Intracellular Killing: Where's the Bug? / Where's the Drug?

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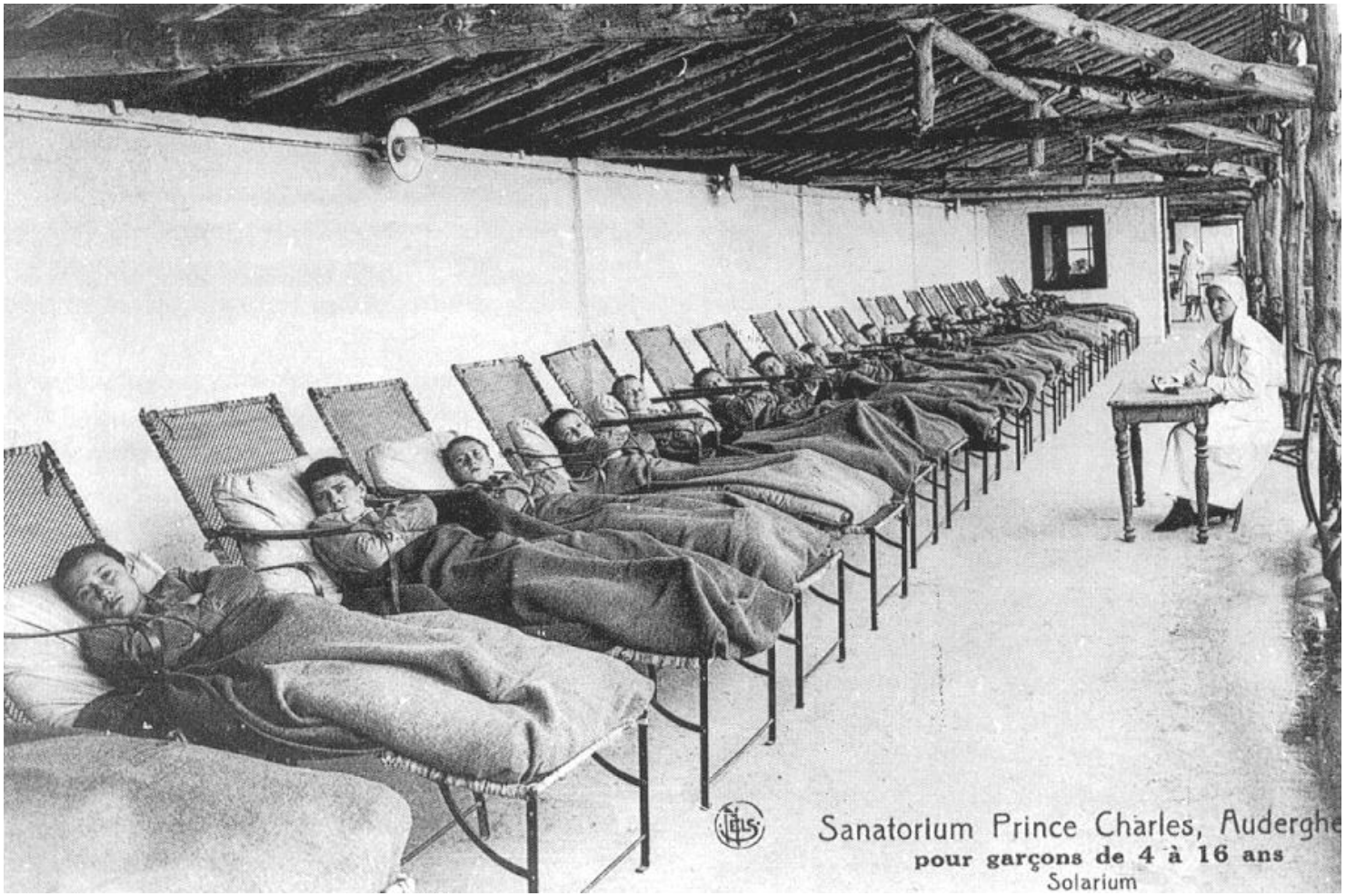
Intracellular infections: examples over the years

1930

Mycobacterium tuberculosis epidemies



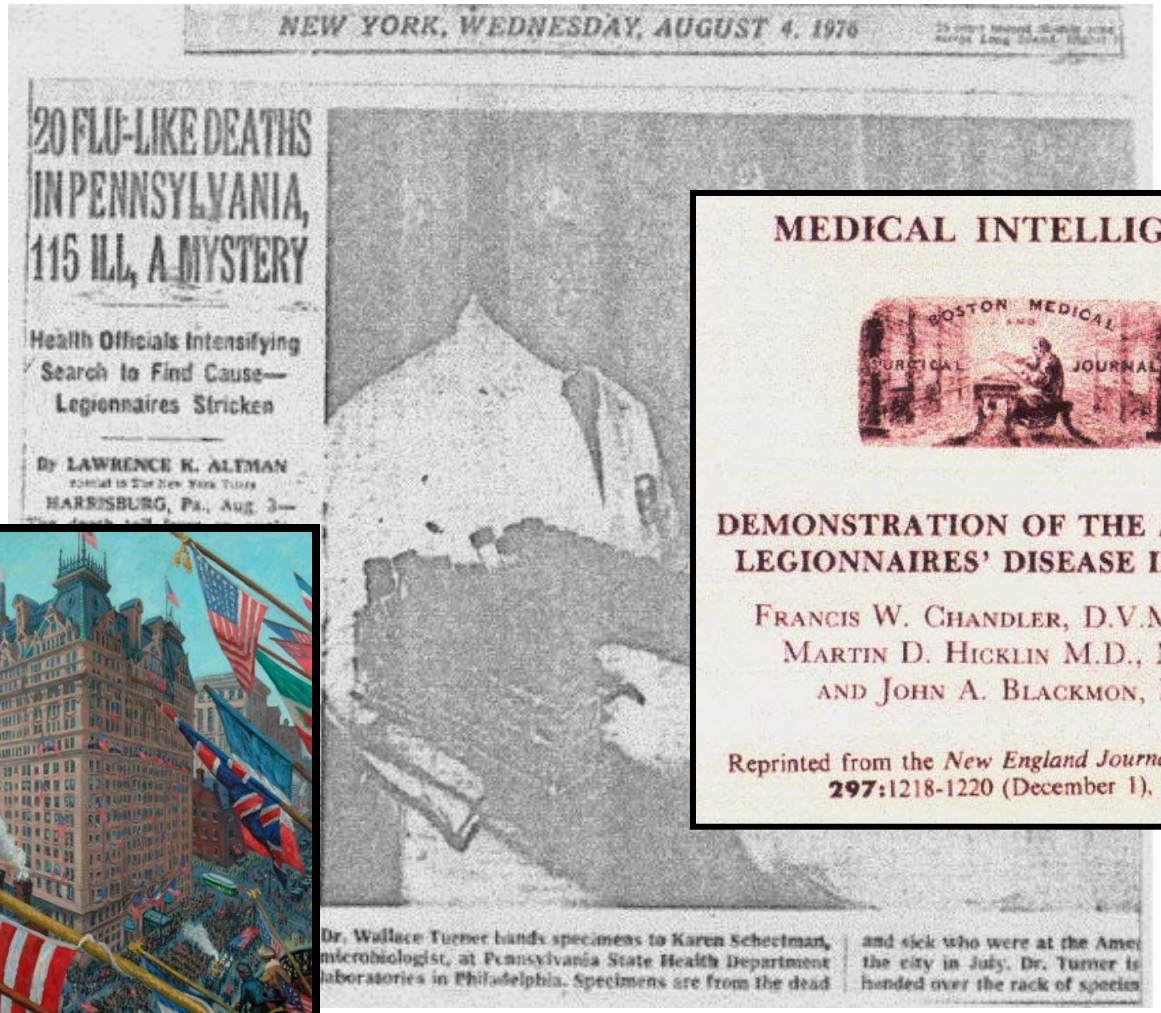
→ intracellular infections can spread widely

Intracellular infections: examples over the years

1976

Legionella pneumophila attacking legionnaires

→ intracellular infections can affect fragile hosts


NEW YORK, WEDNESDAY, AUGUST 4, 1976

20 FLU-LIKE DEATHS IN PENNSYLVANIA, 115 ILL, A MYSTERY

Health Officials Intensifying Search to Find Cause— Legionnaires Stricken

By LAWRENCE K. ALTMAN

HARRISBURG, Pa., Aug. 3—

Dr. Wallace Turner hands specimens to Karen Scheetman, microbiologist, at Pennsylvania State Health Department laboratories in Philadelphia. Specimens are from the dead and sick who were at the Amer the city in July. Dr. Turner is handed over the rack of specim

MEDICAL INTELLIGENCE

BOSTON MEDICAL AND SURGICAL JOURNAL

DEMONSTRATION OF THE AGENT OF LEGIONNAIRES' DISEASE IN TISSUE

FRANCIS W. CHANDLER, D.V.M., PH.D., MARTIN D. HICKLIN M.D., M.P.H., AND JOHN A. BLACKMON, M.D.

Reprinted from the *New England Journal of Medicine* **297**:1218-1220 (December 1), 1977

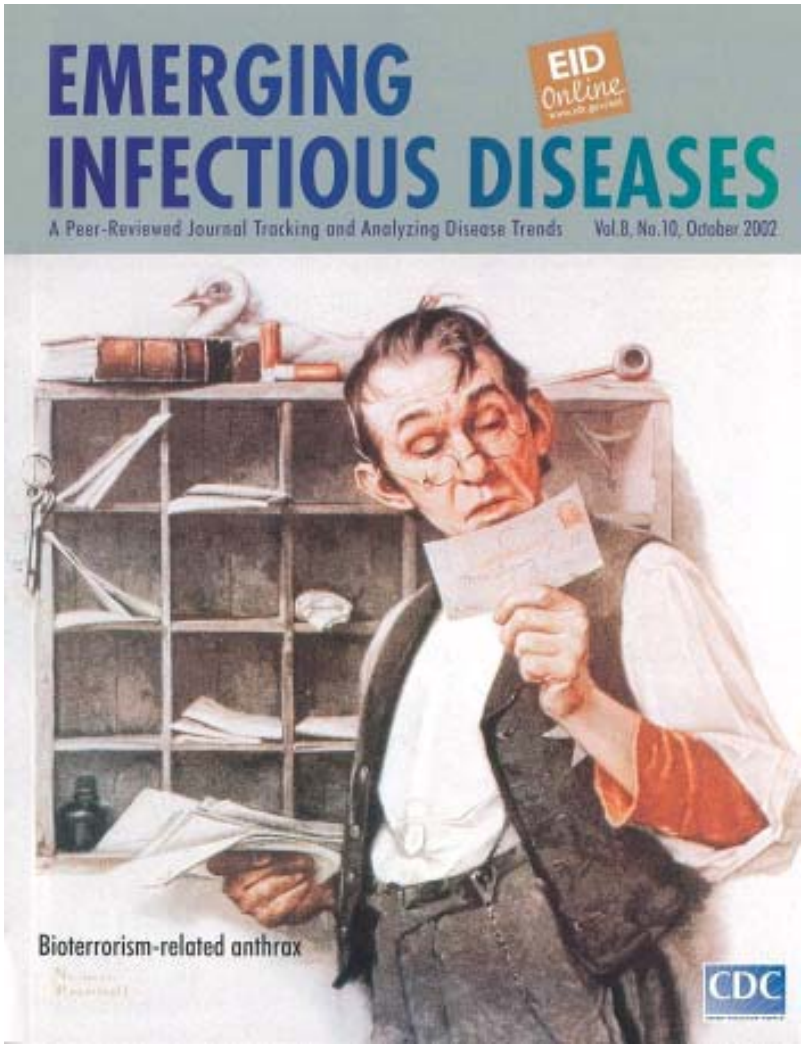
Gov. Shapp To Stay at 'Legion' Hotel

PHILADELPHIA (UPI) — Pennsylvania Gov. Milton J. Shapp planned to eat and sleep Monday at the Bellevue Stratford Hotel — investigated as a possible source of the "Legionnaires' Disease" — to show it poses no health hazard.

Intracellular infections: examples over the years

2002

Bacillus anthracis : biological weapon ?



→ intracellular infections can be highly severe

Intracellular infections watch for us in our daily life …

Listeria monocytogenes : contamination by food

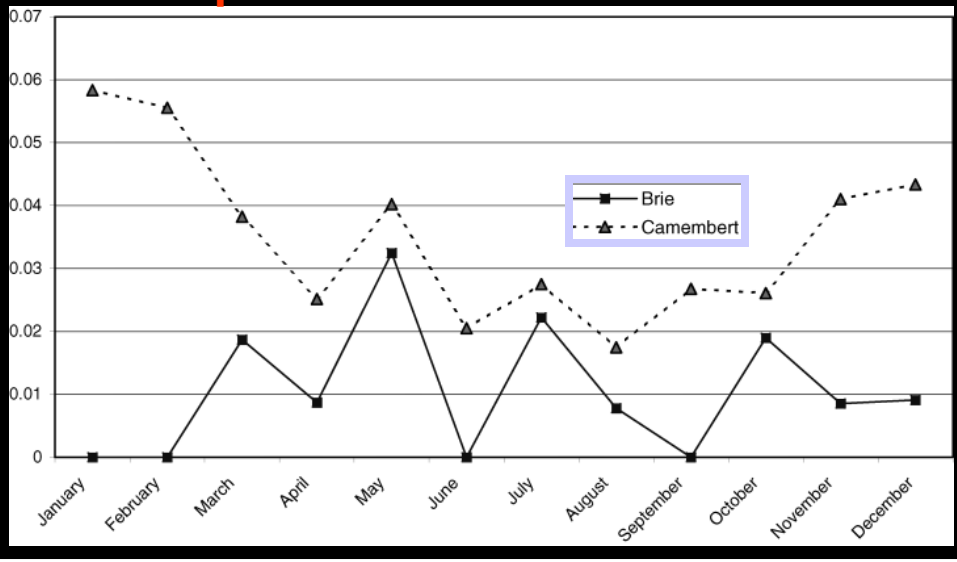
→ frequent contamination but rare infections

Risk Analysis, Vol. 24, No. 2, 2004

Risk Assessment of Listeriosis Linked to the Consumption of Two Soft Cheeses Made from Raw Milk: Camembert of Normandy and Brie of Meaux

