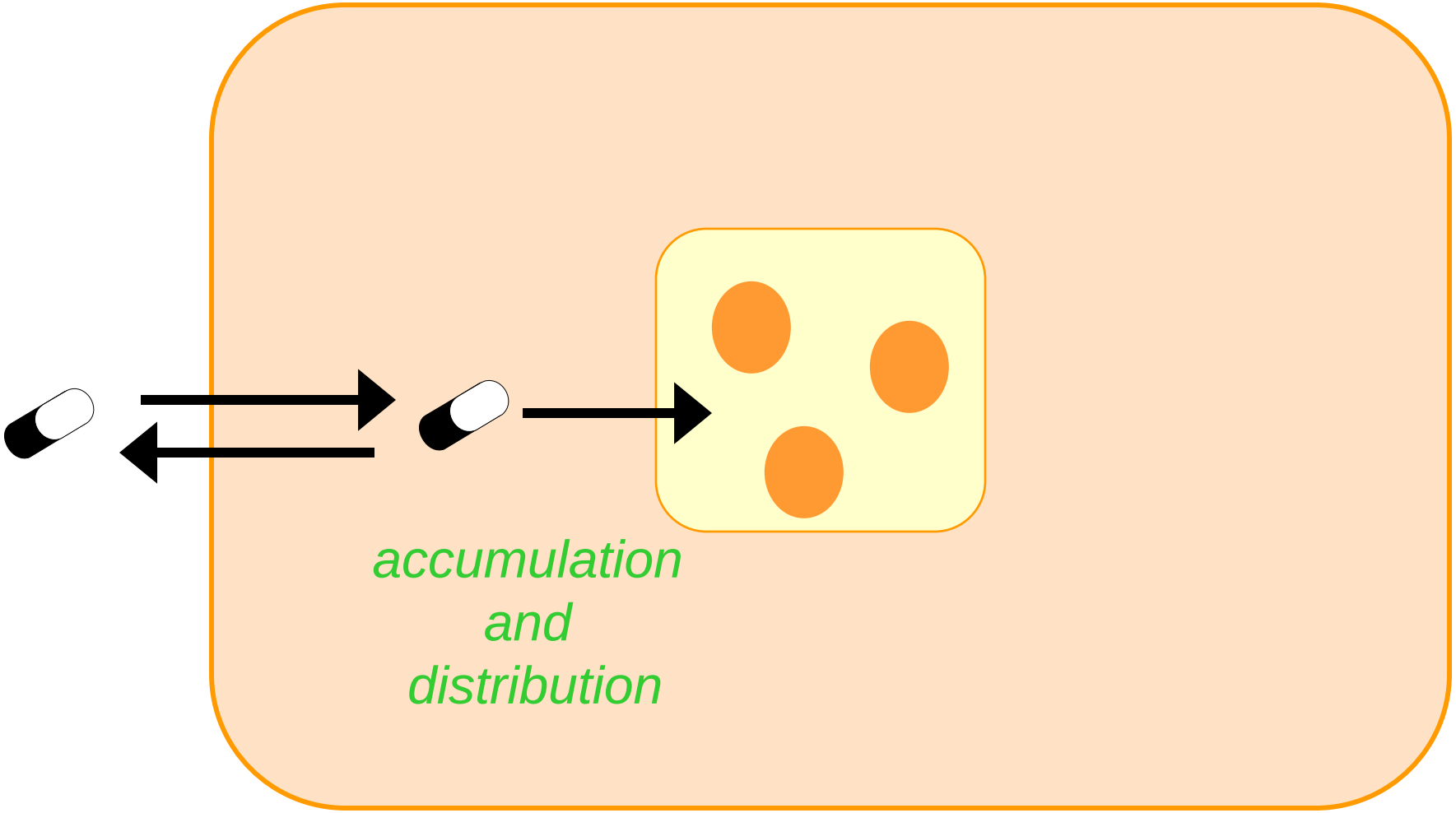


Antibiotic properties for intracellular activity



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

Cellular pharmacokinetics ….



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

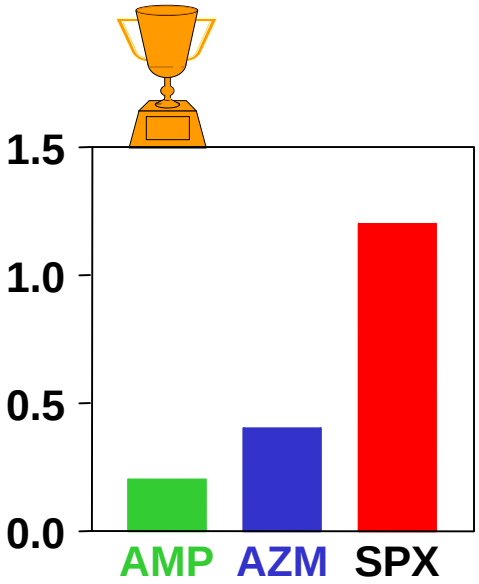
macrolides: fast
ERY: 4-10 x
CLR, ROX, TEL: 10-50x
AZM: > 50 x
CEM-101: 350 x

oxazolidinones: fast
RDZ : 10 x

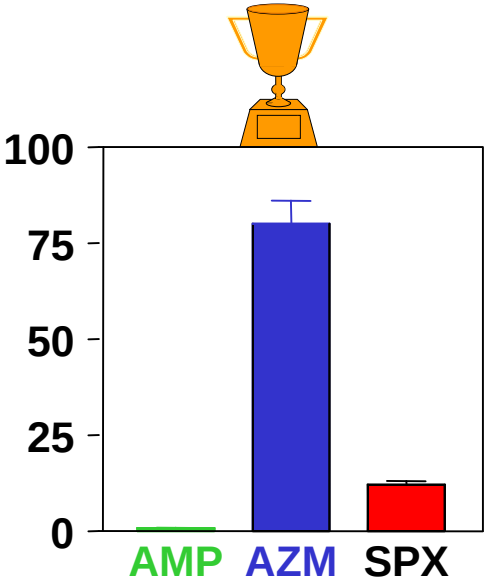
diffusion/
segregation

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

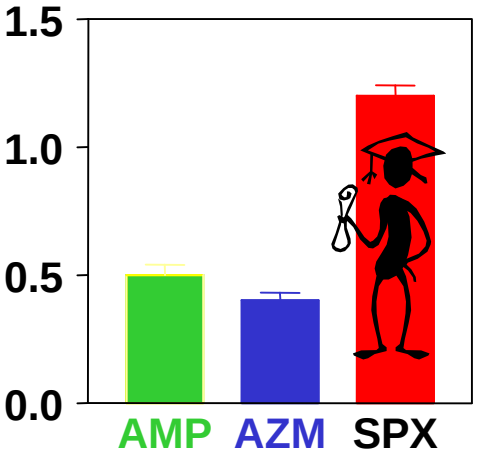
MIC
(L. monocytogenes)



antibiotic accumulation

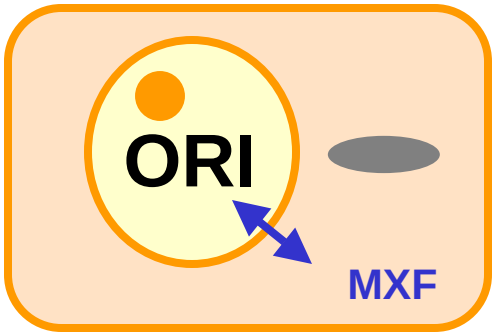


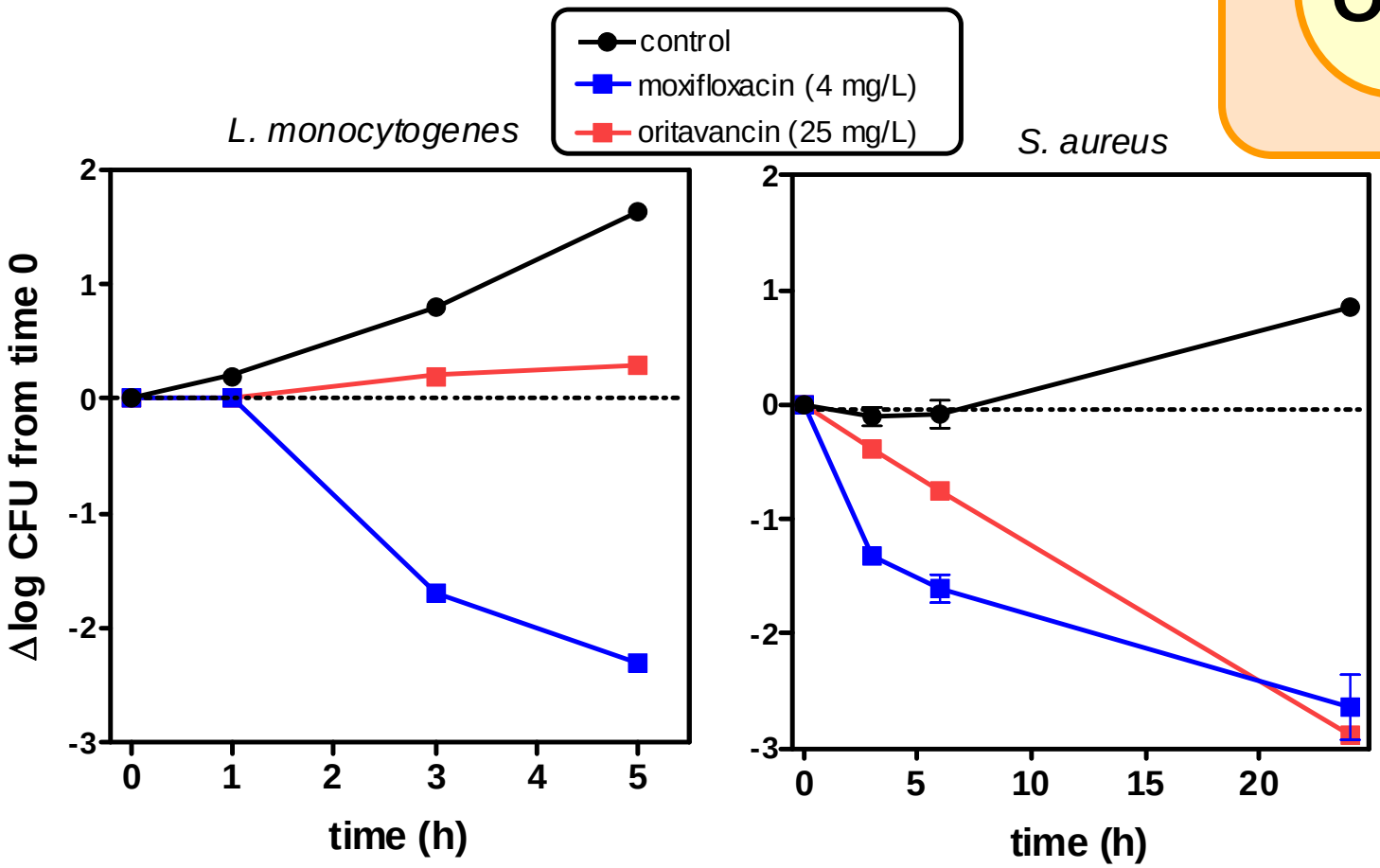
activity on intracellular *Listeria*
(5 h; 10 x MIC)



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

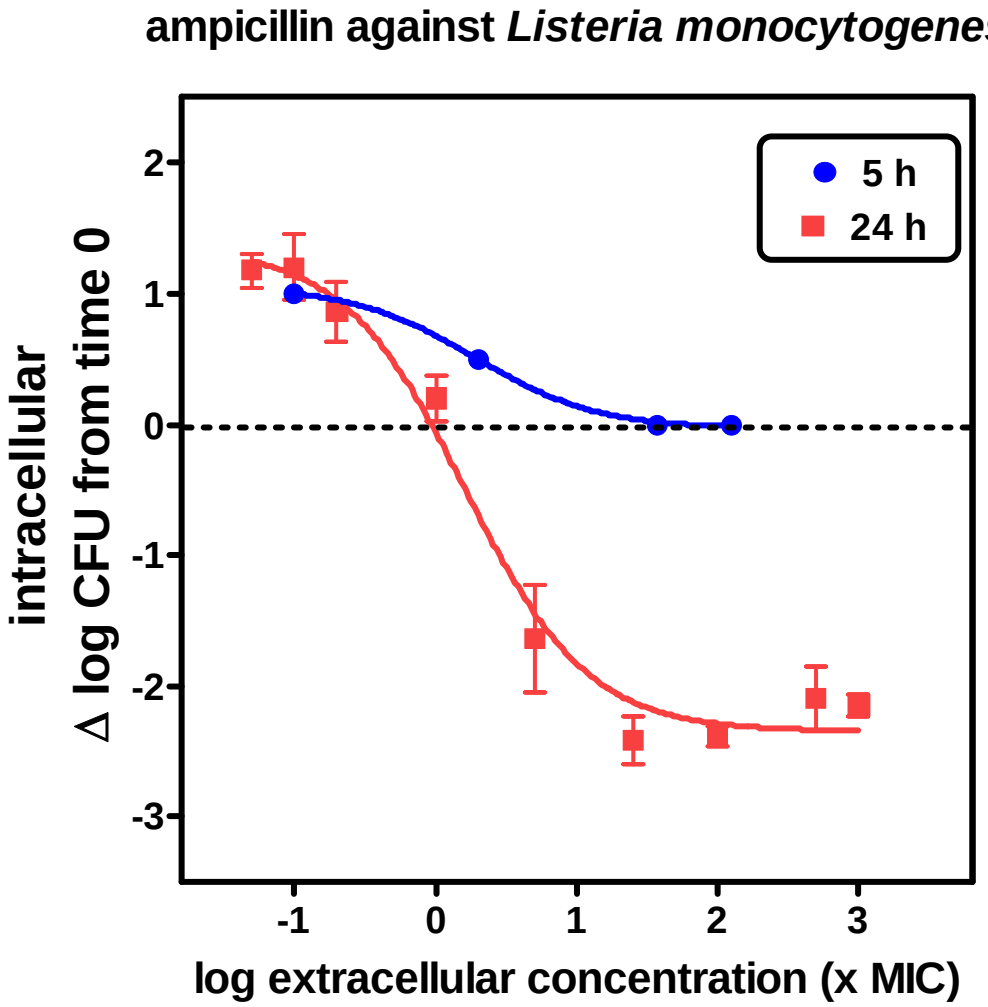
Importance of reaching intracellular bacteria …