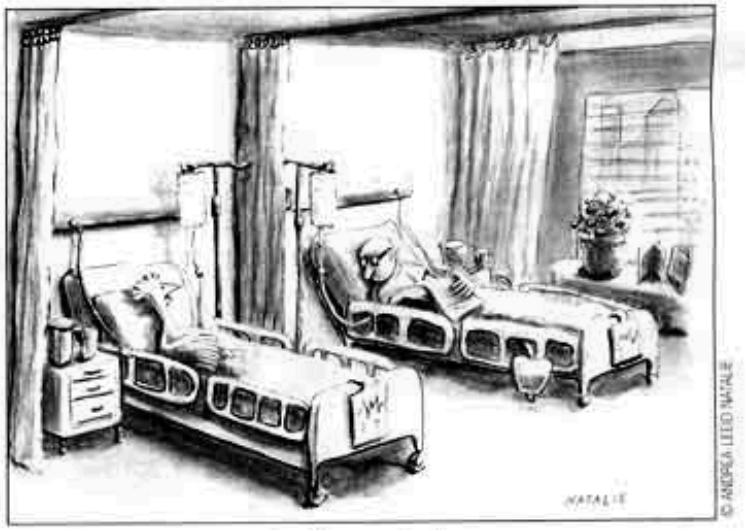
Moez Sanaa,[1]* Louis Coroller,[1] and Olivier Cerf[1]

proportion of tank milks found positive for L.m. in 2000



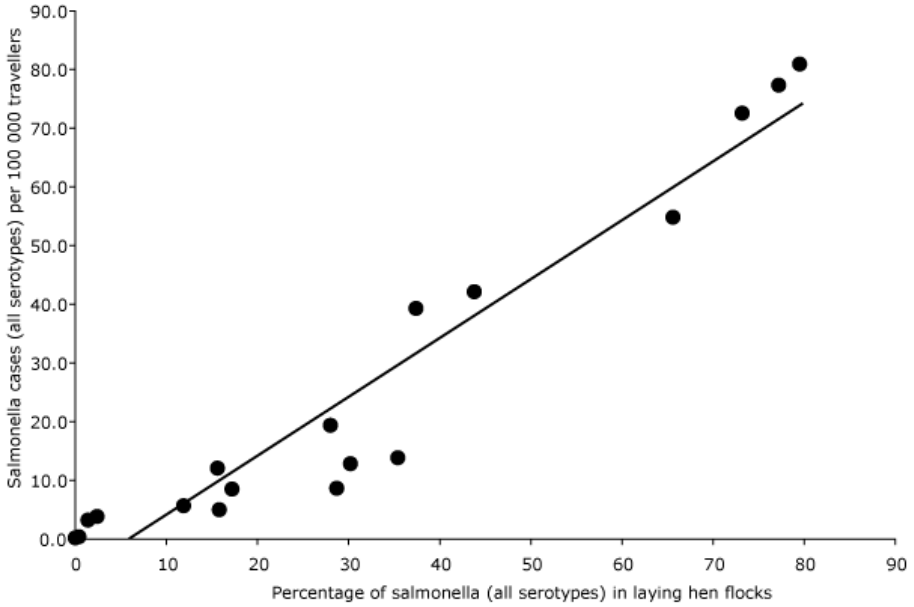
Intracellular infections watch for us in our daily life …

Salmonellosis:
one of the most prevalent food-borne pathology



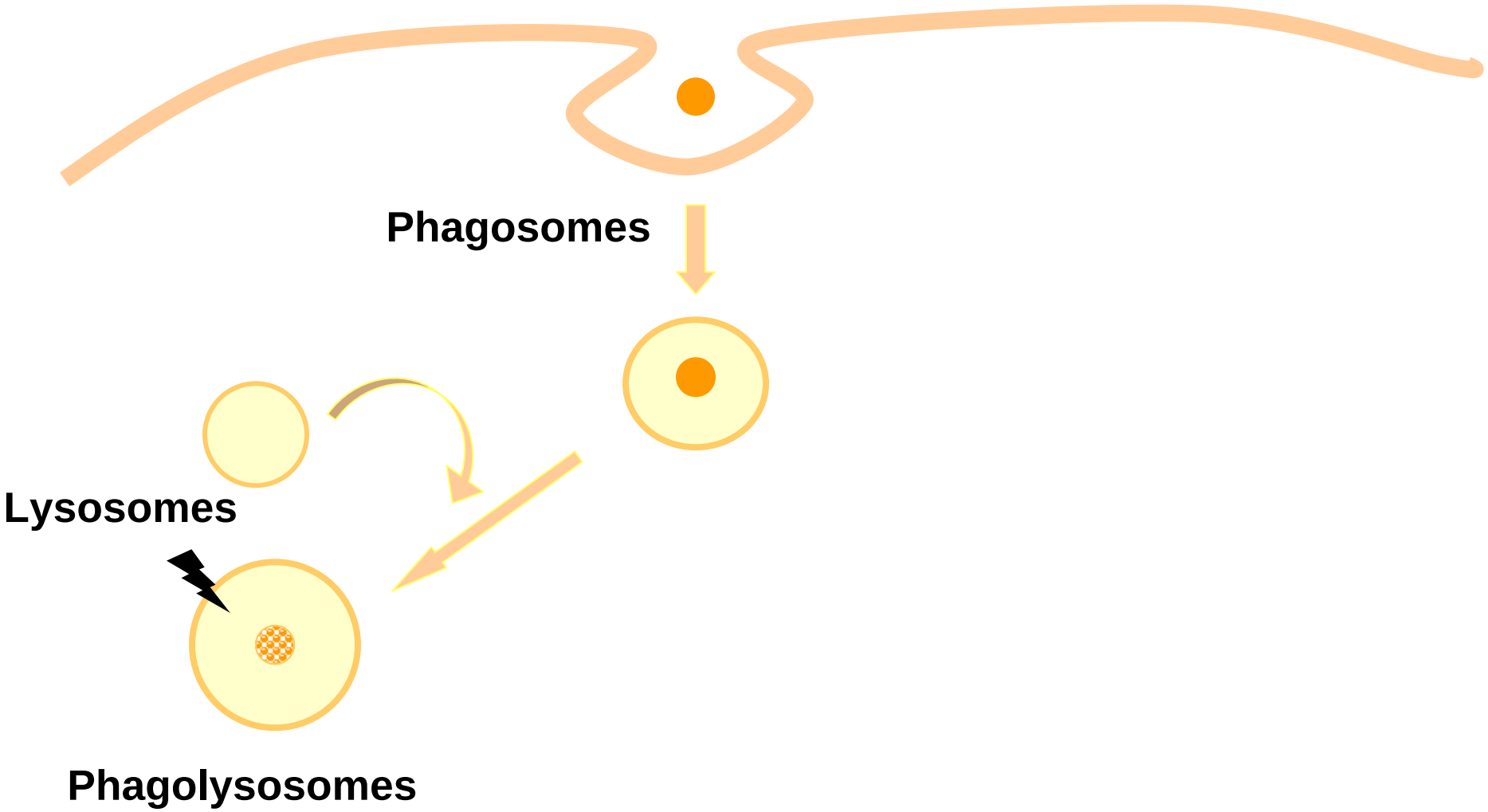
→ frequent contamination and frequent infections

Figure. Travel-related salmonellosis (all serotypes) as a function of prevalence of salmonella in laying hen flocks of *Gallus gallus*. Each dot represents a country.

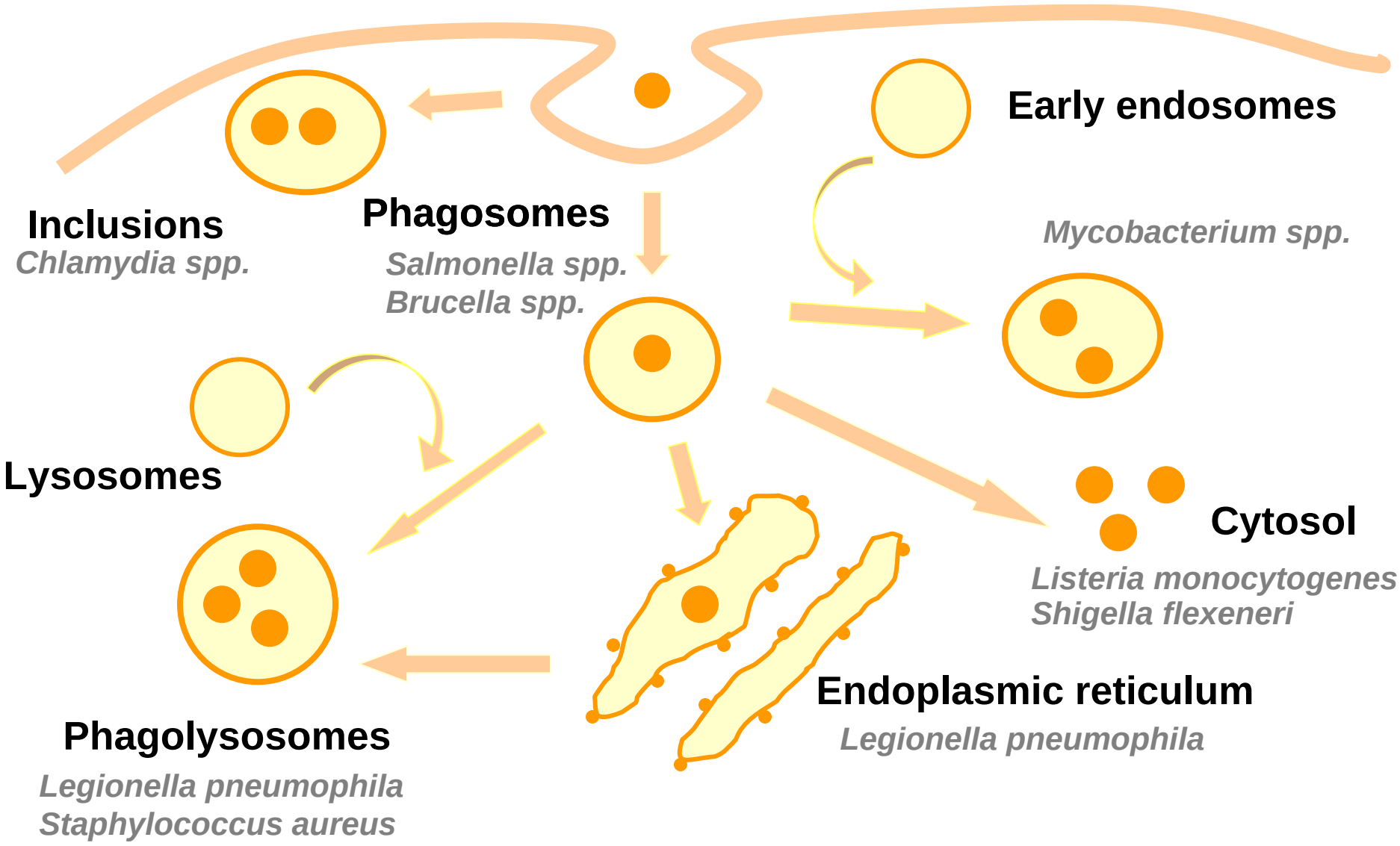


De Jong et al. Euro Surveill. 2006 Jul 6;11(7):E060706.1

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

Where is the bug ?

Where is the bug ?

Obligate intracellular bacteria

bacteria	localisation	infection
Chlamydia pneumoniae	inclusions	pneumonia
Chlamydia trachomatis	inclusions	STD, trachoma
Coxiella brunetii	(phago)lysosomes	Q fever, pneumonia, encephalitis, endocarditis, …
Mycoplasma pneumoniae	cytosol	pneumonia
Rickettsia spp	cytosol	cat scratch; fevers, …

Where is the bug ?

Facultative intracellular bacteria

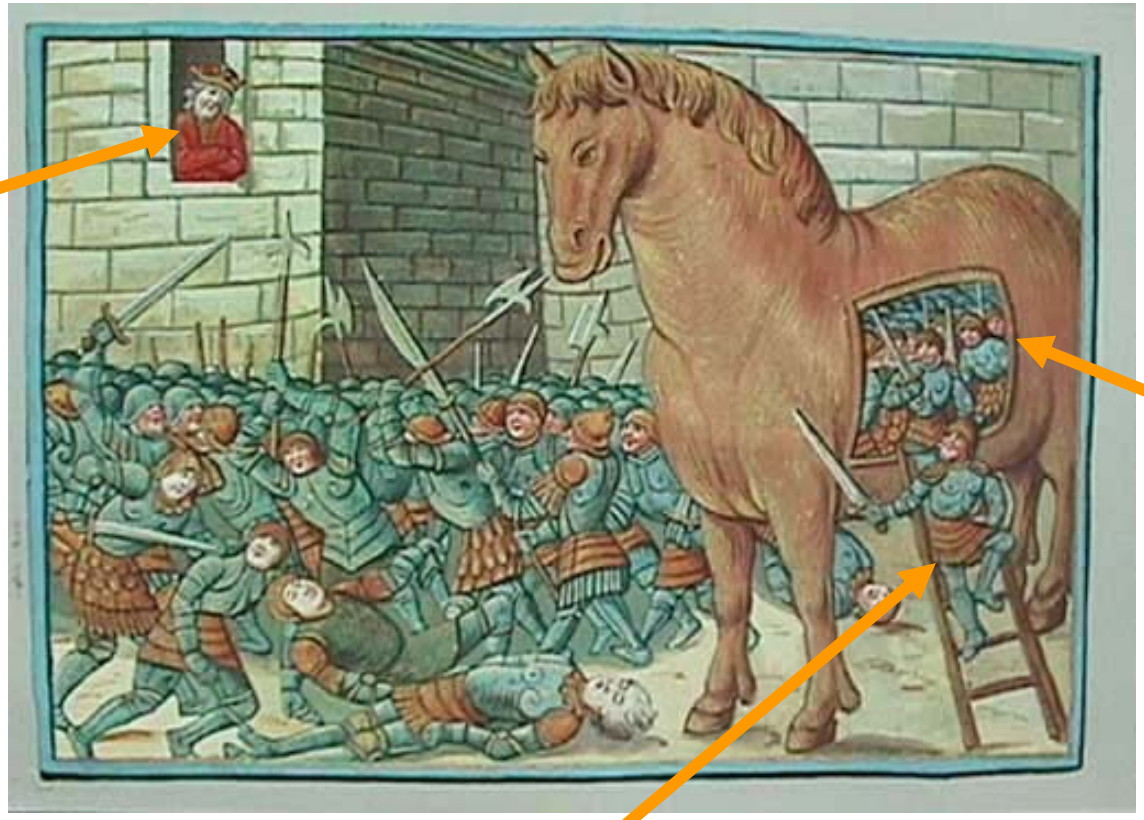
bacteria	localisation	Infection
Brucella spp	phagosomes	brucellosis
Francisella tularensis	phagosomes	tularemia
Legionella pneumophila	RE, lysosomes	pneumonia
Listeria monocytogenes	cytosol	meningitis
Mycobacterium tuberculosis	phagosomes	tuberculosis
Salmonella spp	phagosomes	diarrhoea
Shigella flexneri	cytosol	diarrhoea

Where is the bug ?

Opportunistic intracellular bacteria

bacteria	infection
Bacillus anthracis	anthrax
Borrelia burgdorferi	Lyme disease
Campylobacter jejuni	diarrhoea
Escherichia coli	urinary and digestive tract infections
Helicobacter pylori	ulcer
Staphylococcus aureus	skin infection, osteomyelitis, endocarditis, pneumonia, …
Yersinia spp	pestis, diarrhoea

Benefits of intracellular life

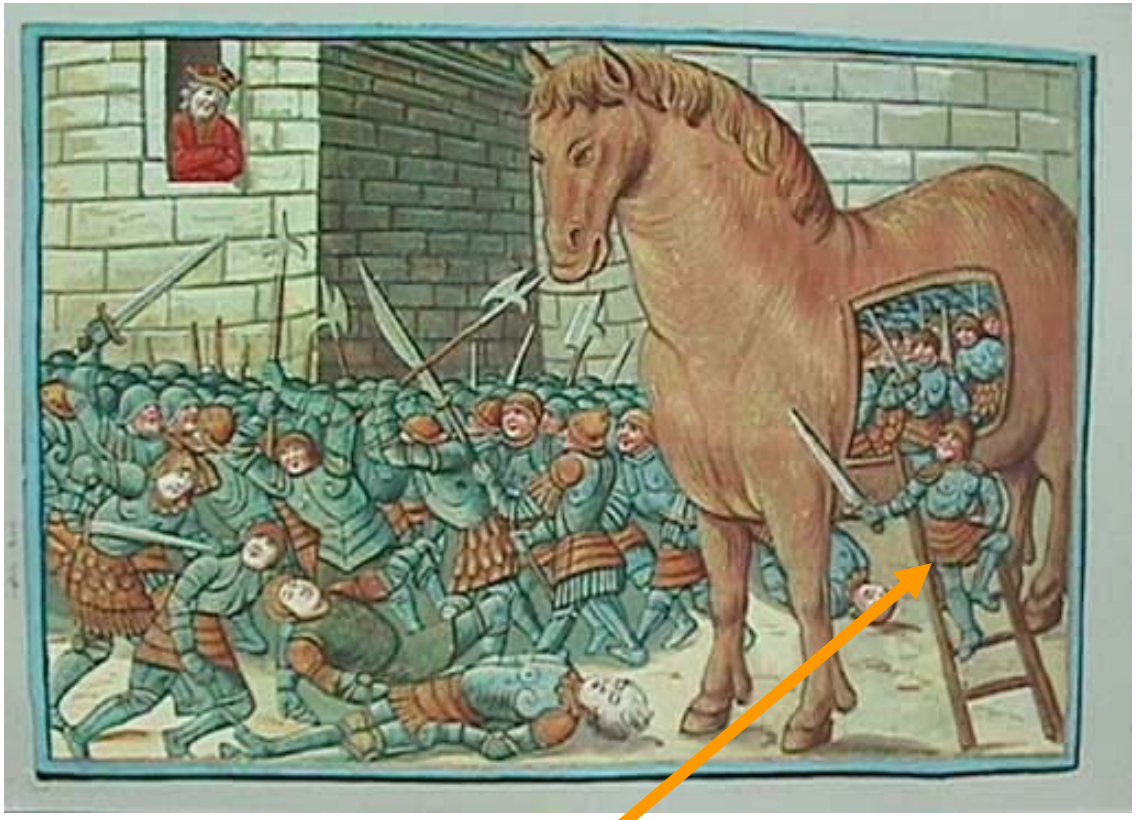


protection

persistence

invasion

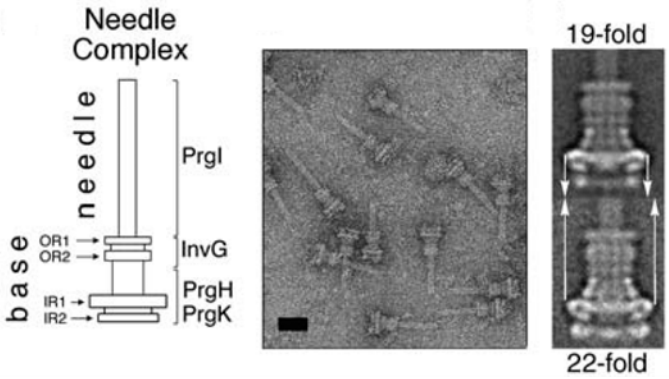
Benefits of intracellular life

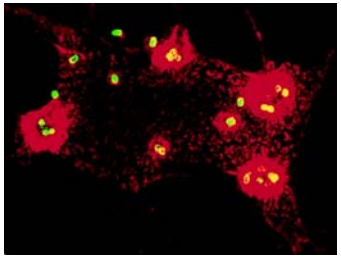


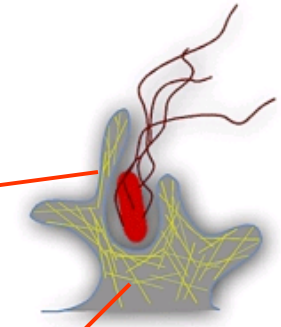
invasion

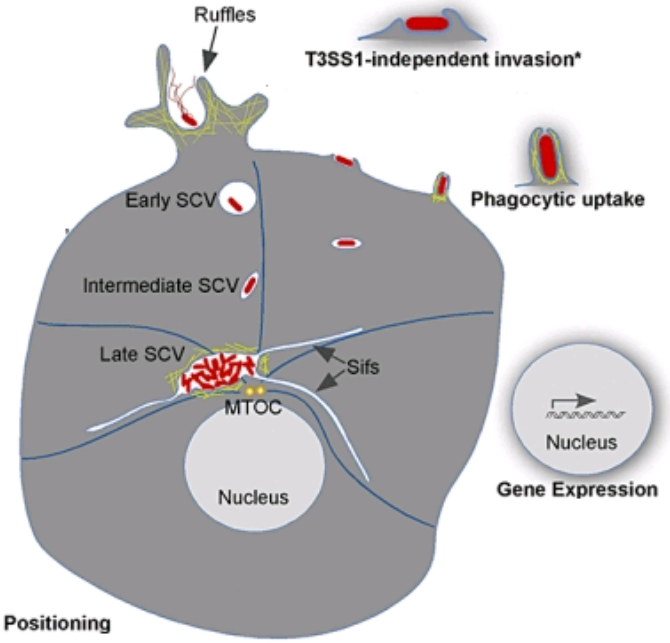
Invasion via the digestive tract

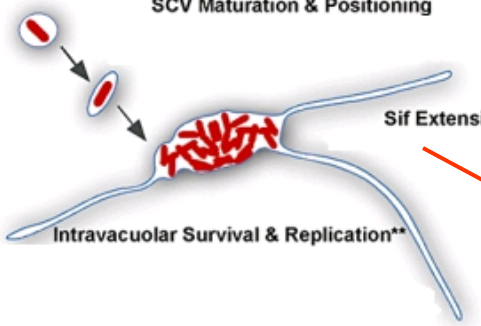
Cell invasion by *Salmonella*

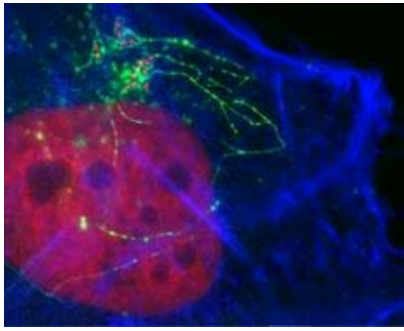










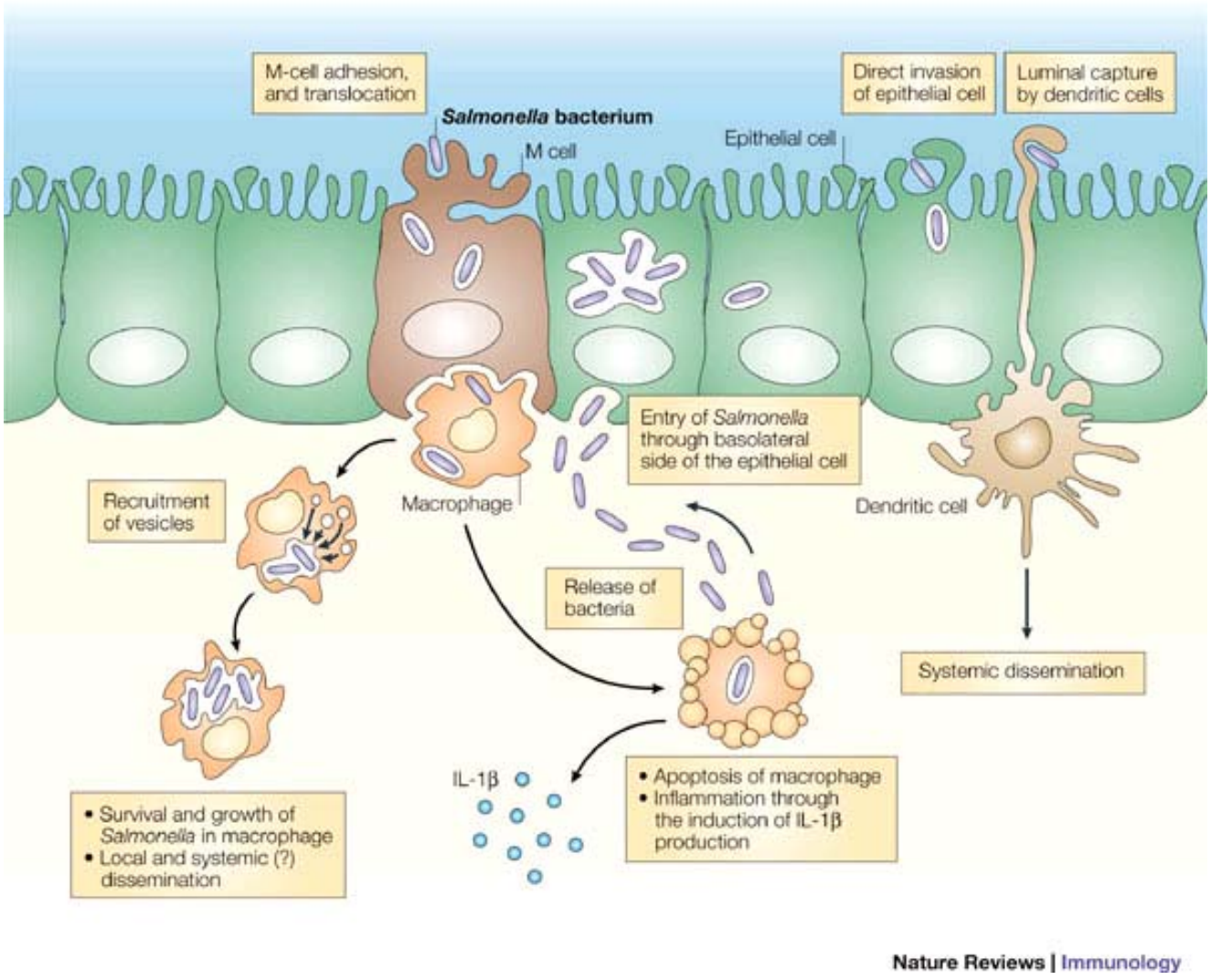


Salmonella establishes its SCV niche in a human cell (stained by cytoskeleton and nucleus)

Patel & Galan, Curr Op Microbiol. (2005) 8:10-5
Maltovits et al., Science (2004) 306:1040-2
Ibarra & Steele-Mortimer, Cell Microbiol. (2009) 11: 1579–158
Humphreys et al, Cell Host Microbe (2009) 19:225-33