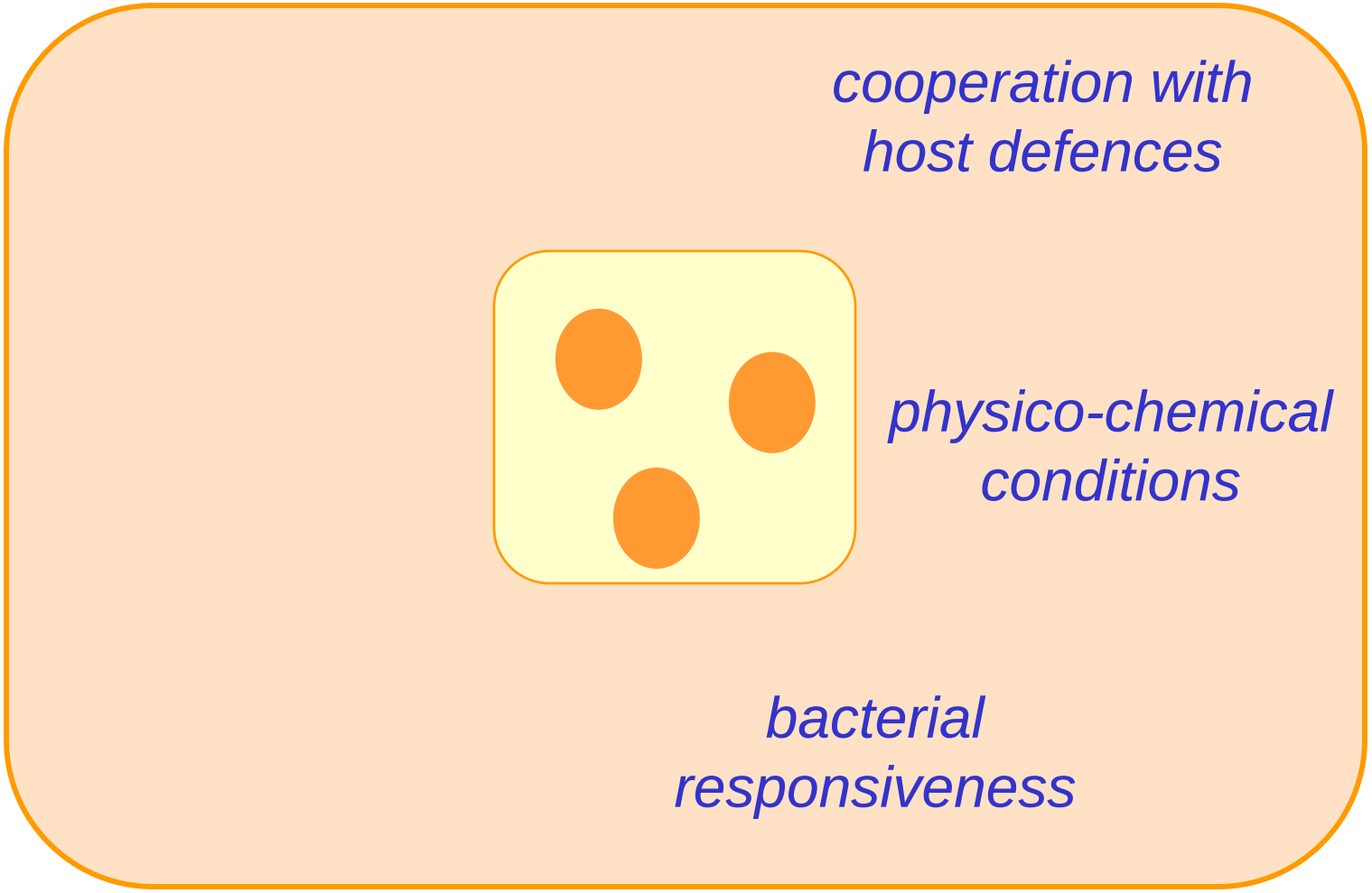
adapted from Carryn et al., AAC (2002) 46:2095-2103
Van Bambeke et al., AAC (2004) 48:2853-60
Barcia-Macay et al., AAC (2006) 50:841-51

Importance of optimizing time and concentration …



adapted from Lemaire et al., JAC (2005) 55:897-904

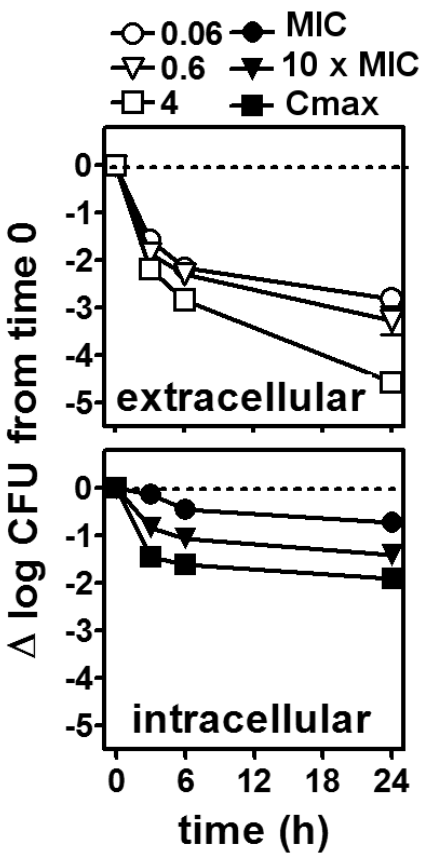
Cellular pharmacodynamics

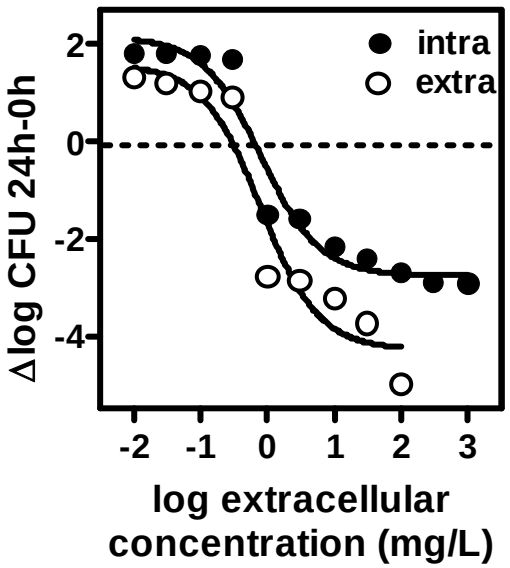


Carryn et al., Infect Dis Clin North Am. (2003) 17:615-34

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

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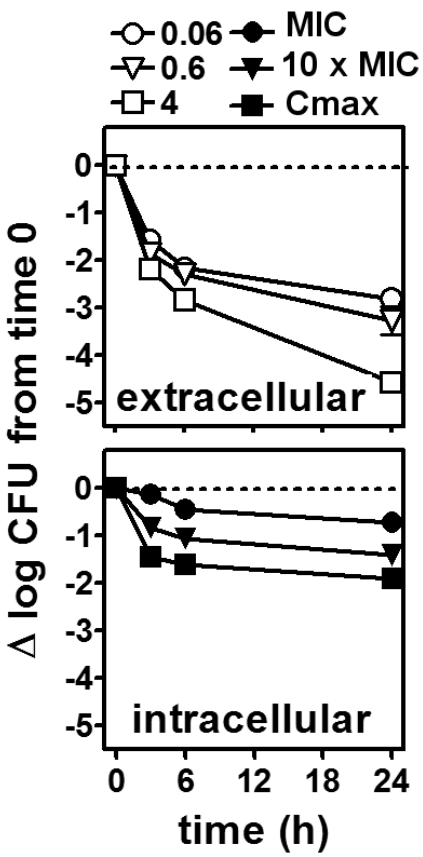
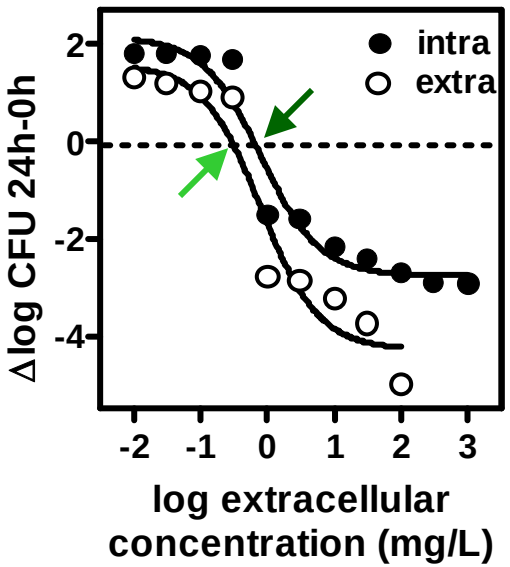




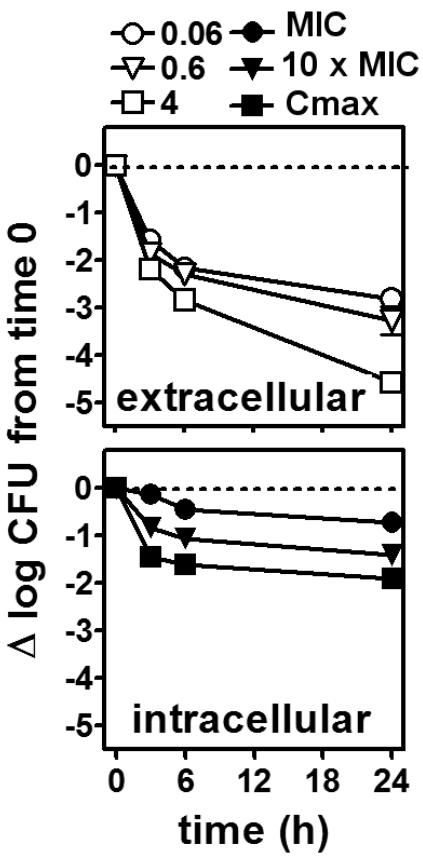
model	C_{stat} (x MIC)
extra	0.27
intra	0.63

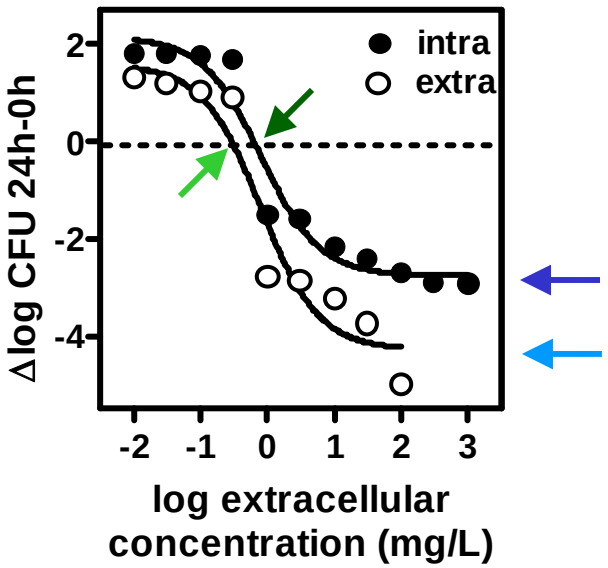
relative potency

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)

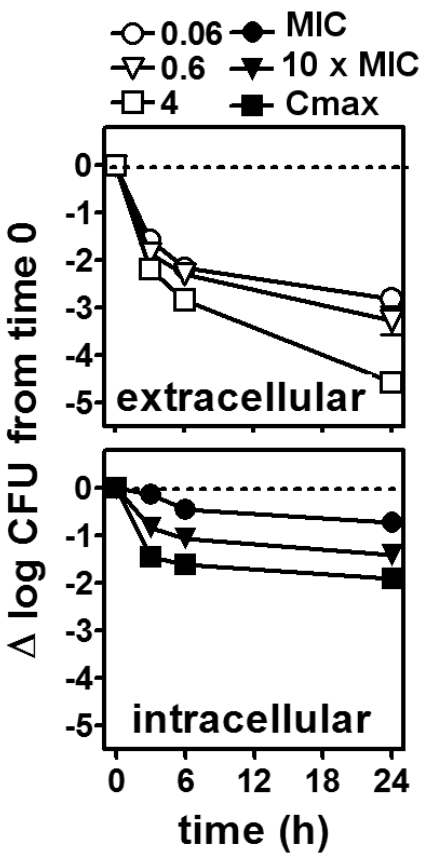
relative potency

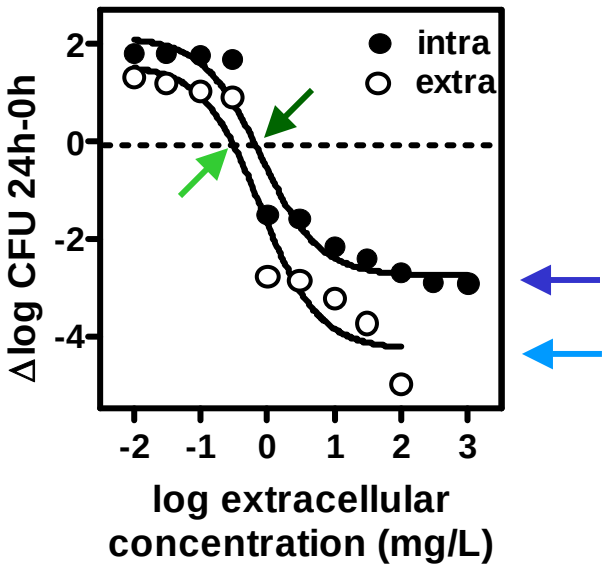
maximal efficacy

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)