Invasion via the digestive tract

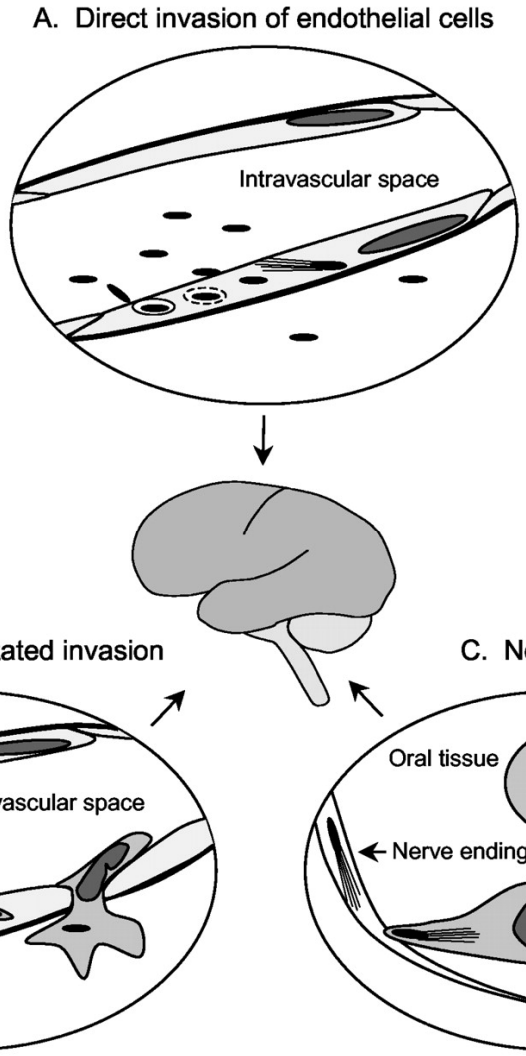
Host invasion by *Salmonella*

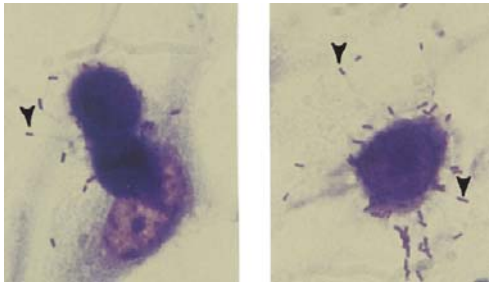


Sansonetti, Nat. Rev. Immunol. (2004) 4:953-64

Migration to the CNS

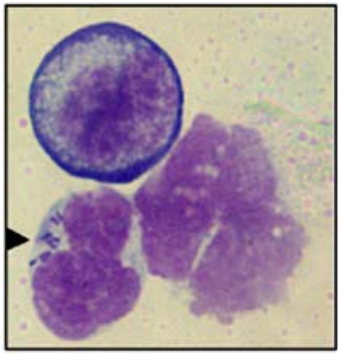
Listeria:
from the gut to the CNS

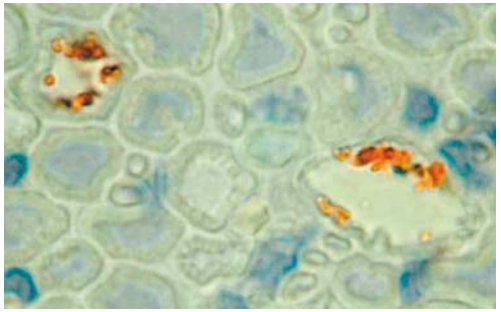




adherence and transfert
from monocytes to endothelial cells

bone-marrow monocyte

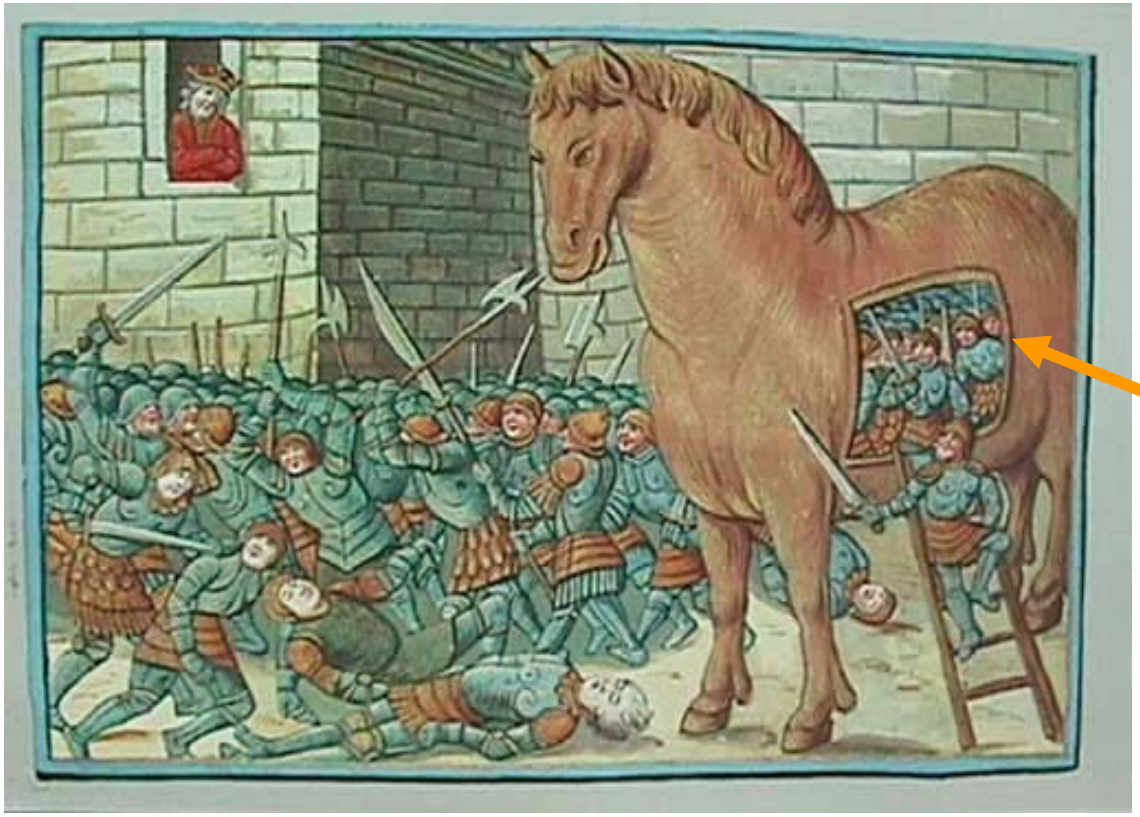




intra-axonal labeling
by anti-listeria antibodies

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

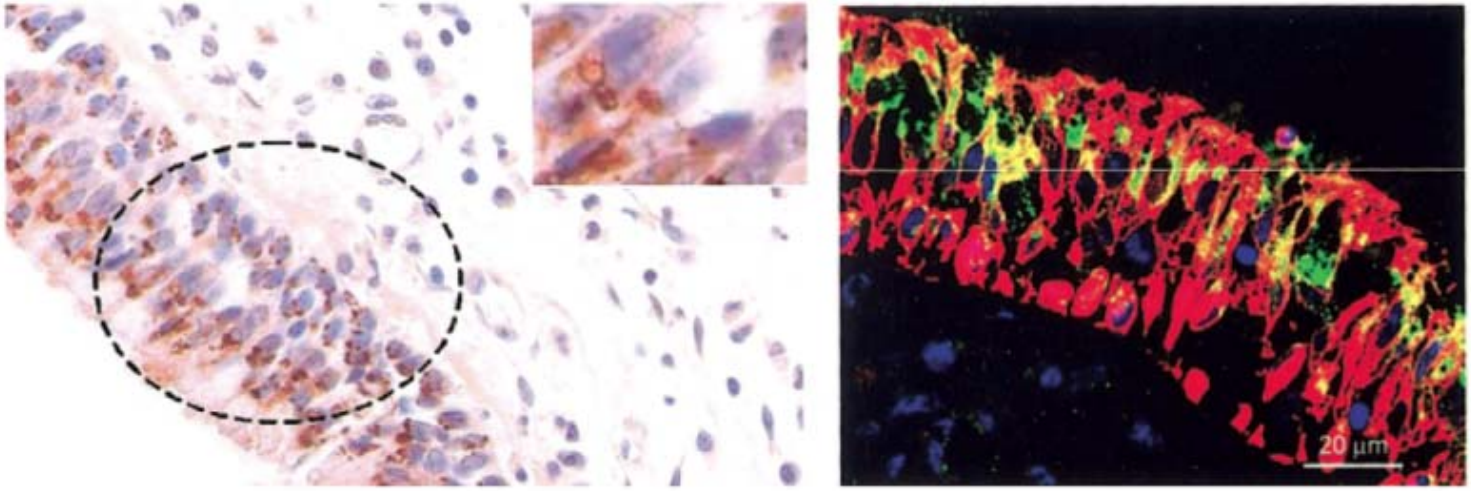
Benefits of intracellular life



persistence

S. aureus persistent infections

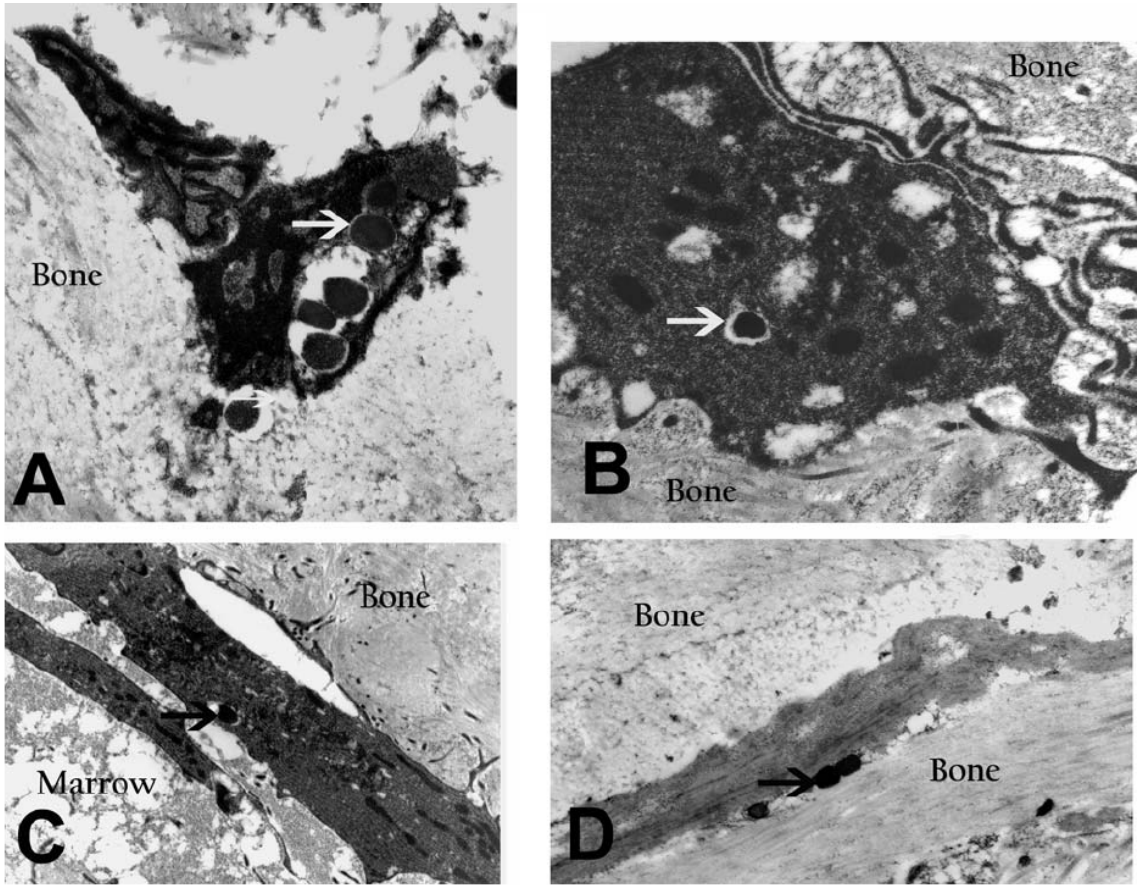
Evidence of an intracellular reservoir in the nasal mucosa of patients with recurrent *Staphylococcus aureus* rhinosinusitis



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

S. aureus persistent infections

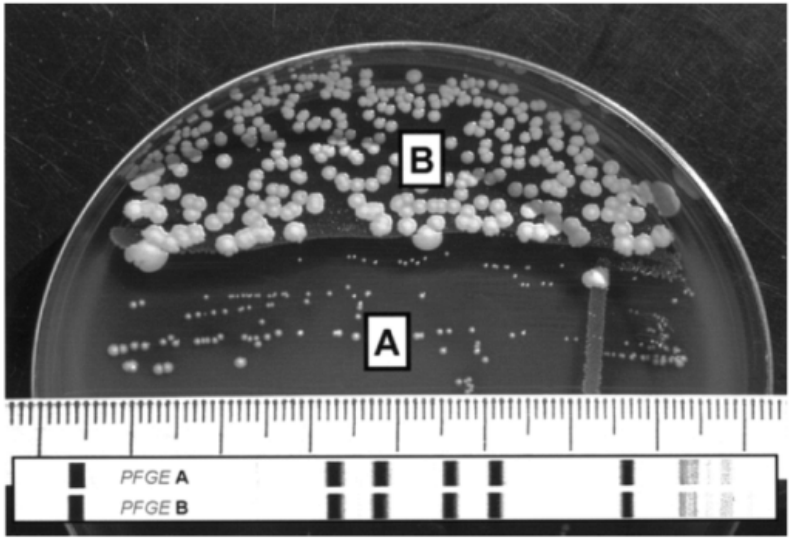
Evidence of an intracellular reservoir in osteocytes (A,B), osteoblasts (C) and bone matrix (D) of a patient with recurrent osteomyelitis



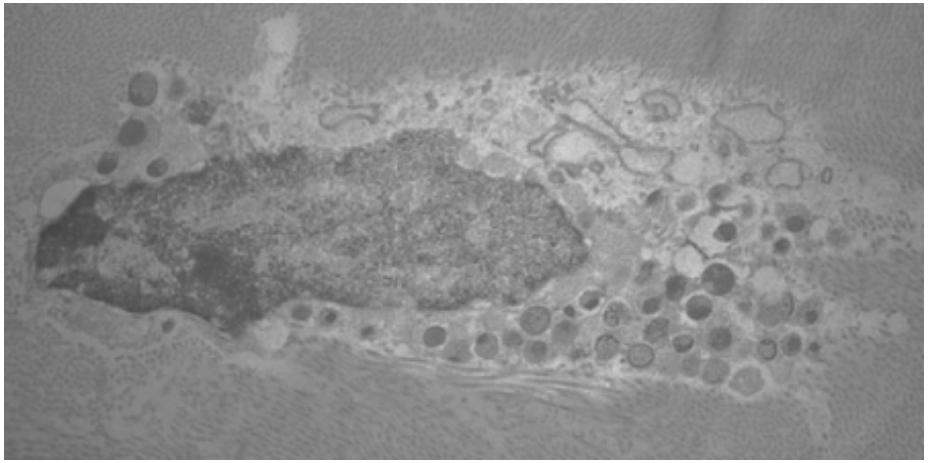
Bosse et al., J Bone Joint Surg Am. (2005) 87:1343-7

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.

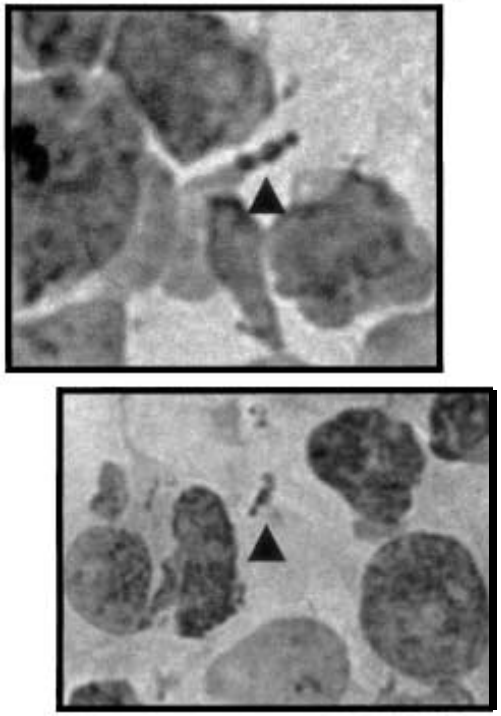


* Fluclox, CIP+ RIF, VAN + FEP

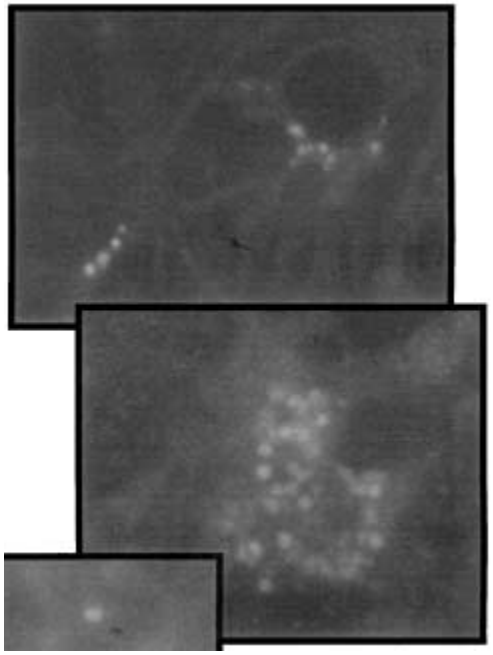
Sendi et al., Clin Infect Dis. (2006) 43:961-7

S. pyogenes persistent infections

Evidence of intracellular group A streptococci in tonsils of recurrently infected adults



HRP-antibody against GAS

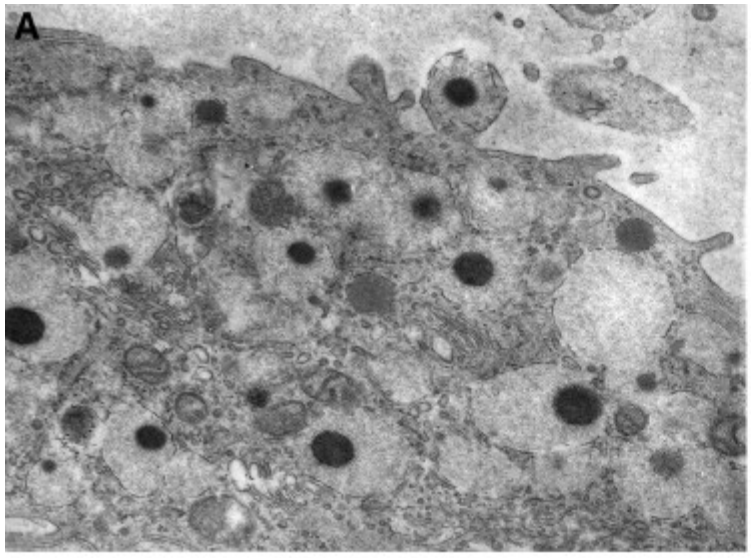


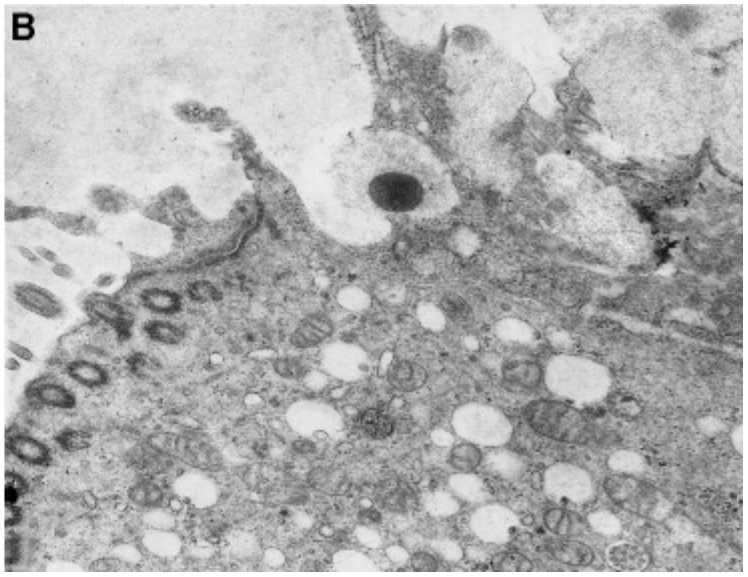
FITC-antibody against GAS

Podbielski et al., Int J Med Microbiol. (2003) 293:179-90.