Quantitative comparison
~ models
~ drugs

relative potency

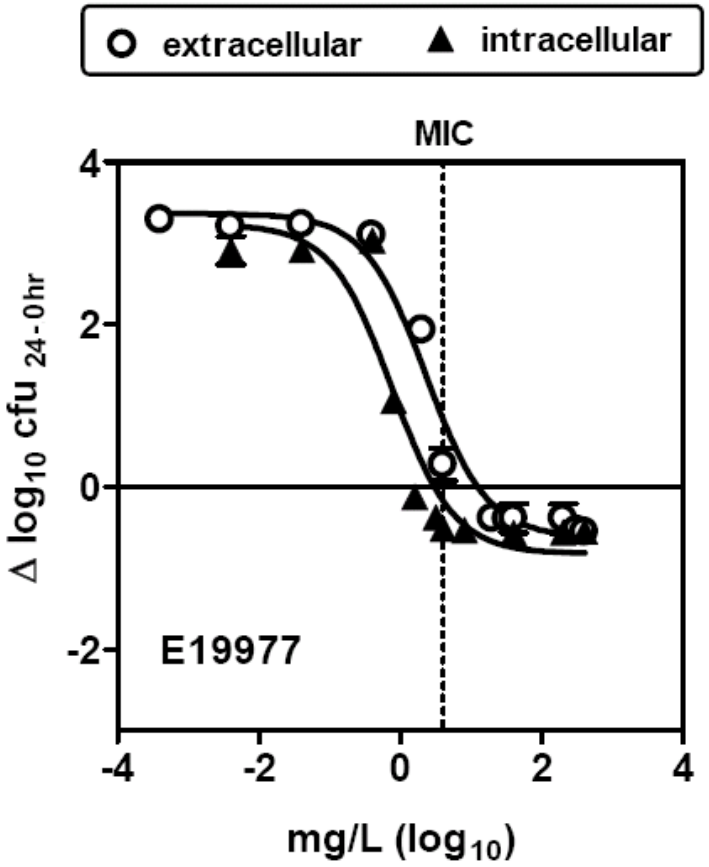
maximal efficacy

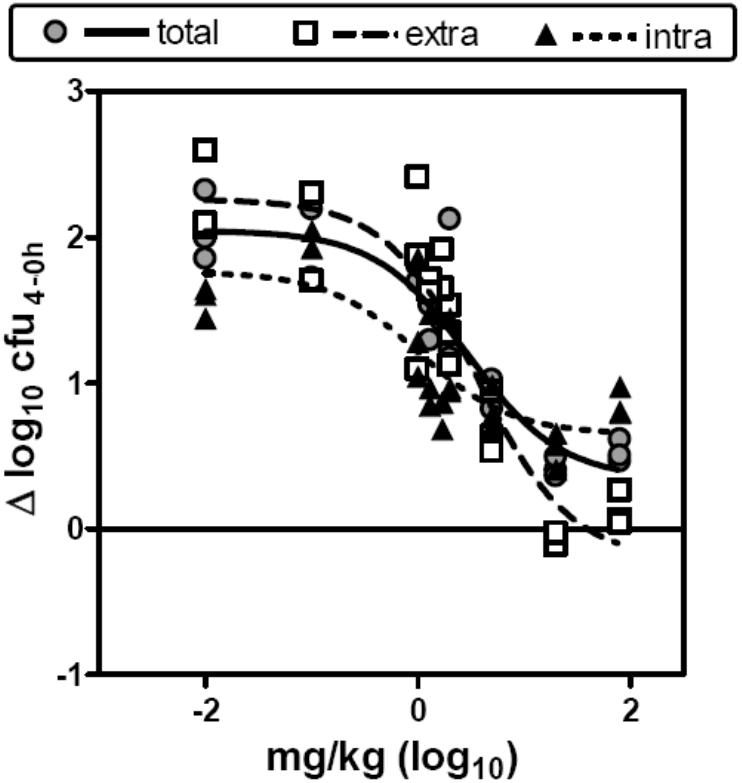
in vitro vs *in vivo*

Linezolid & *S. aureus*

in vitro
(macrophages)

in vivo
(peritonitis)





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

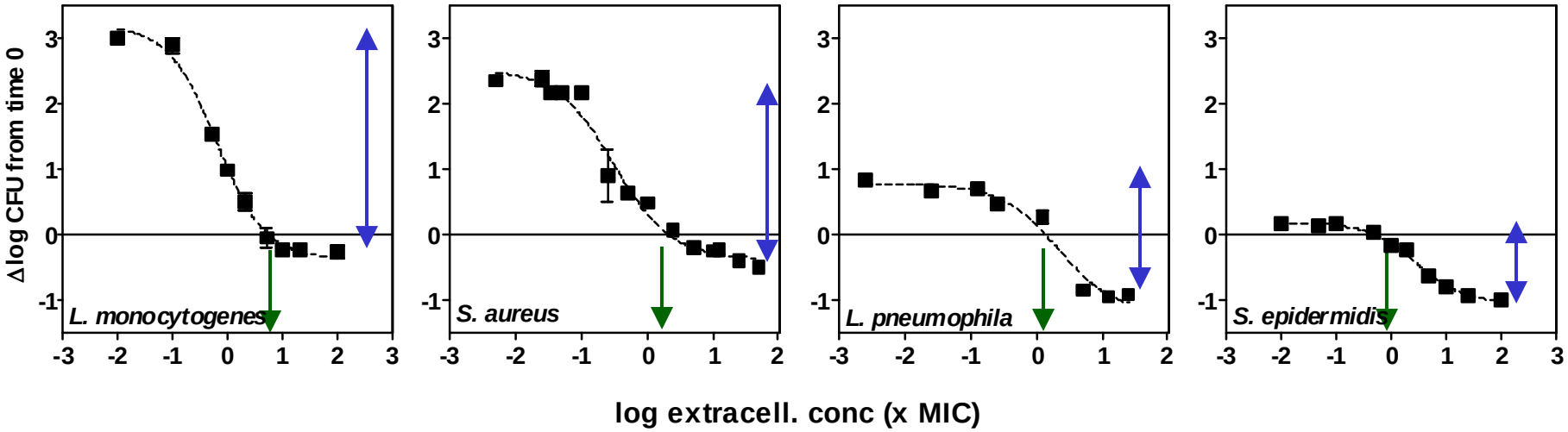
What do these models tell us ?

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

What do these models tell us ?

comparison : 1 drug ~ different models of infection

Linezolid ; THP-1 cells



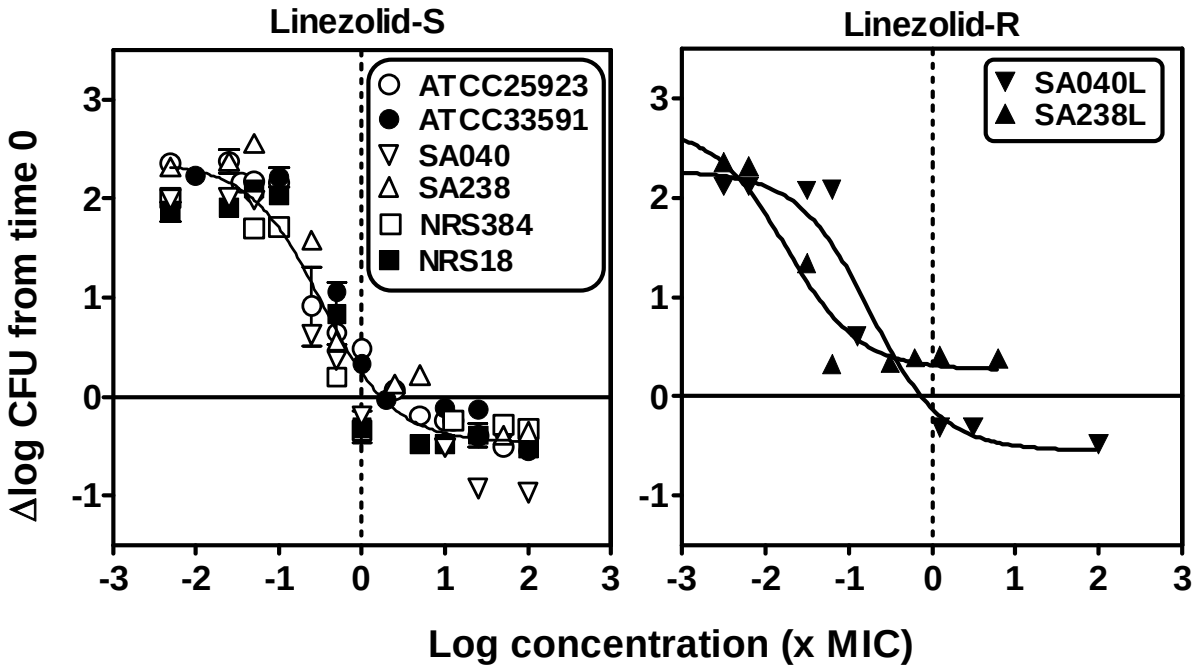
- Cs close to the MIC
- amplitude of the effect depending on intracell. growth

Lemaire et al, AAC (2010) 54:2549-59

What do these models tell us ?

comparison : 1 drug ~ different bacterial strains

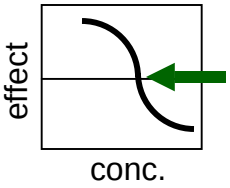
Linezolid ; THP-1 cells & *S. aureus*



- Cs close to the MIC for all susceptible strains
- Resistant strains may show modified Emax

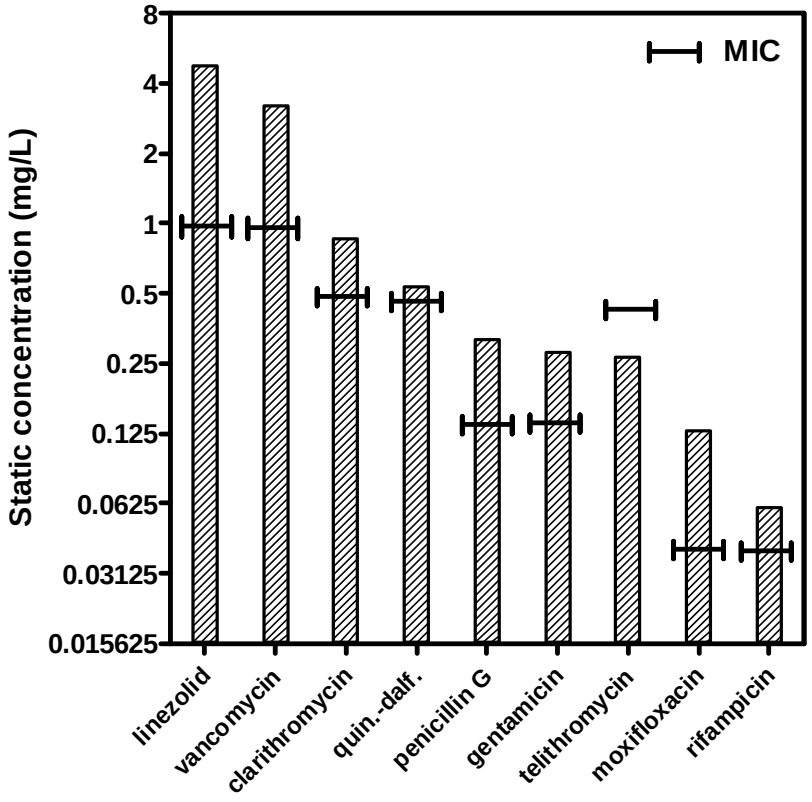
Lemaire et al, AAC (2010) 54:2549-59

What about intracellular **potency** ?