S. pneumoniae persistent infections

Evidence for intracellular persistence of *S. pneumoniae* and other Gram-positive cocci in epithelial cells of ear mucosa of children with otitis media with effusion

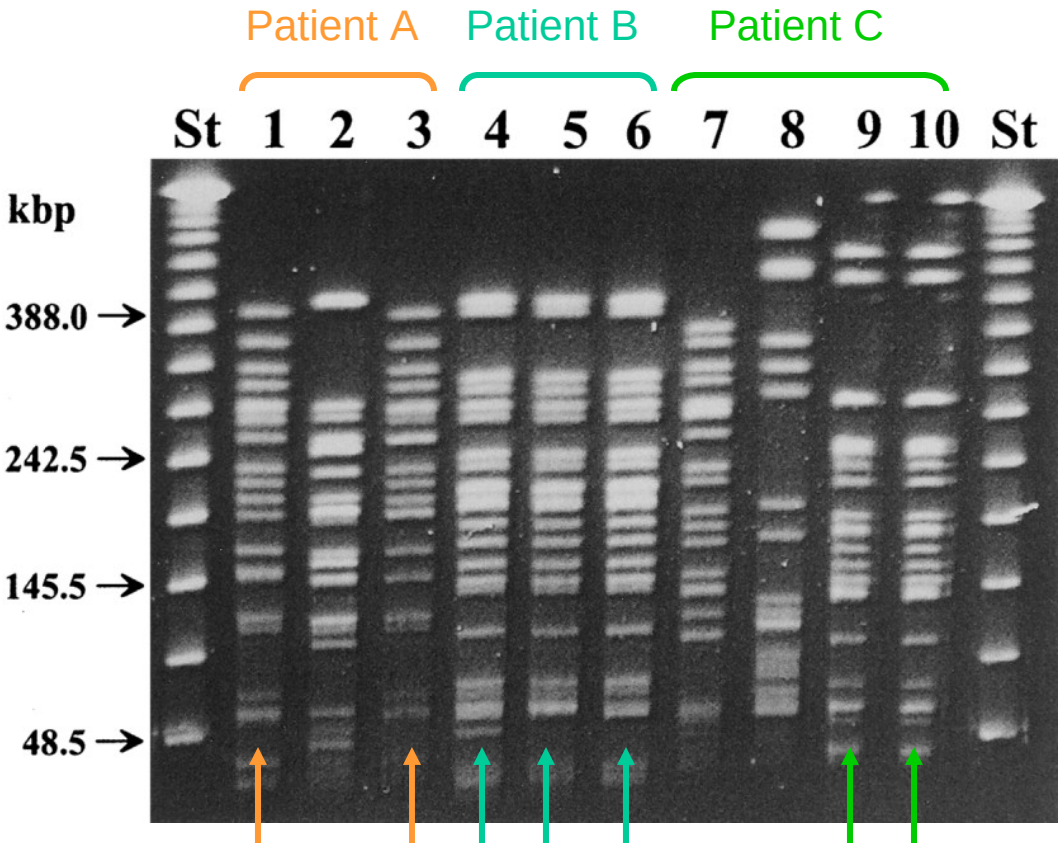




Coates et al., Otolaryngol Head Neck Surg. (2008) 138:778-81

E. coli recurrent infections

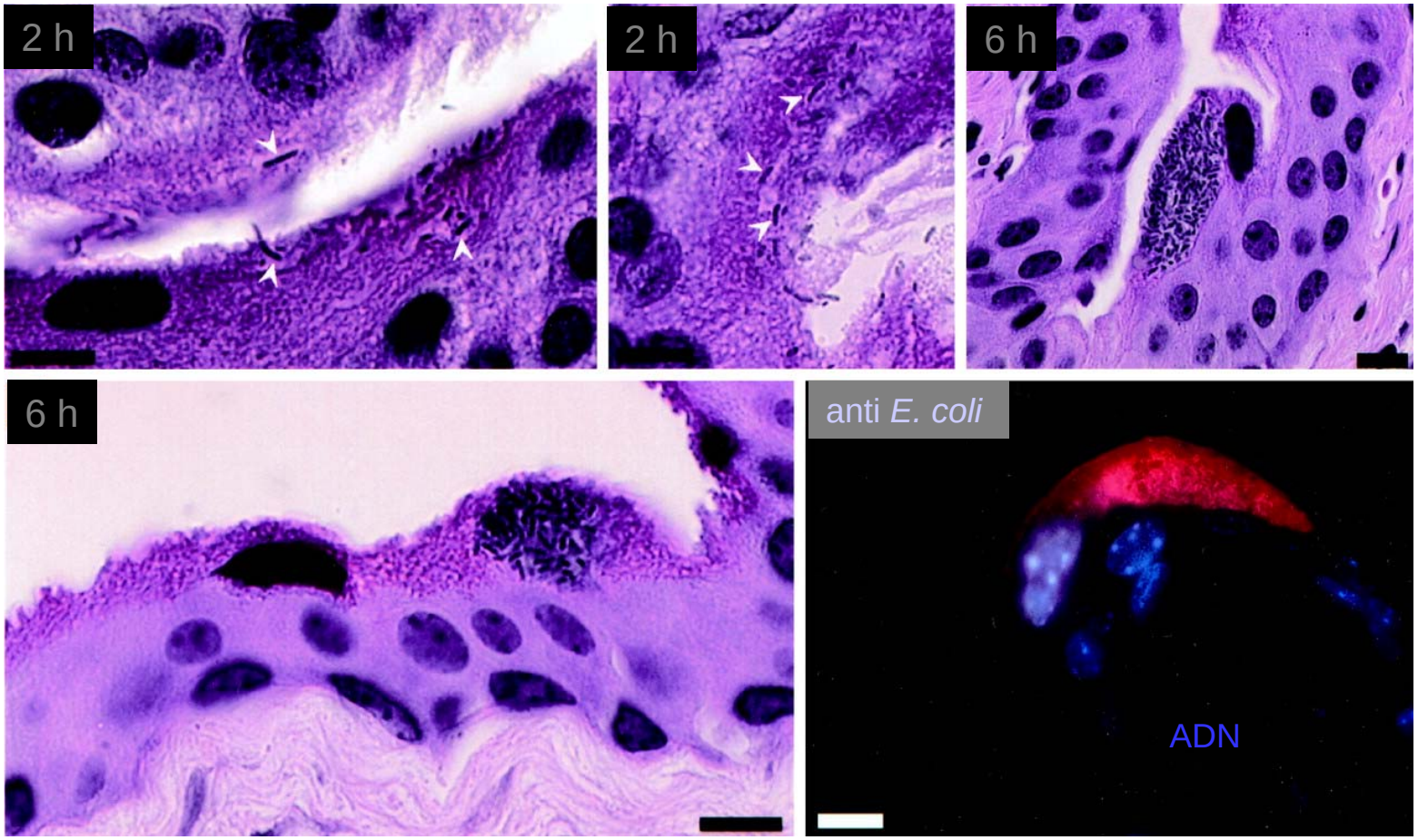
Recurrent infections are often (65 %)
due to the same strain



Jantunen et al., J Infect Dis. (2002) 185:375-9

E. coli recurrent infections

E. coli invades the bladder epithelium

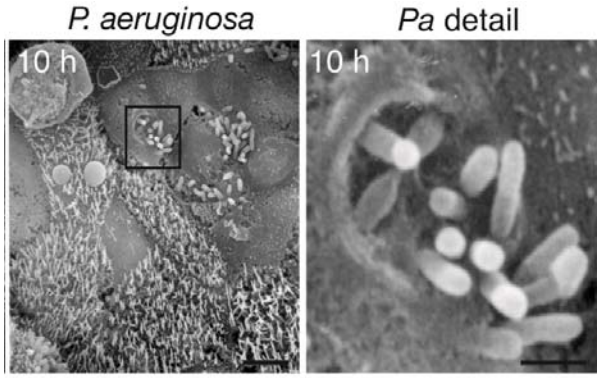


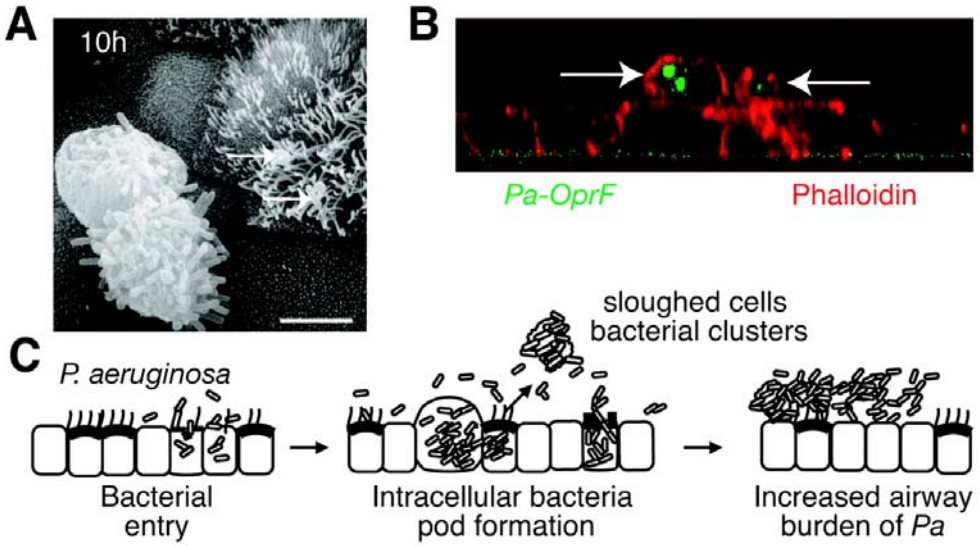
Mulvey et al., infect. Immun. (2001) 69:4572-9