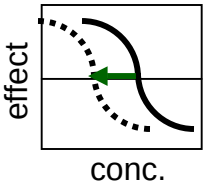
comparison : 1 model ~ different drugs

THP-1 ; *S. aureus*



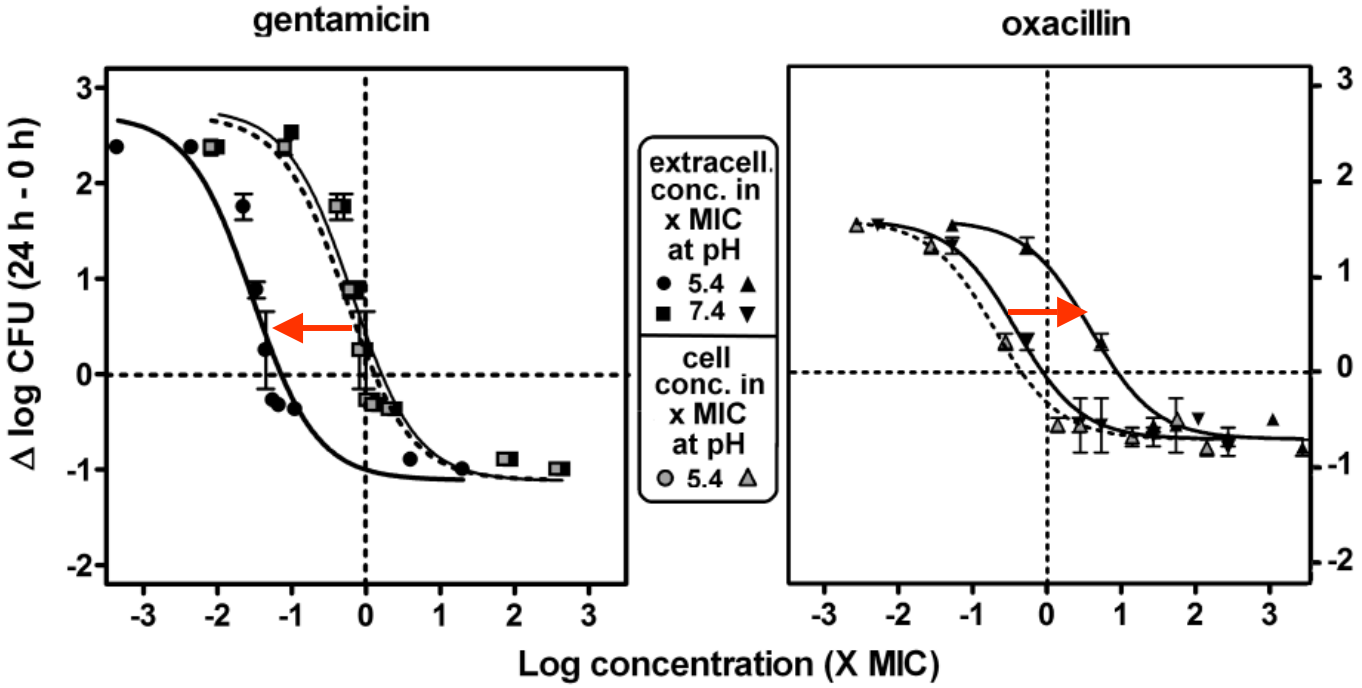
- Cs close or slighthy higher than the MIC for all drugs

How to modulate intracellular **potency** ?



➔ **change pH!**

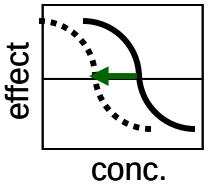
THP-1 ; phagolysosomal *S. aureus*



MIC at acidic pH

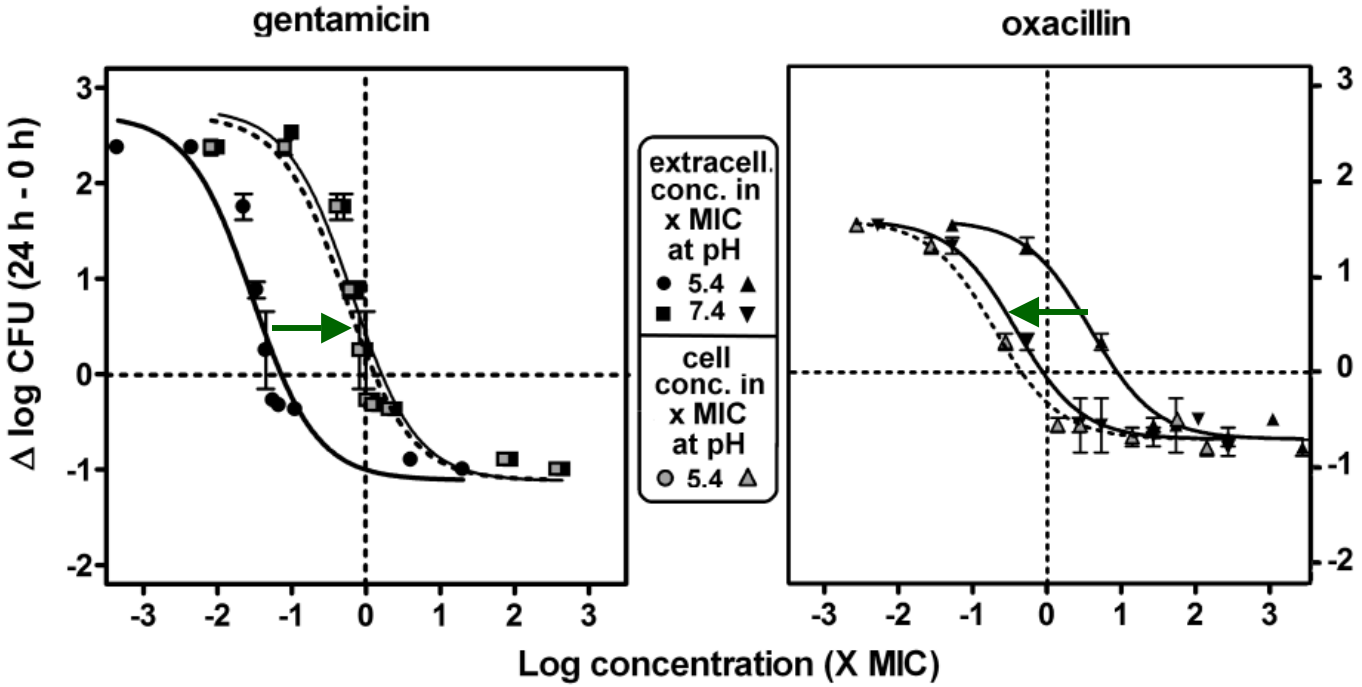
Baudoux et al, JAC (2007) 59:246-53

How to modulate intracellular **potency** ?



➔ **change pH!**

THP-1 ; phagolysosomal *S. aureus*



MIC at acidic pH x lysosomal accumulation

Baudoux et al, JAC (2007) 59:246-53

A quite nice common experience ….

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 283, NO. 19, pp. 12769–12776, May 9, 2008

Restoration of Susceptibility of Methicillin-resistant *Staphylococcus aureus* to β-Lactam Antibiotics by Acidic pH

ROLE OF PENICILLIN-BINDING PROTEIN PBP 2a[*S]

Received for publication, January 4, 2008, and in revised form, March 11, 2008 Published, JBC Papers in Press, March 12, 2008, DOI 10.1074/jbc.M800079200

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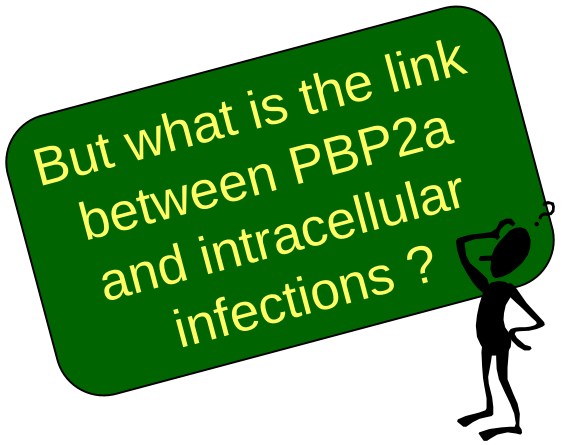
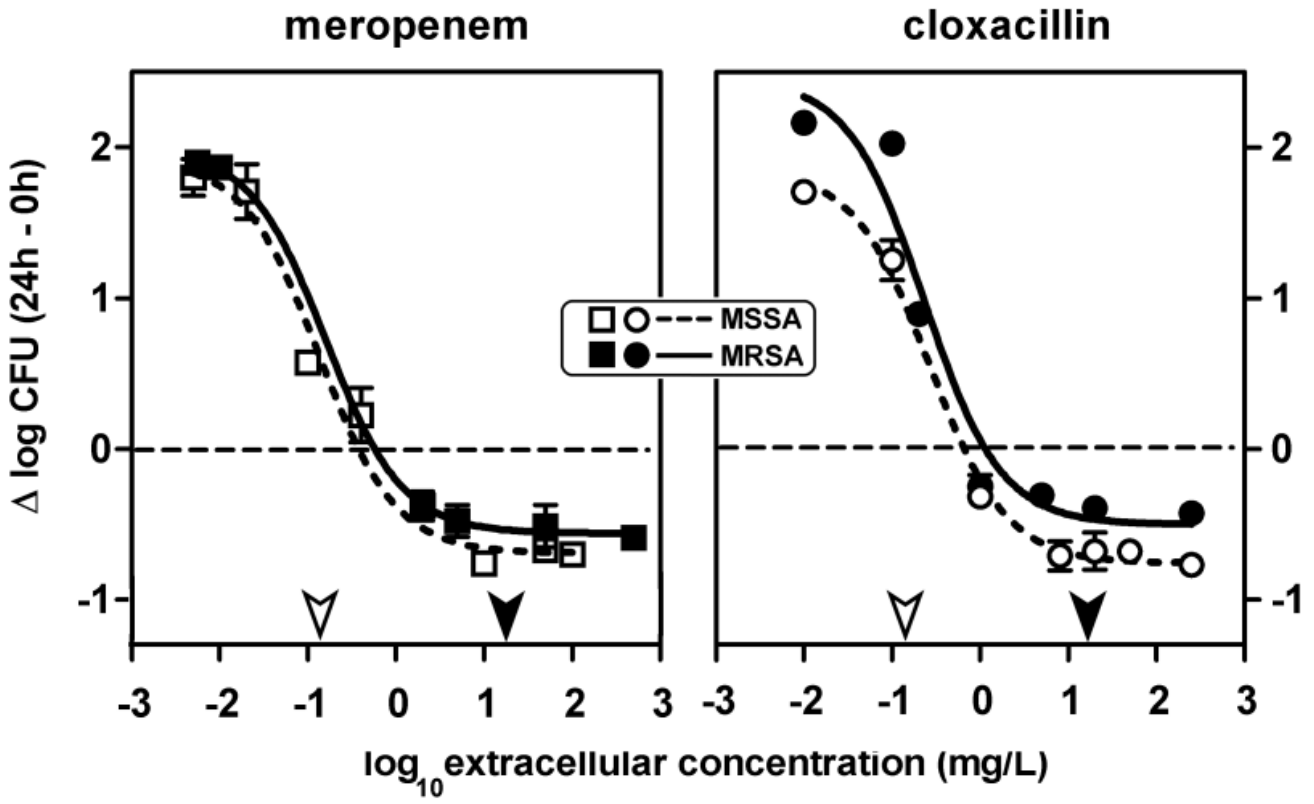


MRSA *vs.* MSSA: intracellular activity of β-lactams

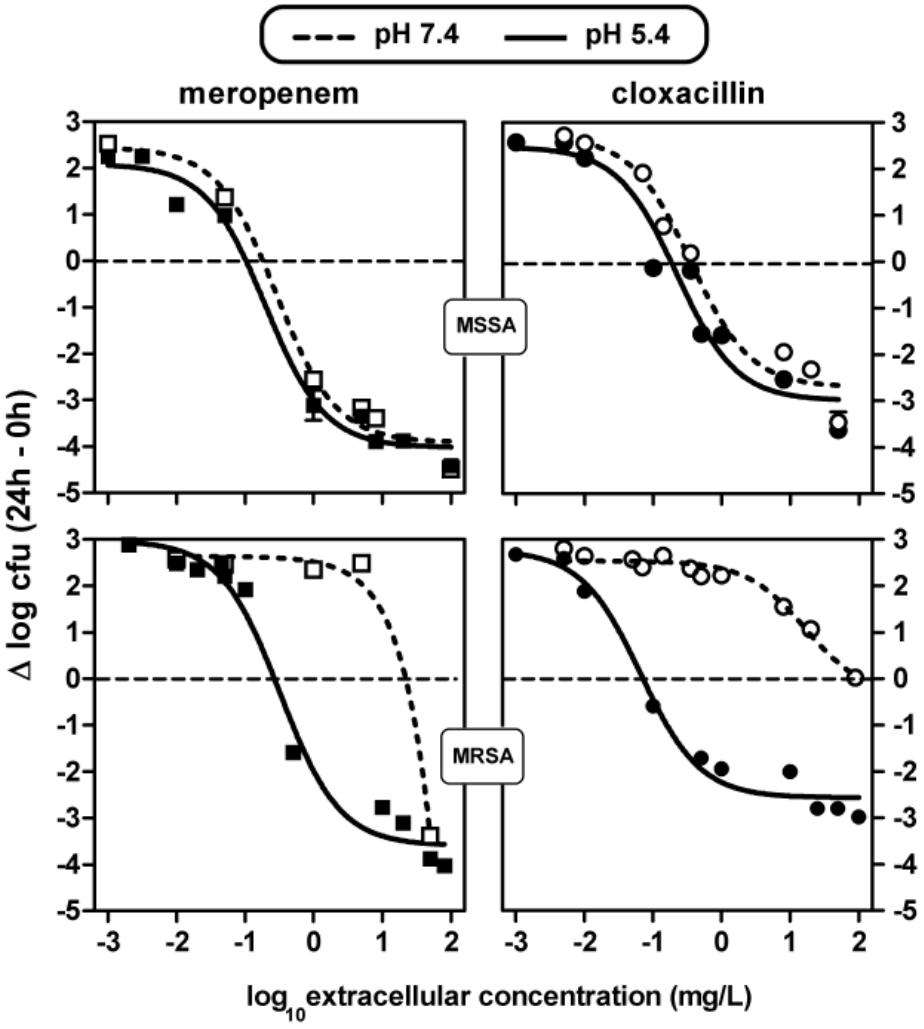
MRSA are as susceptible as MSSA to β-lactams when intracellular !



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

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

MRSA are as susceptible as MSSA in broth at acidic pH

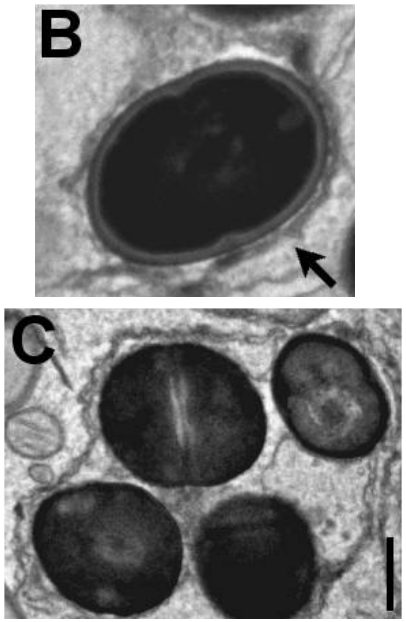


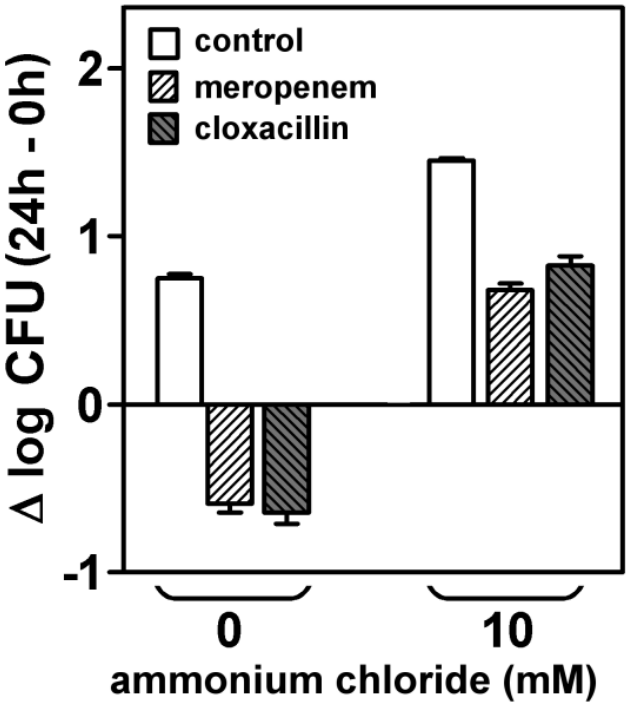
Lemaire et al., AAC (2007) 51:1627-32

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

Neutralization of lysosomes makes intracellular MRSA resistant to β-lactams !

MRSA are inside [acidic] vacuoles





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

PBP2a conformation is modified by acidic pH

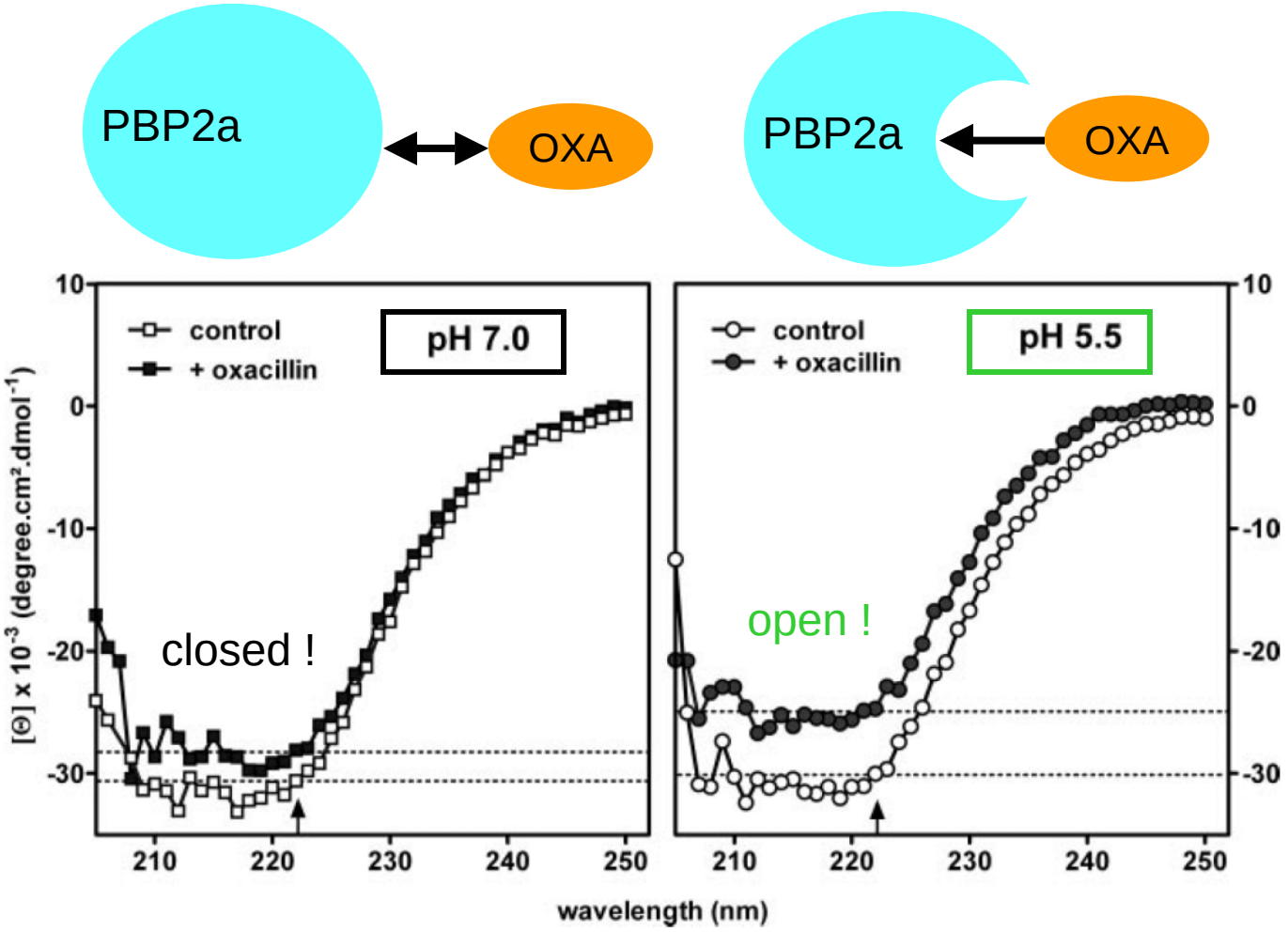
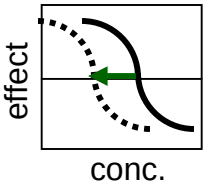


FIGURE 4. **Circular dichroic spectra of PBP 2a at pH 7. 0 (*left panel*) and pH 5.5 (*right panel*) in the absence (*open symbols*) and in the presence (*closed symbols*) of oxacillin (30 μM) for 30 min at 25 °C.** The *thin dotted lines* in each graph represent minima of PBP 2a molar ellipticity at 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

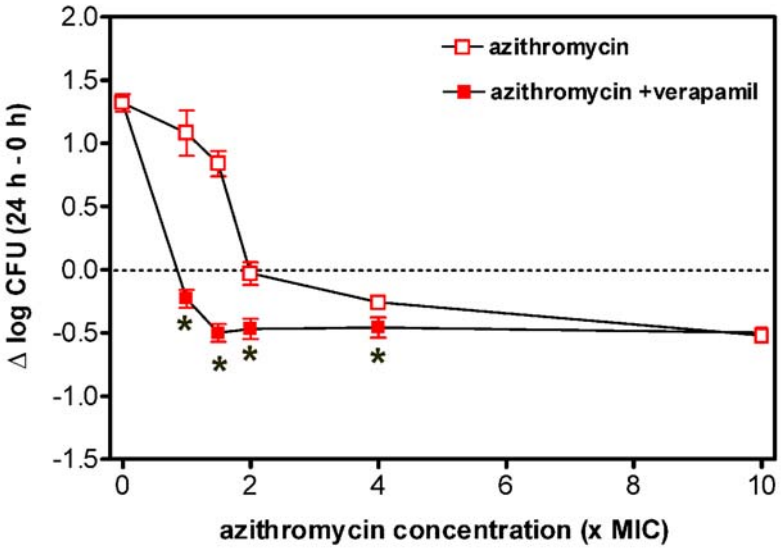
How to modulate intracellular **potency** ?

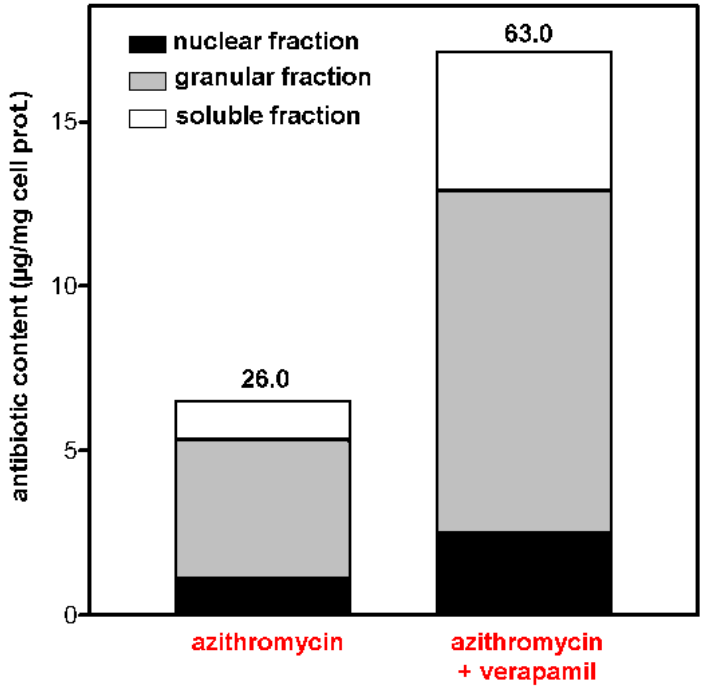


➔ **increase concentration !**

- intracellular potency
- accumulation in lysosomes

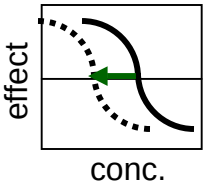
of **azithromycin** are increased by P-glycoprotein inhibitors





Seral et al., JAC (2003) 51:1167-73

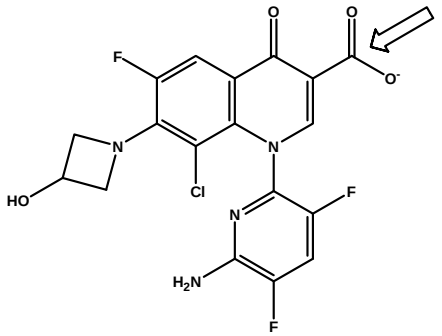
How to modulate intracellular **potency** ?

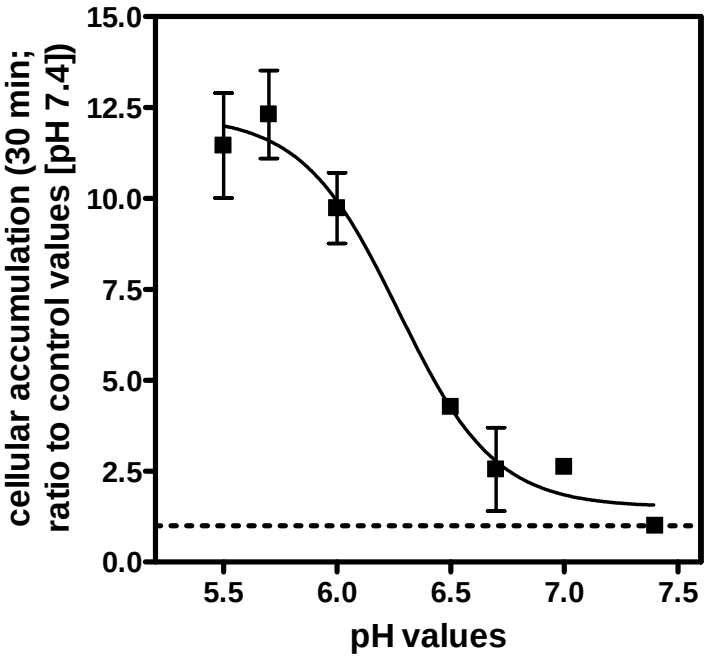


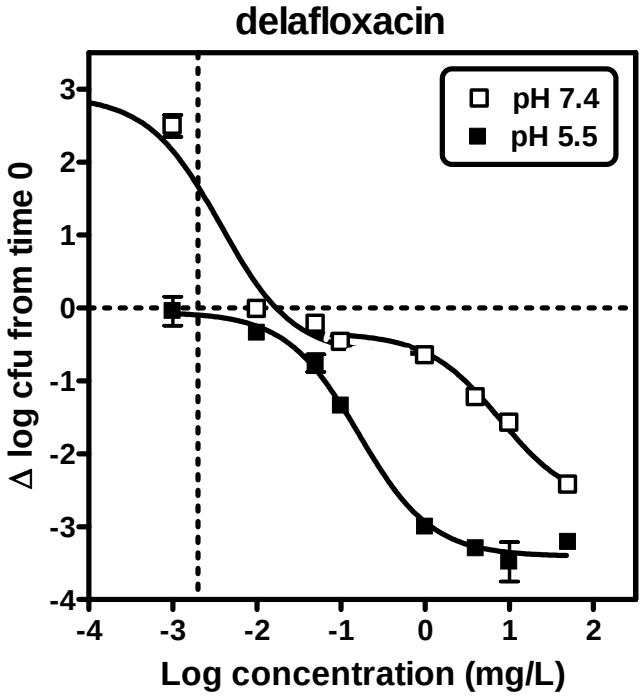
➔ **increase concentration by modulating pH !**