P.aeruginosa recurrent infections

P. aeruginosa invades alveolar epithelial cells

P. aeruginosa PAO1 localized to membrane blebs induced in human alveolar epithelial cells

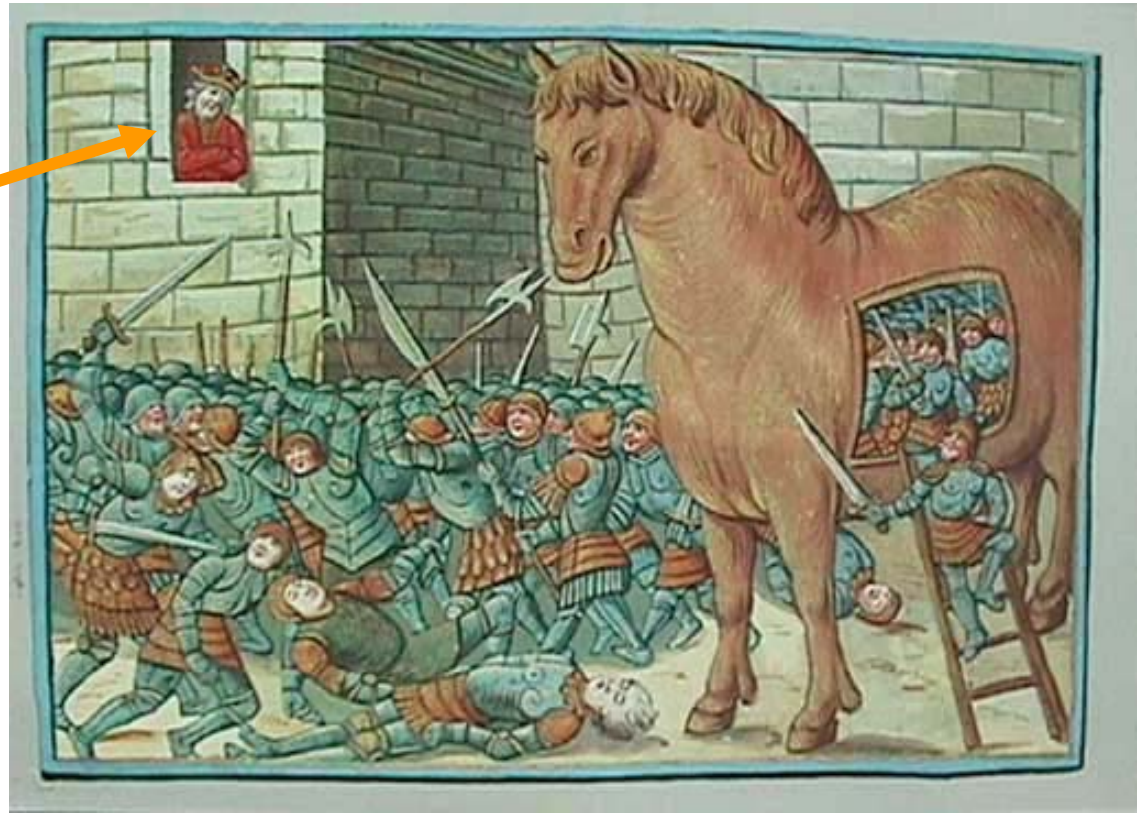




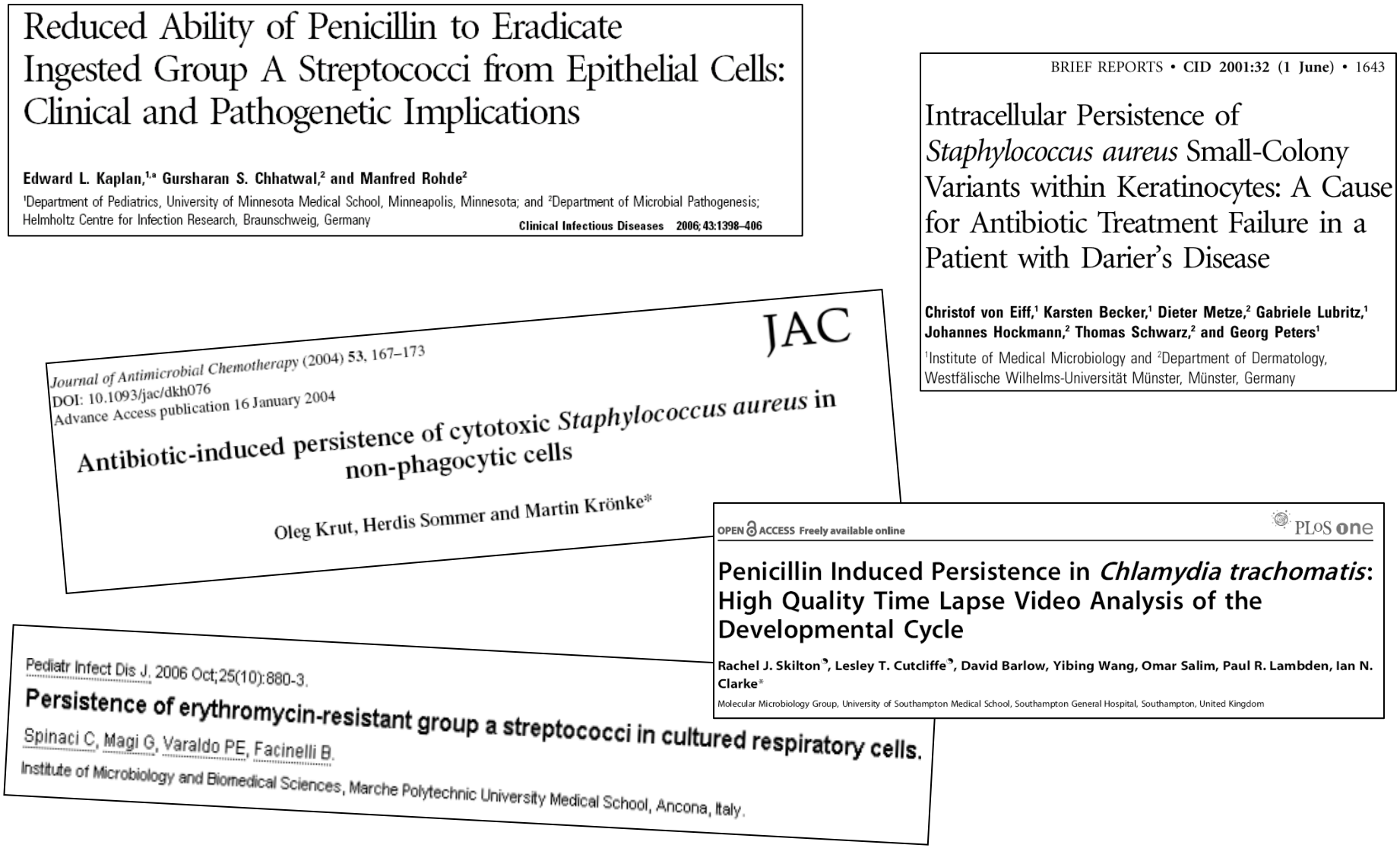
Garcia-Medina et al., Infect Immun. (2005) 73:8298-305
Angus et al. Infect Immun. (2008) 76:1992-2001

Benefits of intracellular life

protection



Failure to eradiate 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

BRIEF REPORTS • CID 2001:32 (1 June) • 1643

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

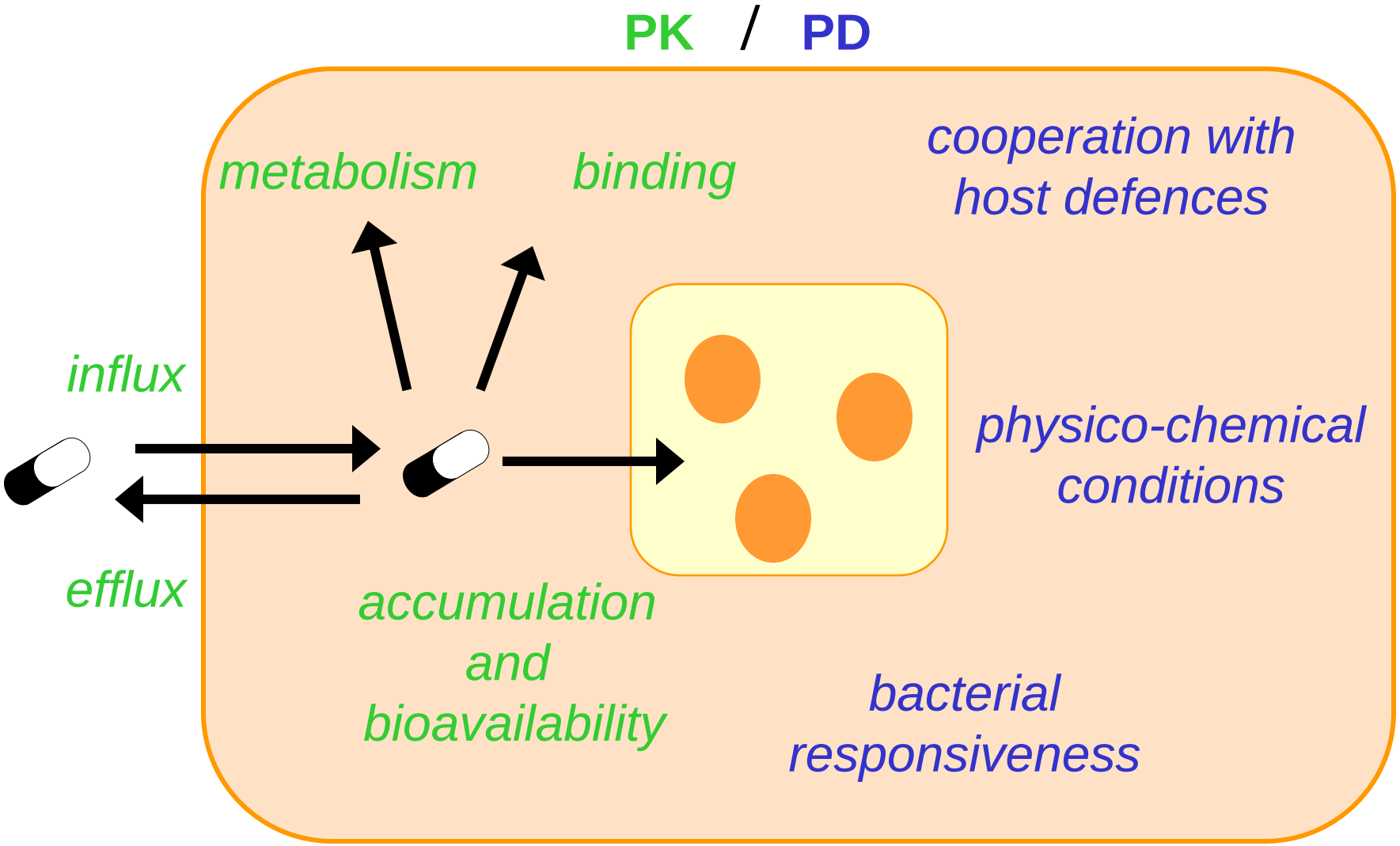
Journal of Antimicrobial Chemotherapy (2005) **55**, 383–386

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*

Optimisation of antibiotic treatments

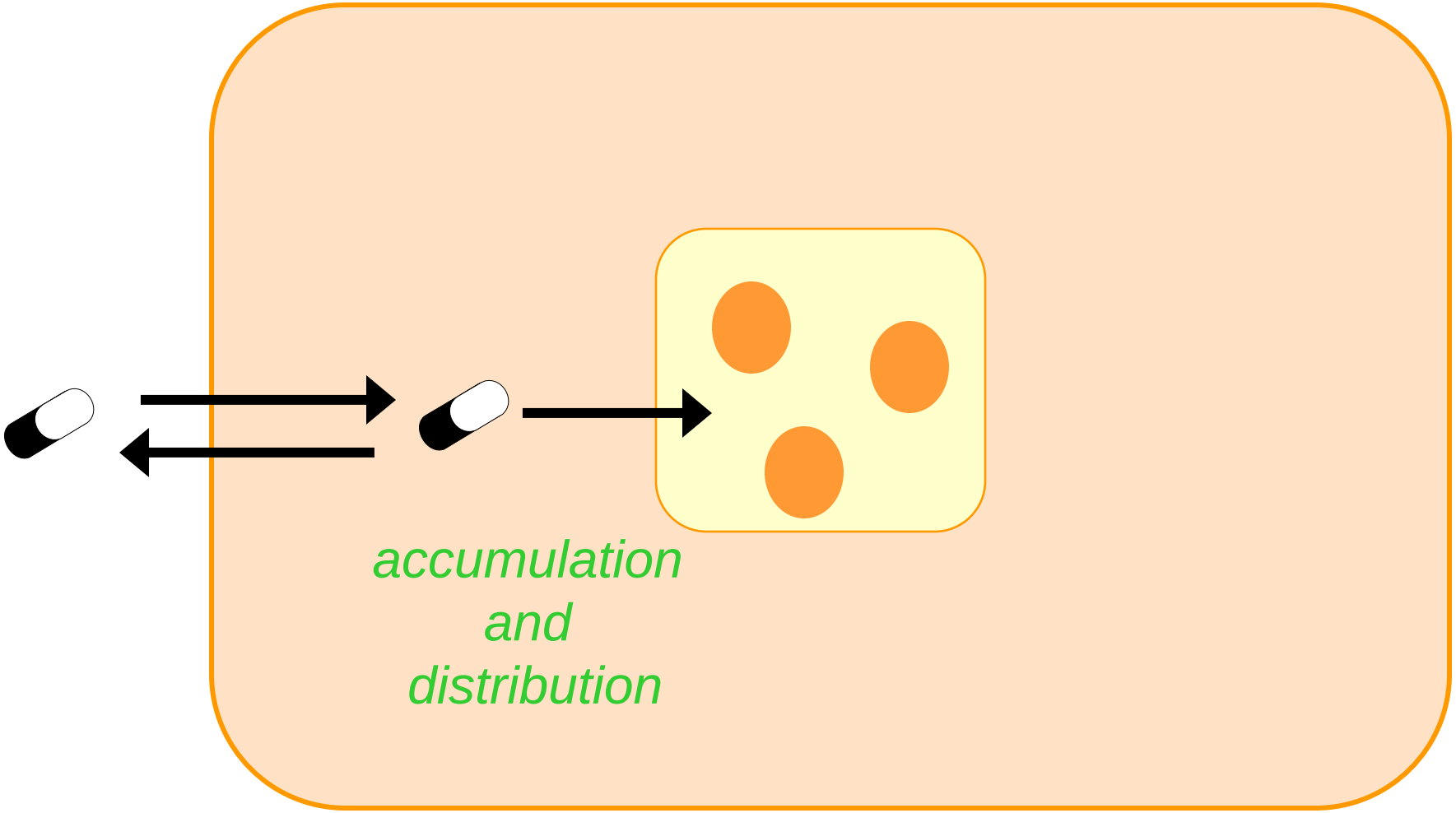


Carryn et al., Infect Dis Clin North Am. (2003) 17:615-34

Where is the drug ?

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

Where is the drug ?



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

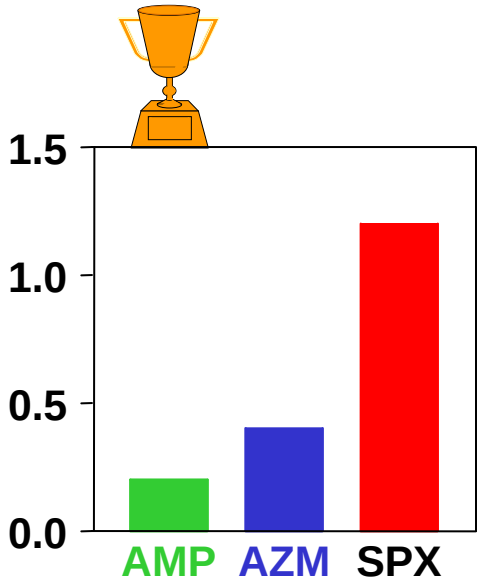
macrolides: fast
ERY: 4-10 x
CLR, ROX, TEL: 10-50x
AZM: > 50 x
CEM-101: 350 x

oxazolidinones: fast
RDZ : 10 x

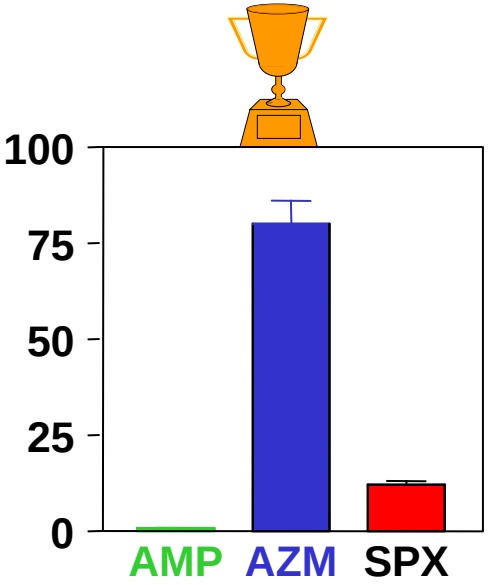
diffusion/
segregation

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

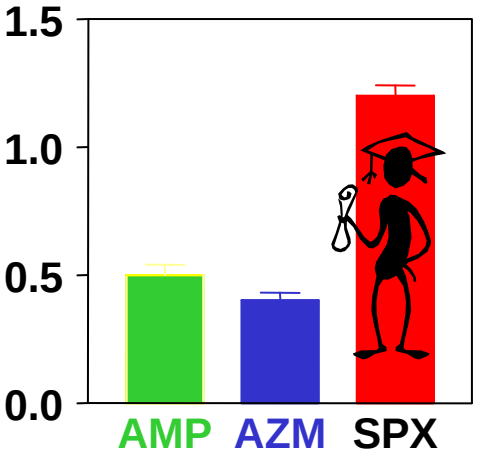
MIC
(L. monocytogenes)



antibiotic accumulation

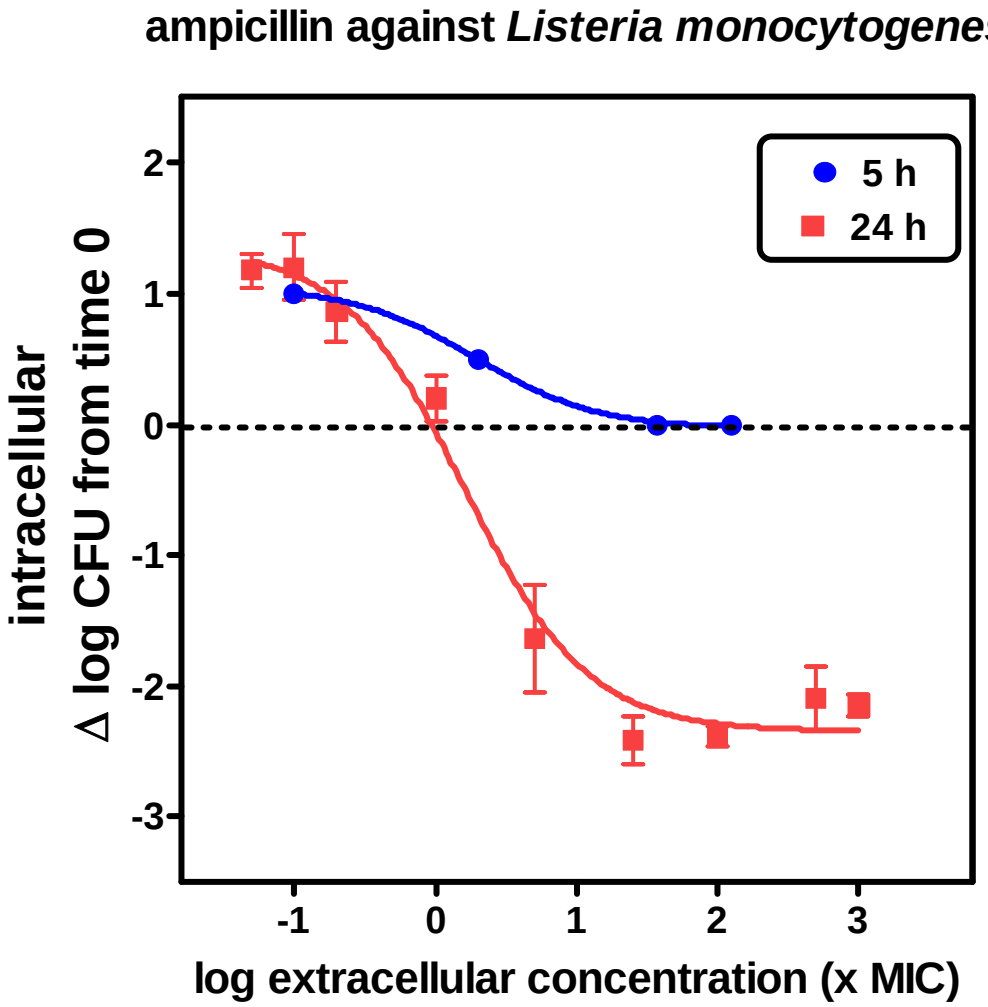


activity on intracellular *Listeria*
(5 h; 10 x MIC)