- cellular accumulation
- intracellular potency

of **delafloxacin** are increased in medium at acidic pH

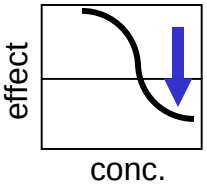






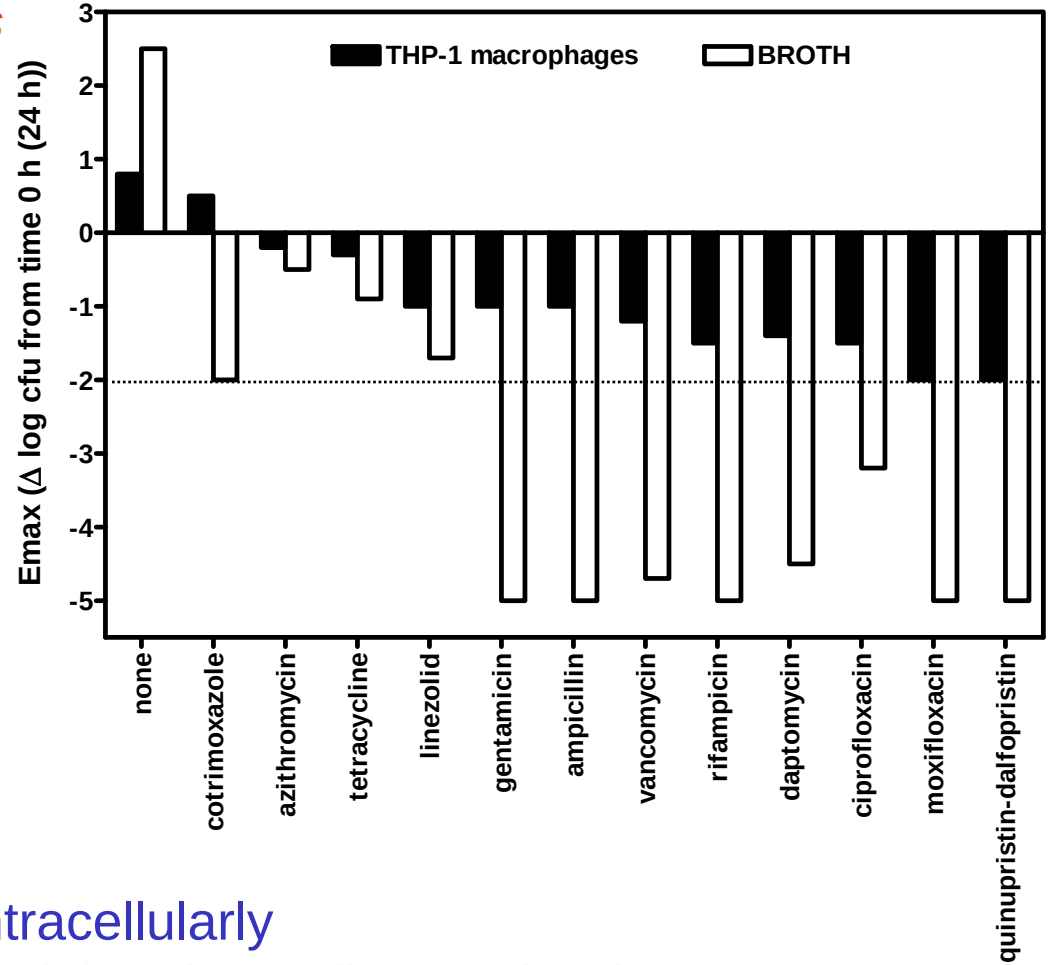
Lemaire et al., ICAAC (2010) A1-677

What about intracellular **efficacy** ?



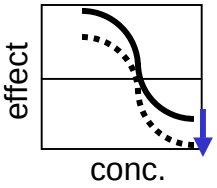
comparison : 1 model ~ different drugs

THP-1 ; *S. aureus*

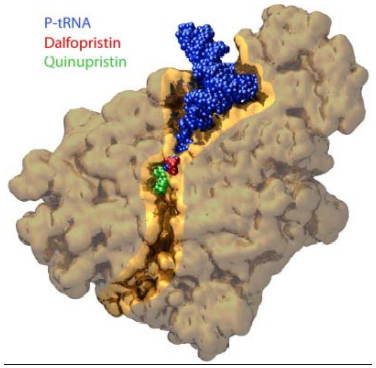


- Emax lower intracellularly
  highly variable depending on the drug

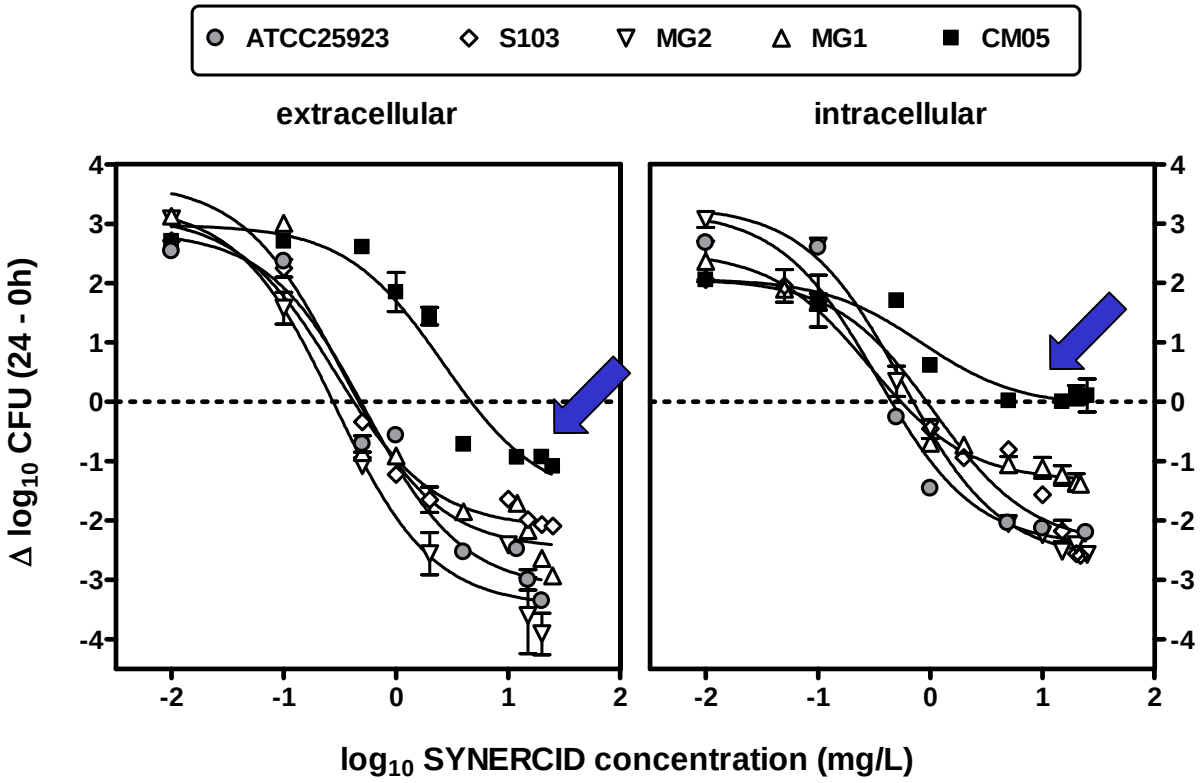
How to modulate intracellular **efficacy** ?



comparison : 1 drug ~ S vs R strains



THP-1 ; *S. aureus*

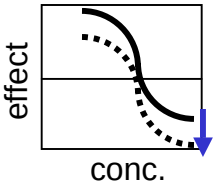


strain	geno	Res	MIC
ATCC 25923	-	-	0.45
S103	*vatB*	S_A	0.40
MG2	*msrA/B*	S_B	0.50
MG1	*ermA*	S_B	0.40
CM05	*ermA/B cfr*	S_B S_A	**5**

- Emax lower for a resistant strain

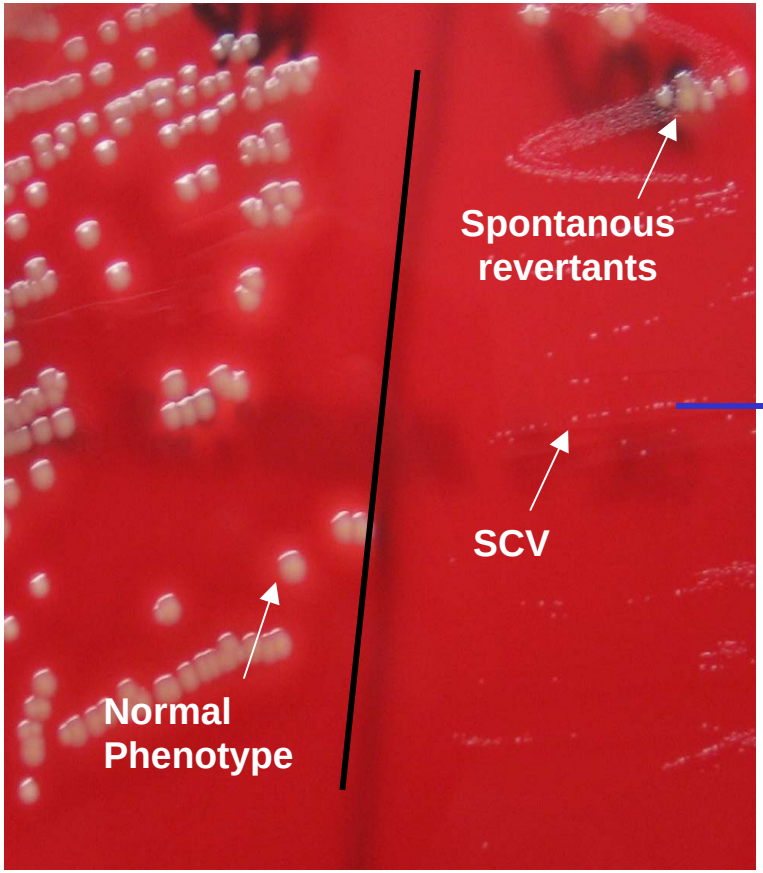
Baudoux et al., JAC (2010) 65:1228-36

How to modulate intracellular **efficacy** ?

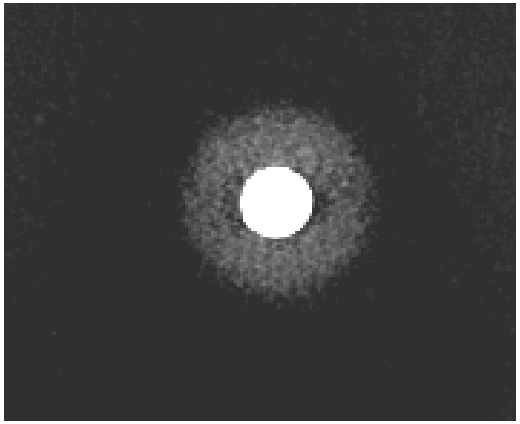


comparison : isogenic strains with different phenotypes

S. aureus : SCV vs normal phenotype

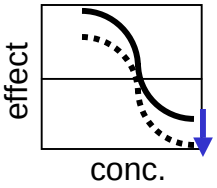


Thymidine dependent



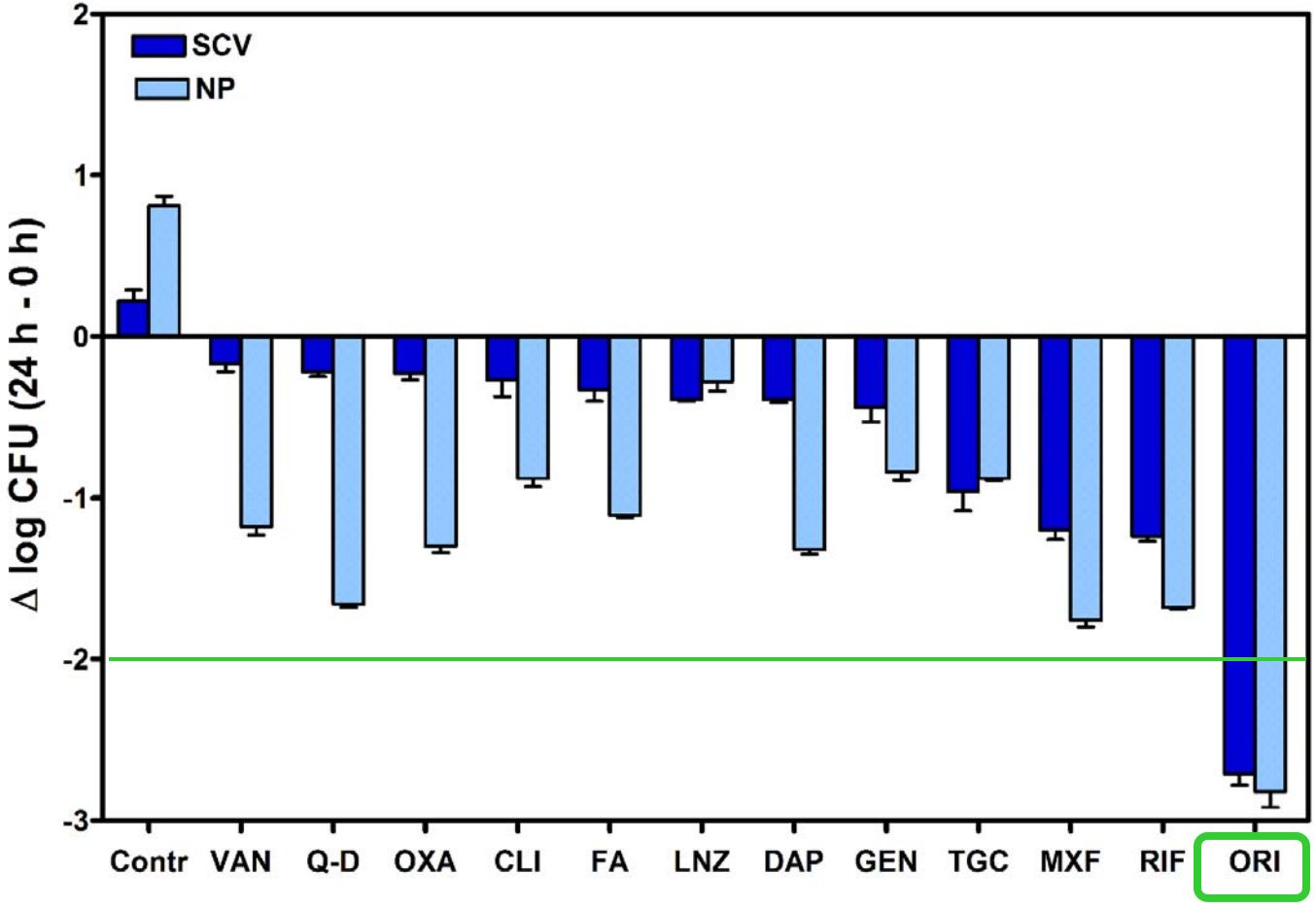
Vergison et al. JAC (2007) 59:893-9

How to modulate intracellular **efficacy** ?