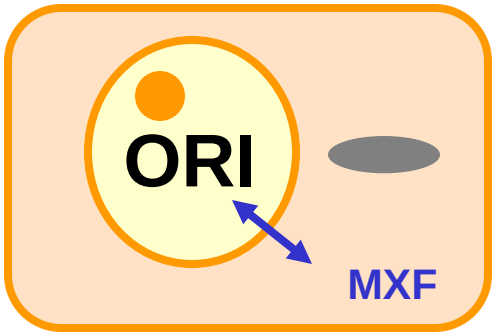
Ouadrhiri et al (1999) AAC 43:1242-51

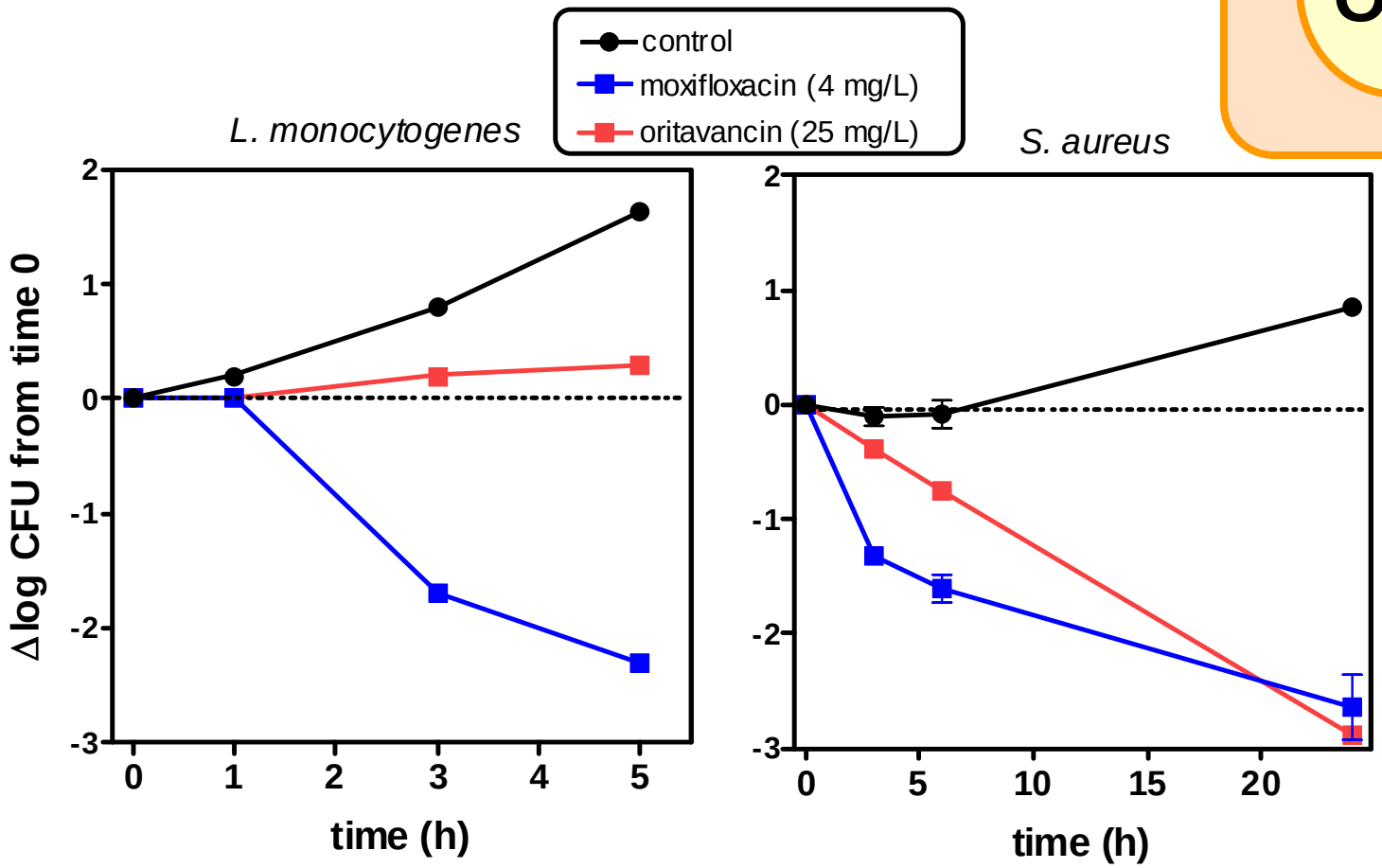
Importance of optimizing time and concentration …



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

Importance of reaching intracellular bacteria …

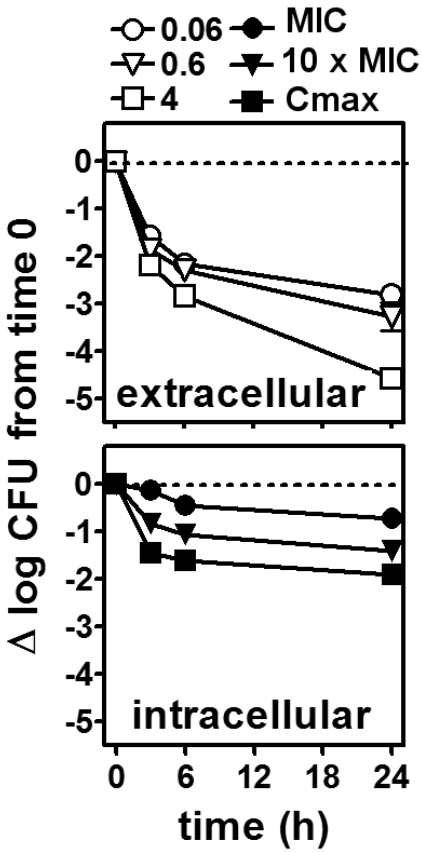


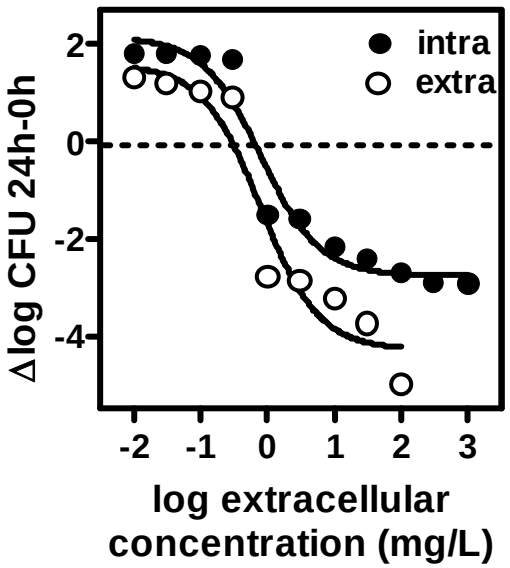


adapted from Carryn et al., AAC (2002) 46:2095-2103
Van Bambeke et al., AAC (2004) 48:2853-60
Barcia-Macay et al., AAC (2006) 50:841-51

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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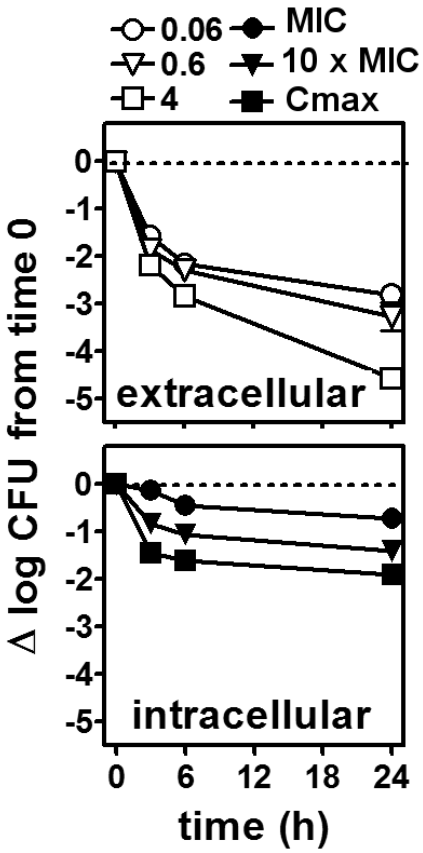
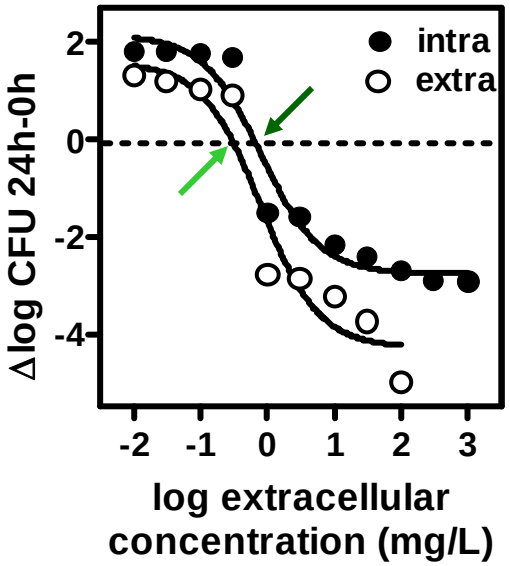




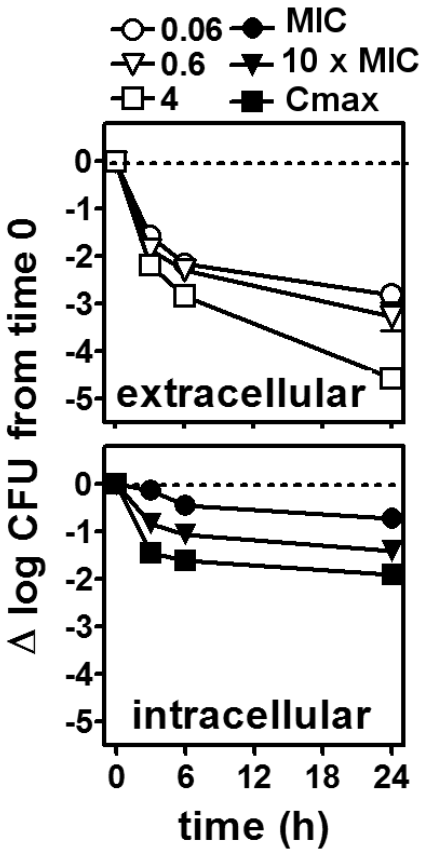
model	C_{stat} (x MIC)
extra	0.27
intra	0.63

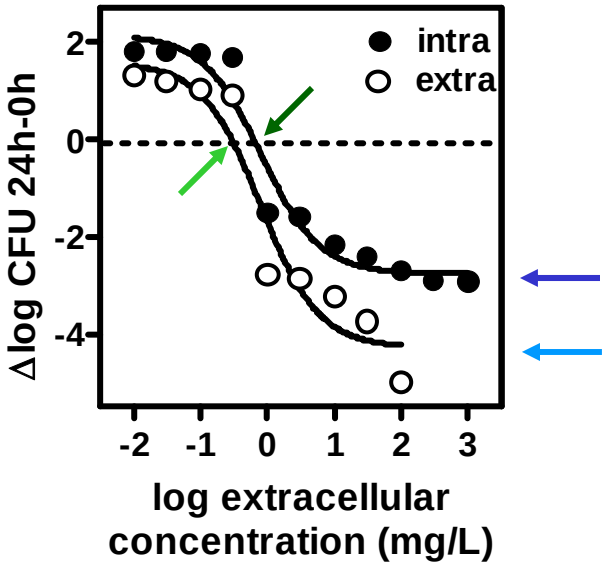
relative potency

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)