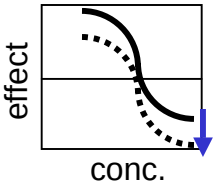
comparison : isogenic strains with different phenotypes

S. aureus – THP1



Nguyen et al, AAC (2009) 53:1434–42

How to modulate intracellular **efficacy** ?



➔ Use combinations: Fractional maximal effect (FME) approach

- Handle the nonlinear pharmacodynamics exhibited by antibiotics
- Analyse the combinations with calculated and not arbitrarily chosen concentrations

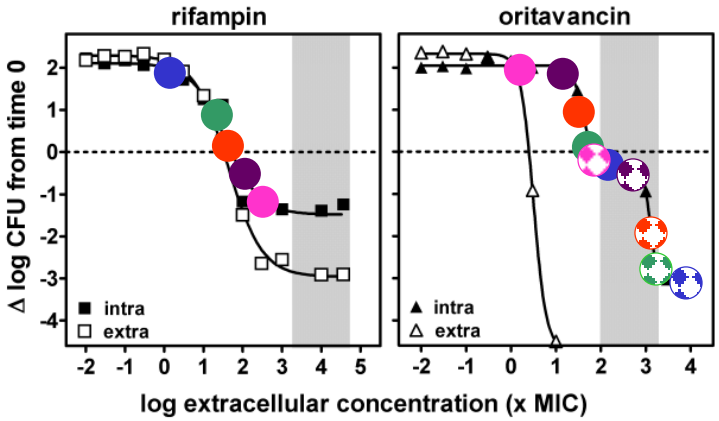
Effect (E): decrease of inoculum after 24 h. Sigmoid E_{max} model ⇒ E_{max}, EC_{50}

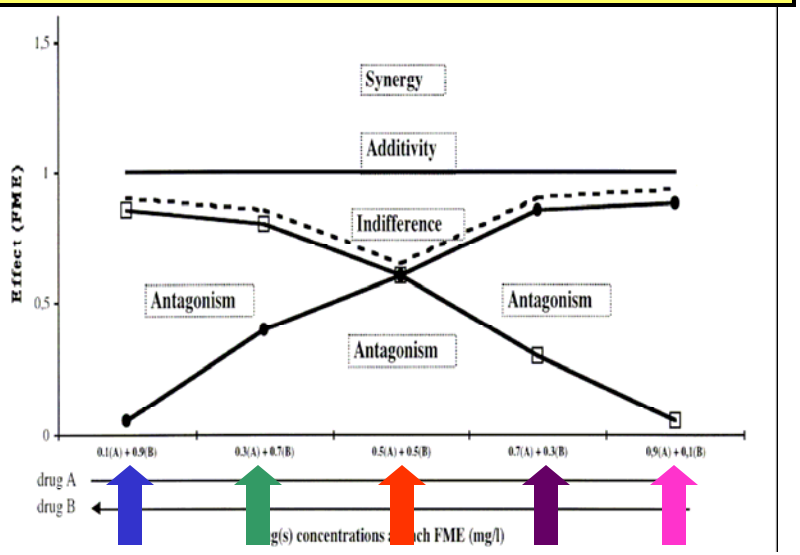
$$E = \frac{E_{max} \cdot C^n}{EC_{50}^n + C^n}$$

ATBs (A et B) are combined to a FME =1.

5 pairs: 0.1 FME_A + 0.9 FME_B, 0.3 FME_A + 0.7 FME_B, 0.5 FME_A + 0.5 FME_B, 0.7 FME_A + 0.3 FME_B, 0.9 FME_A + 0.1 FME_B

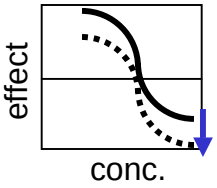
Correspoding concentration to be tested alone and in combination:



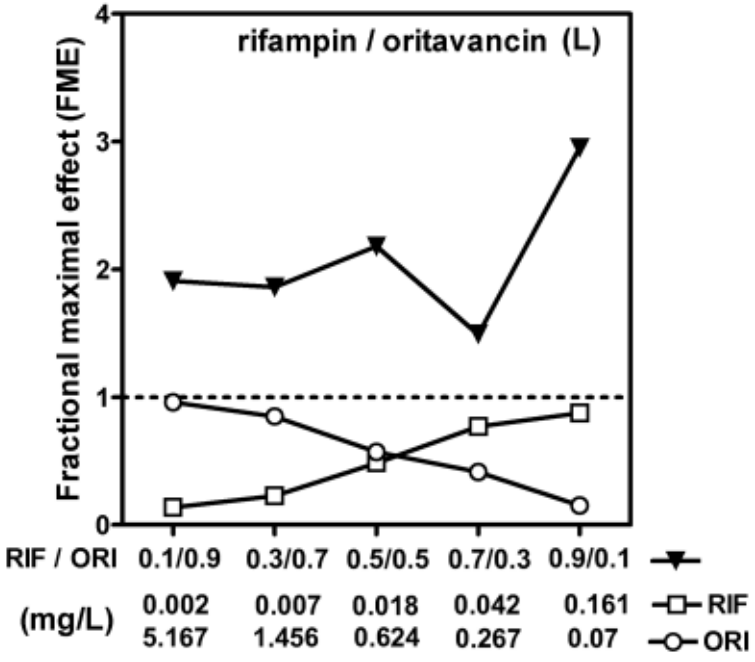


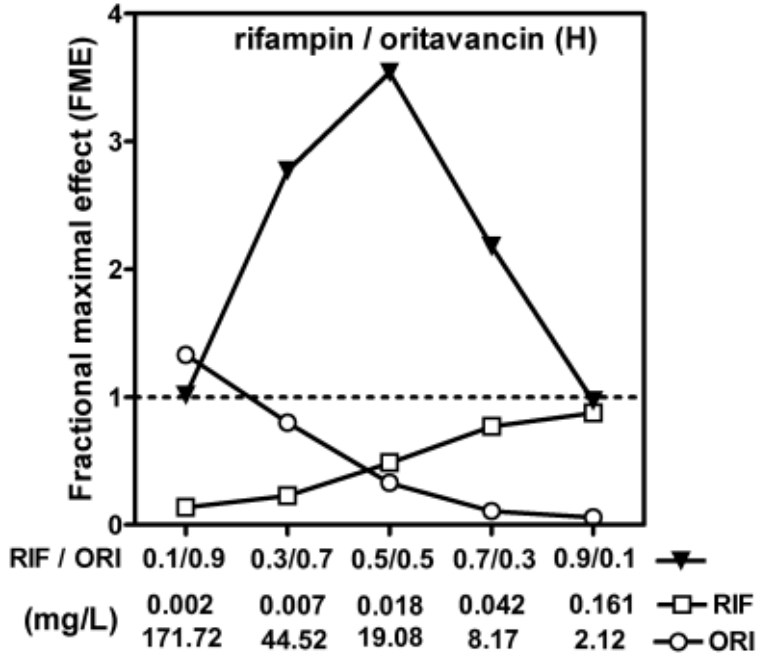
Desbiolles et al, AAC (2001) 45: 3328-33

How to modulate intracellular **efficacy** ?



Fractional maximal effect (FME) approach: RIF – ORI vs SCV





FME > 1 : synergistic; = 1: additive

RIF-ORI combination is highly synergistic over a wide range of concentration ratios

Nguyen et al, AAC (2009) 53:1443-49

Conclusion : what do these models tell us ?

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

Conclusion : what do these models tell us ?

- intracellular drug relative potency (Cs) = intracellular « MIC »

  - ✓ close or slightly higher than MIC in broth even for drugs with high accumulation
  - ✓ reflect of
    - drug concentration in the infected compartment
    - influence of environment on intrinsic activity
    - bioavailability

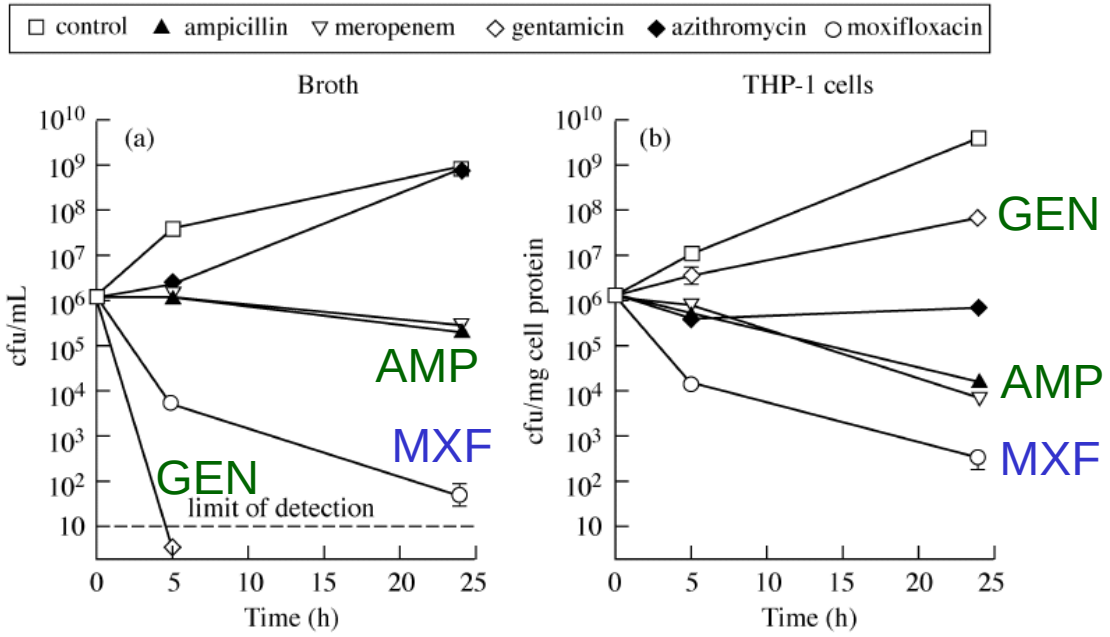
- drug efficacy

  - ✓ lower than extracellularly
  - ✓ highly variable depending on
    - the drug
    - the bacteria
  - ✓ reflect of change in
    - bacterial responsiveness ?
    - metabolism ?
    - persisters ? SCV ?
    - bacterial growth rate ?

Perspectives : what to do with these data ?

Use of these models to suggest new therapeutic options

Listeria in vitro



Listeria in vivo

Bacterial counts in CSF of rabbits during the study period

Group	16 h after induction of meningitis[a]	End of treatment[a]
C	5.340 ± 0.717 ($n = 12$)[b]	6.334 ± 0.634 ($n = 10$)
M	5.375 ± 0.356 ($n = 11$)	3.830 ± 0.518 ($n = 9$)
A2	4.428 ± 0.810 ($n = 5$)	3.520 ± 0.840 ($n = 5$)

[a] Log_{10} cfu/mL.
[b] Number of rabbits.

MXF
GEN+AMP

Carryn et al, JAC (2003) 51:1051-52; Sipahi et al. JAC (2008) 61:670-3

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

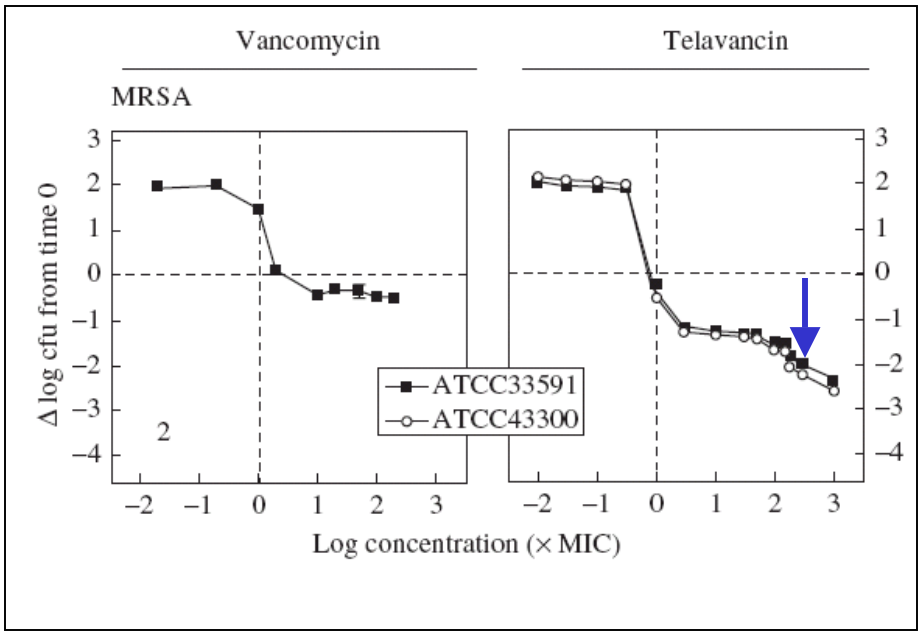
C. Intracellular Antimicrobial Concentration Assessment

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.

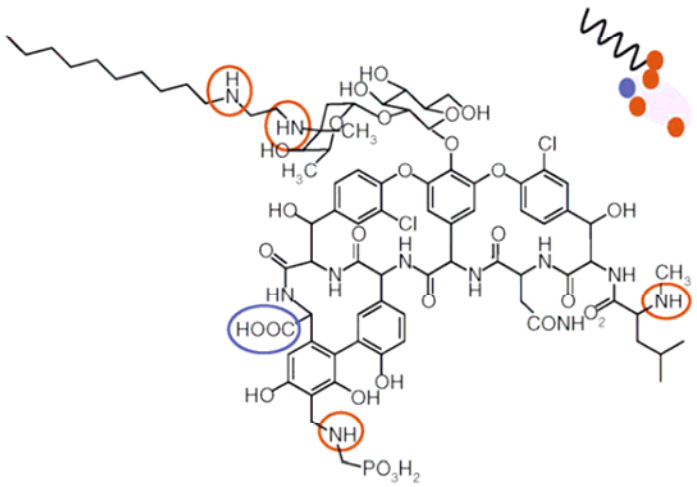
Use of these models to position new molecules

VIBATIV™ (telavancin) for injection

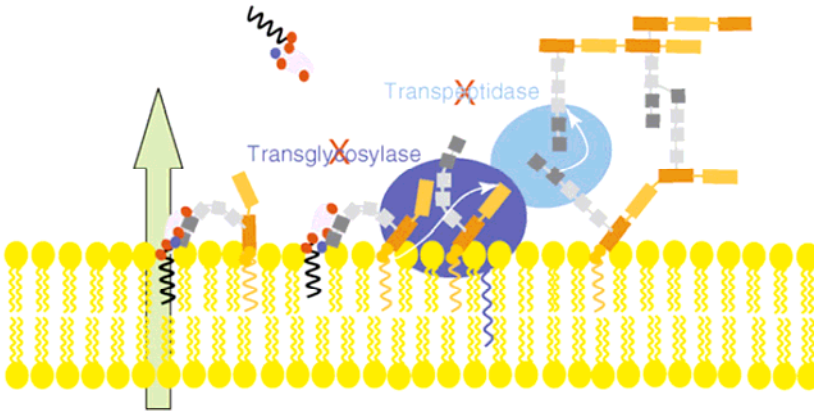
New on
the US market !



(e) Telavancin (lipoglycopeptide)

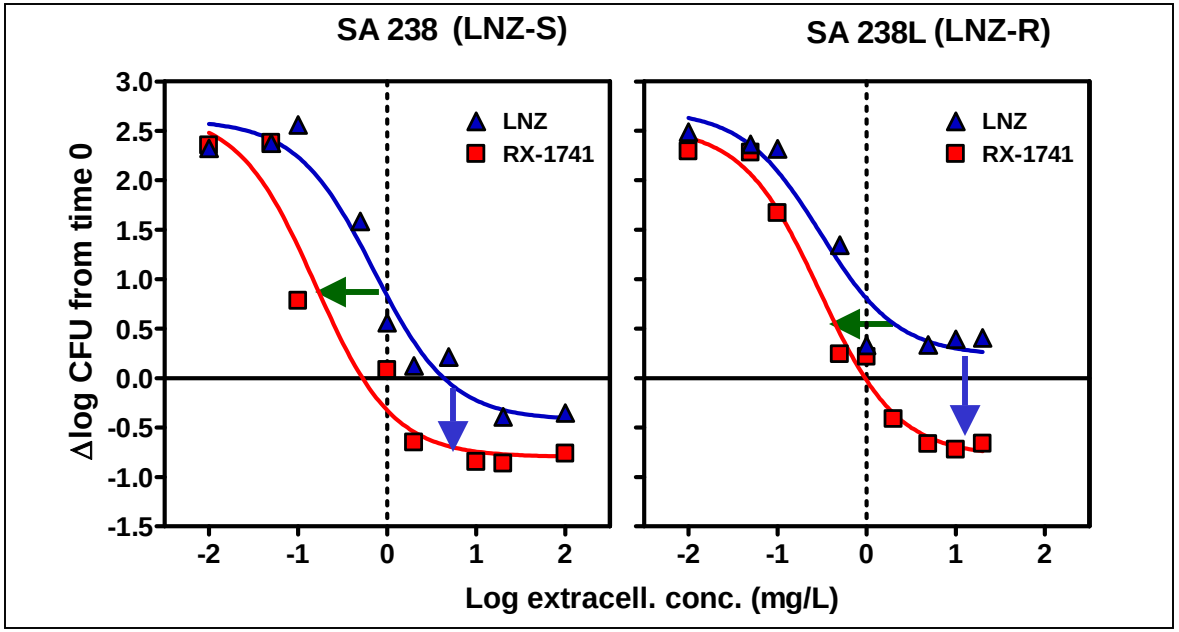


(e) Telavancin (lipoglycopeptide)

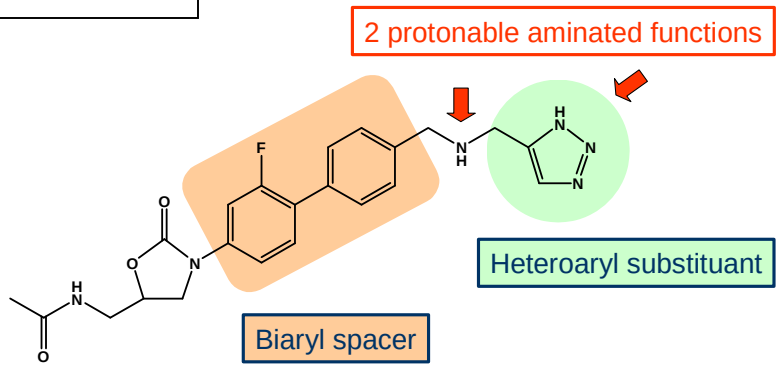


Barcia-Macay et al, JAC (2006) 58:1177–84; Van Bambeke et al., TIPS (2008) 29:124-34

Use of these models to position new molecules



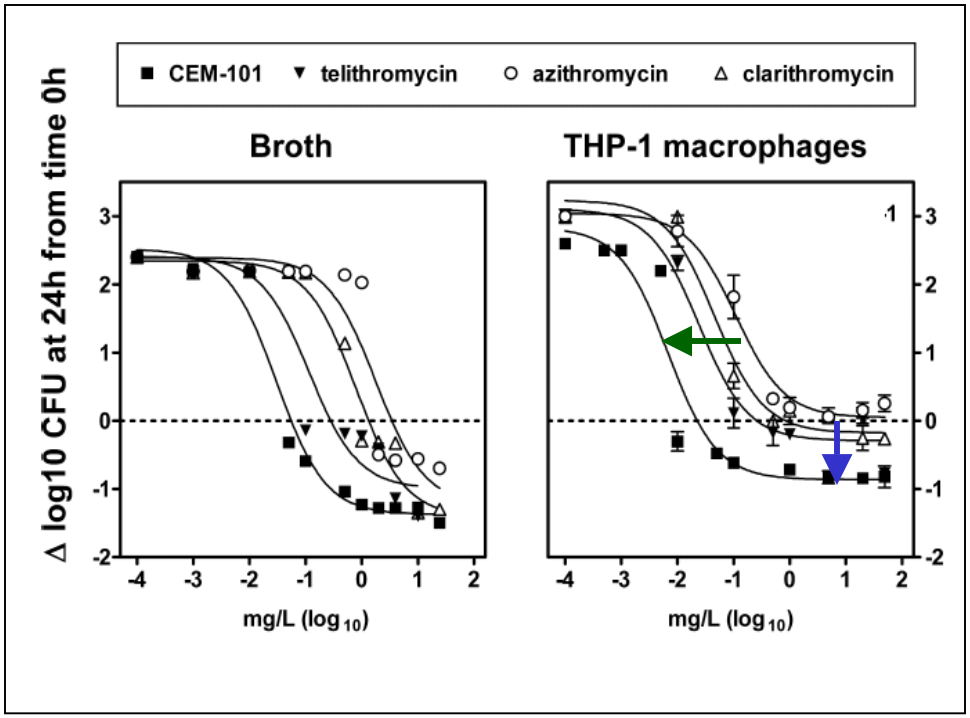
Rib-X PHARMACEUTICALS completed Phase II for radezolid



Improved binding to the target
Increased cellular accumulation

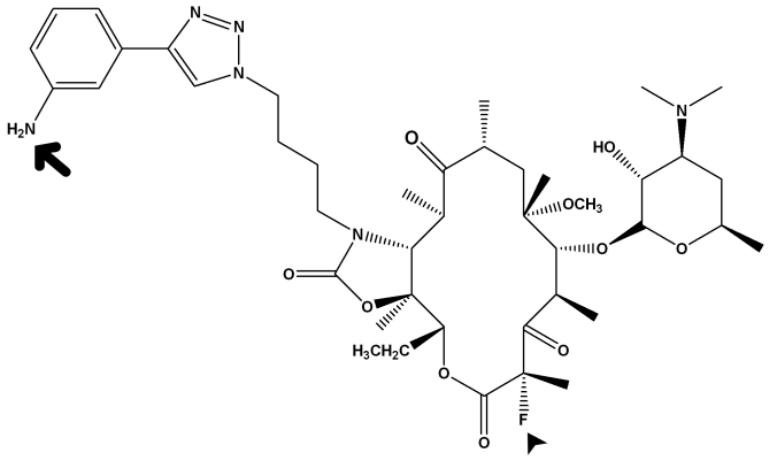
Lemaire et al, AAC (2010) 54:2549-2559

Use of these models to position new molecules



cempra PHARMACEUTICALS entering Phase II

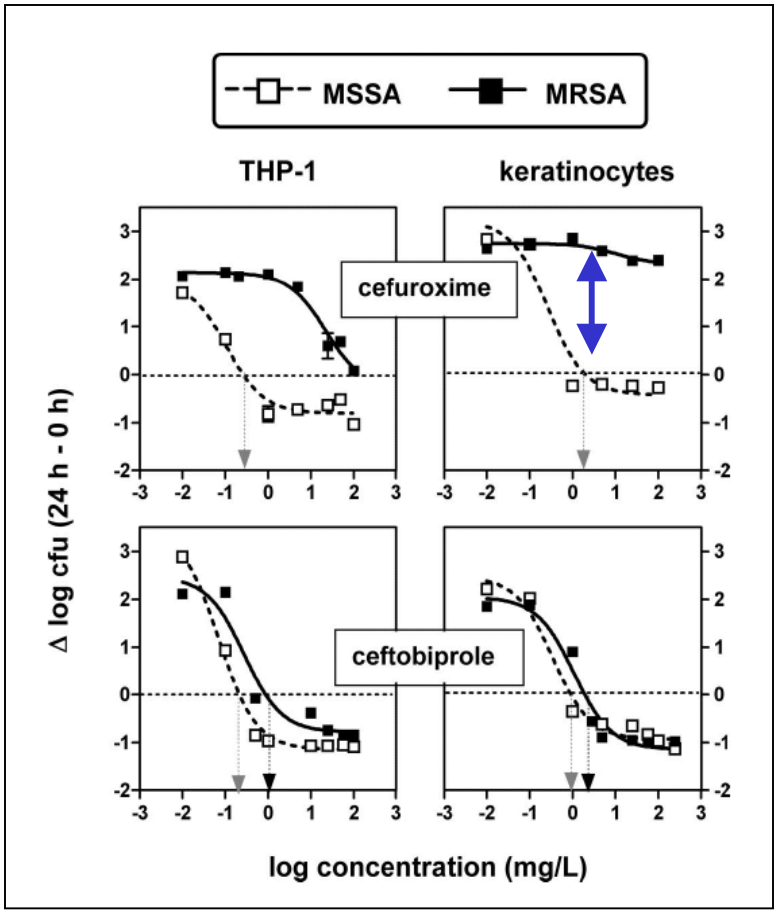
Solithromycin - fluoroketolide

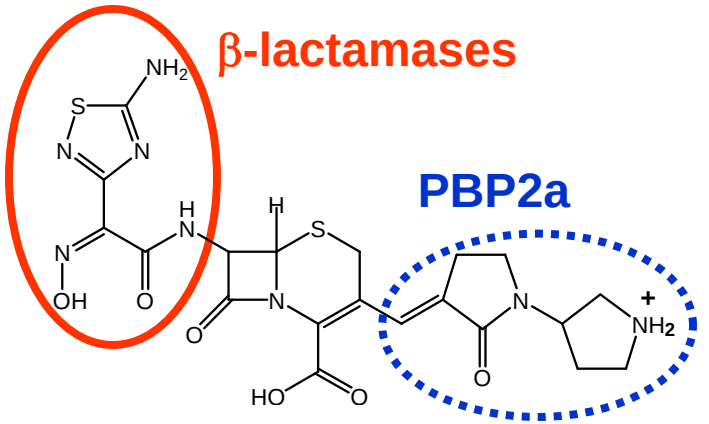


Improved binding to the target
High cellular accumulation

Lemaire et al, AAC (2009) 53:53:3734-43

Use of these models to position new molecules





also binds to PBP2a

basilea PHARMACEUTICA

But failed at FDA …

Yet, ceftaroline received a positive advice on Sept. 7!

CEREXA

Lemaire et al, AAC (2009) 53:2289-97

Which drug is going to win the battle against intracellular bacteria ?

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

L. monocytogenes


Y. Ouadhriri


S. Carryn


S. Vandevelde


A. Lismond

P. aeruginosa


J. Buyck


G. De Laminne