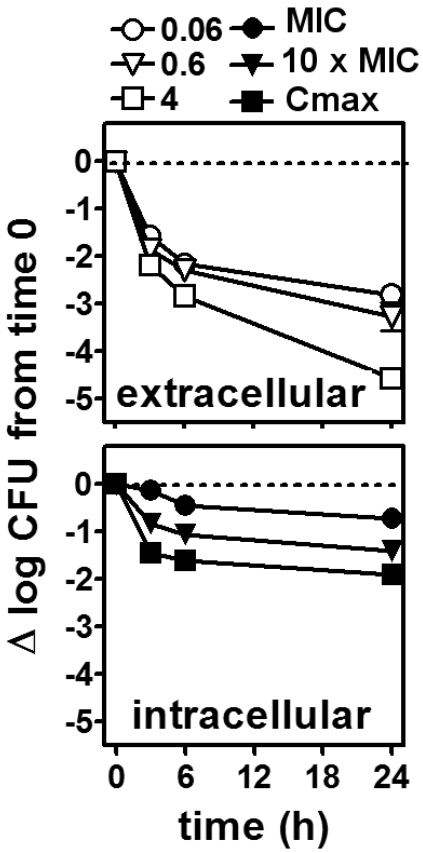
relative potency

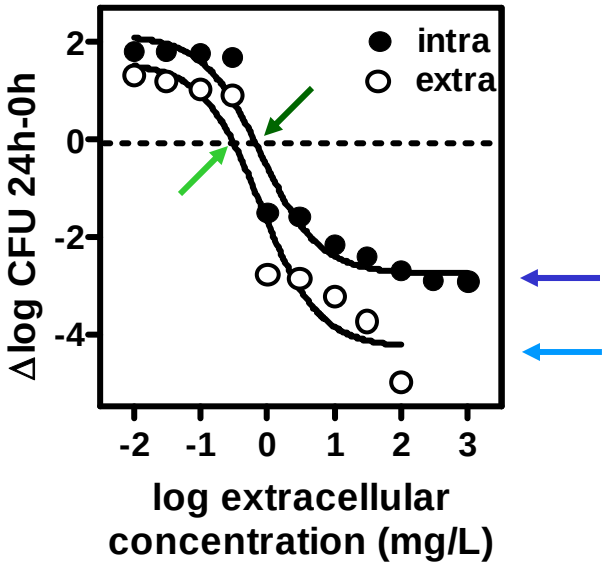
maximal efficacy

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

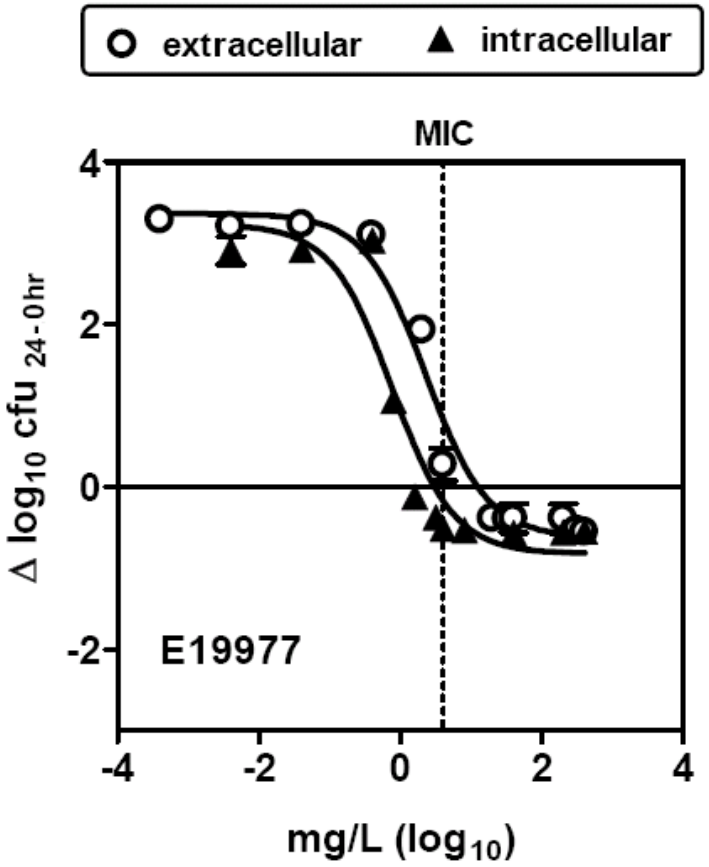
maximal efficacy

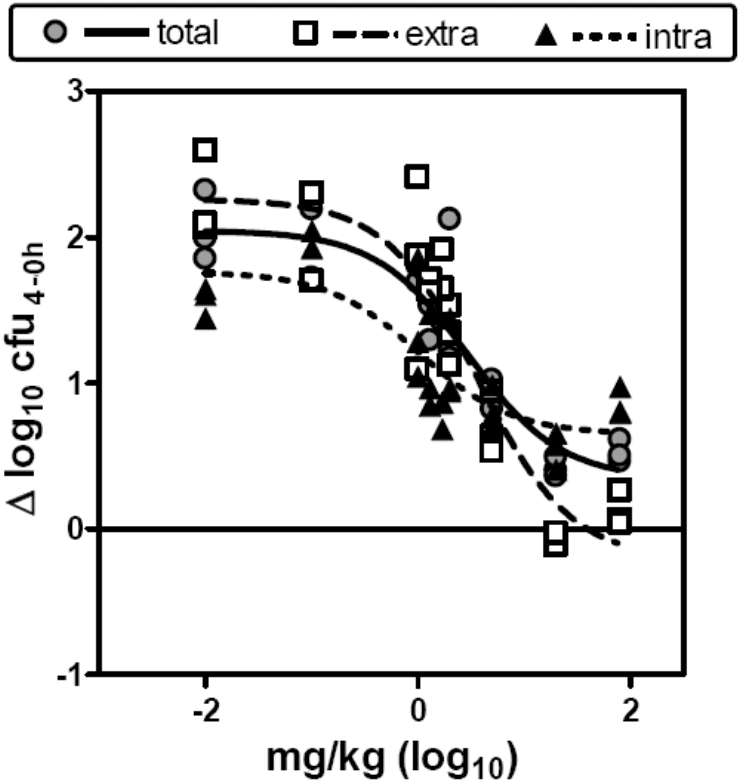
in vitro vs *in vivo*

Linezolid & *S. aureus*

in vitro
(macrophages)

in vivo
(peritonitis)





Sandberg et al. JAC (2010) in press

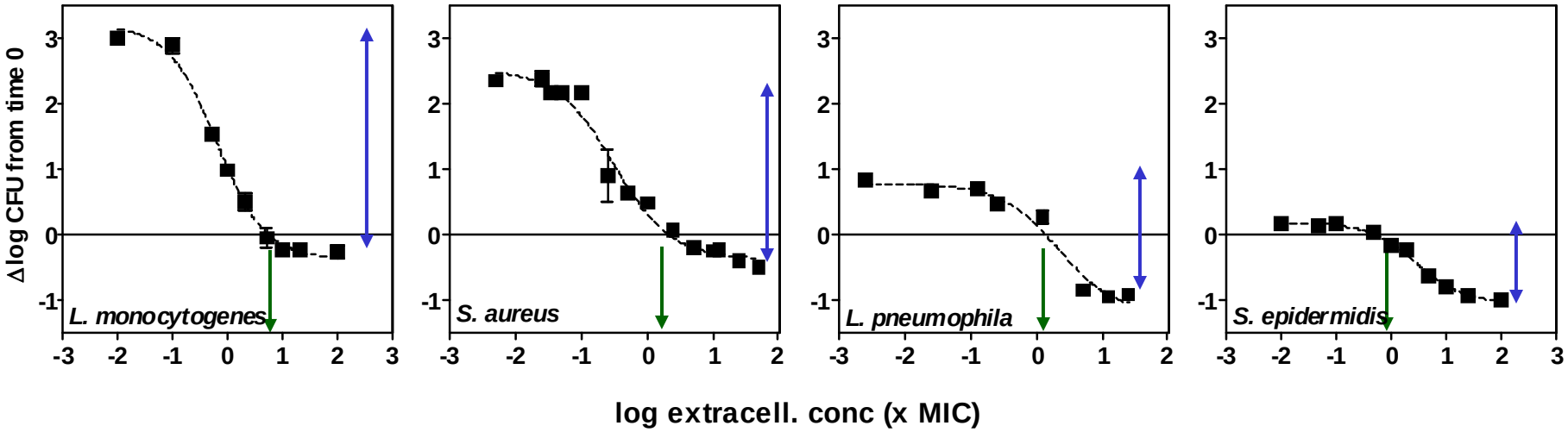
What do these models tell us ?

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

What do these models tell us ?

comparison : 1 drug ~ different models of infection

Linezolid ; THP-1 cells

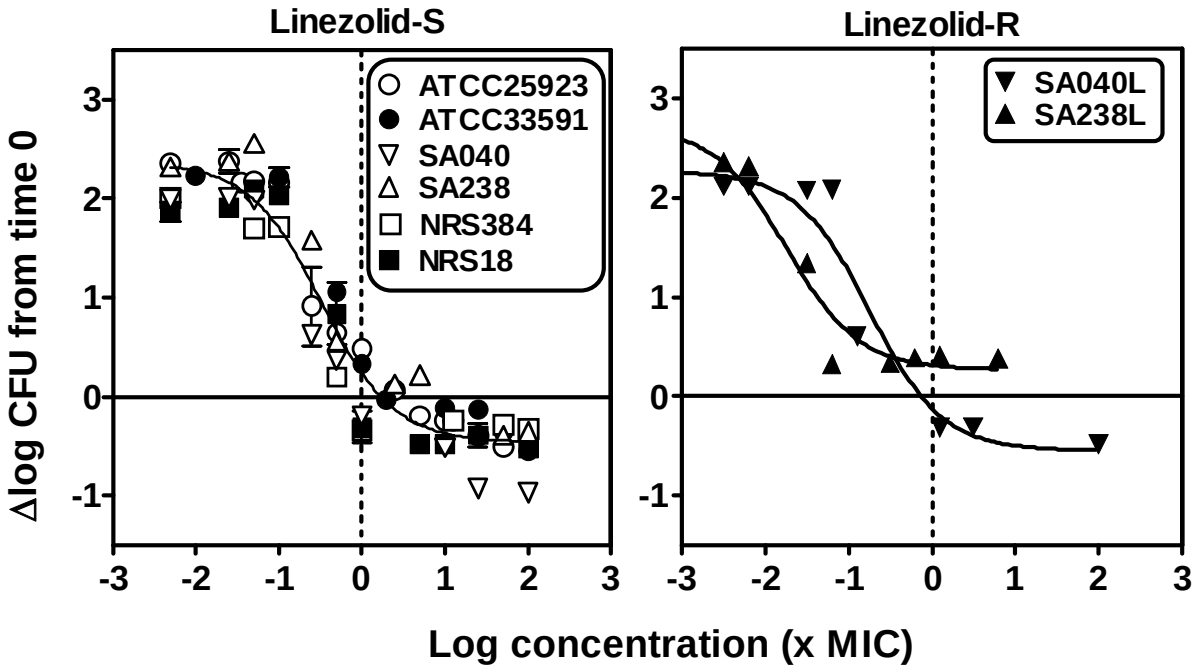


- Cs close to the MIC
- amplitude of the effect depending on intracell. growth

What do these models tell us ?

comparison : 1 drug ~ different bacterial strains

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

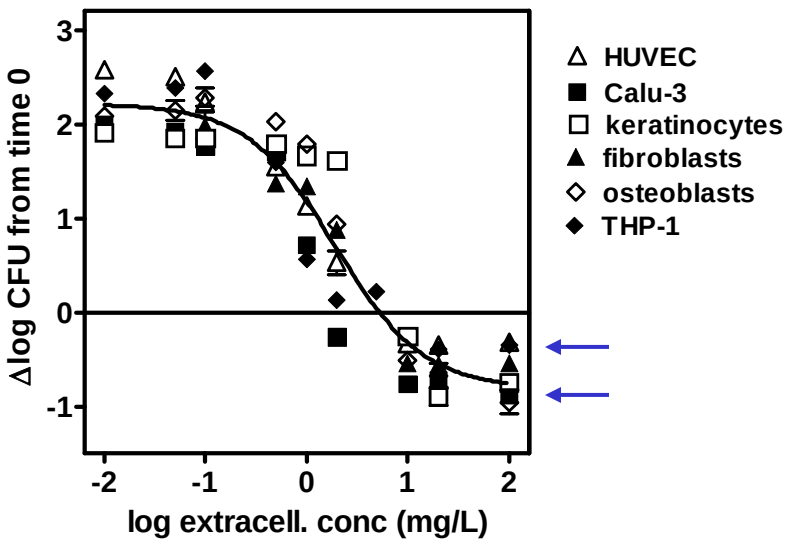


- Cs close to the MIC for all susceptible strains
- Resistant strains may show modified Emax

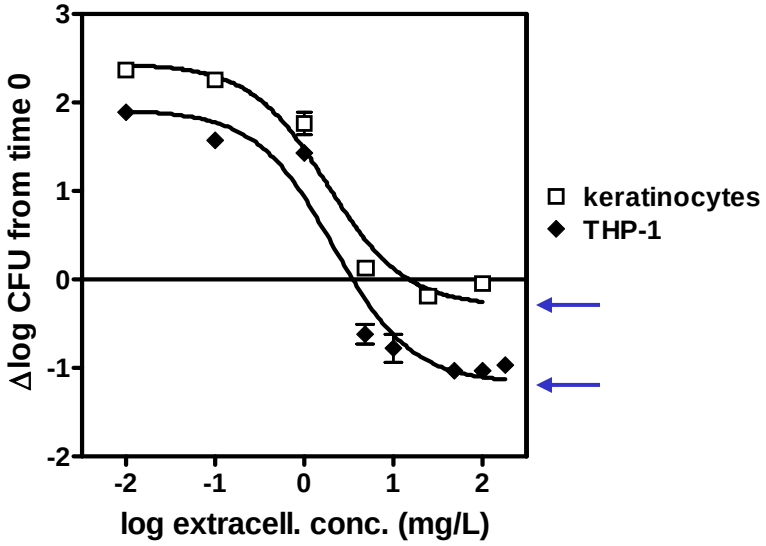
What do these models tell us ?

comparison : 1 drug ~ different cell types

Linezolid ; *S. aureus*



Ceftriaxone ; *S. aureus*



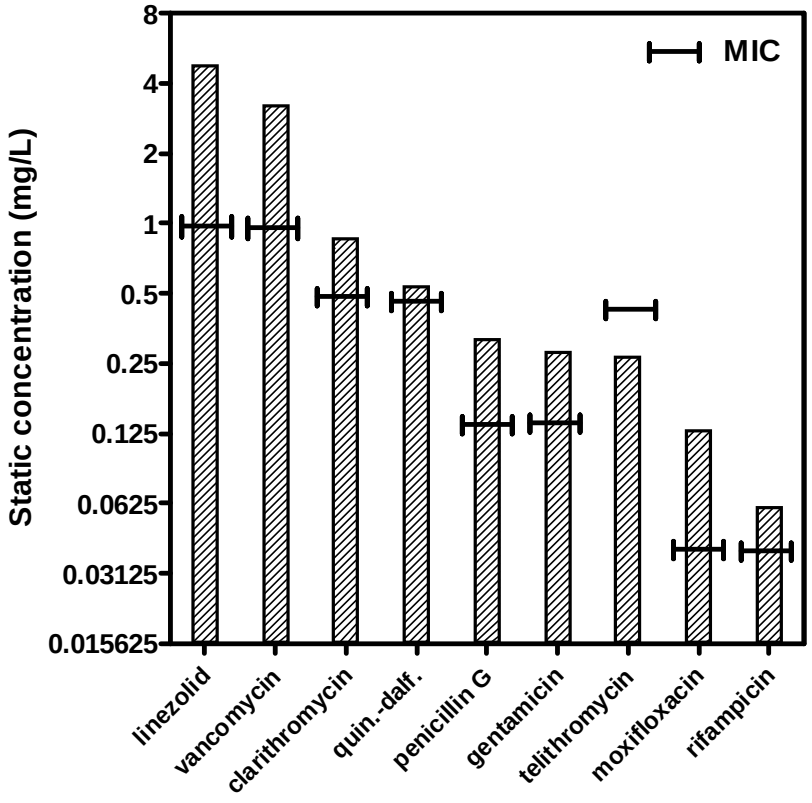
- Cs close to the MIC in all cell types
- Emax may vary ~ 1 log among cell types (depending on the drug !)

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

What do these models tell us ?

comparison : 1 model ~ different drugs

THP-1 ; *S. aureus*

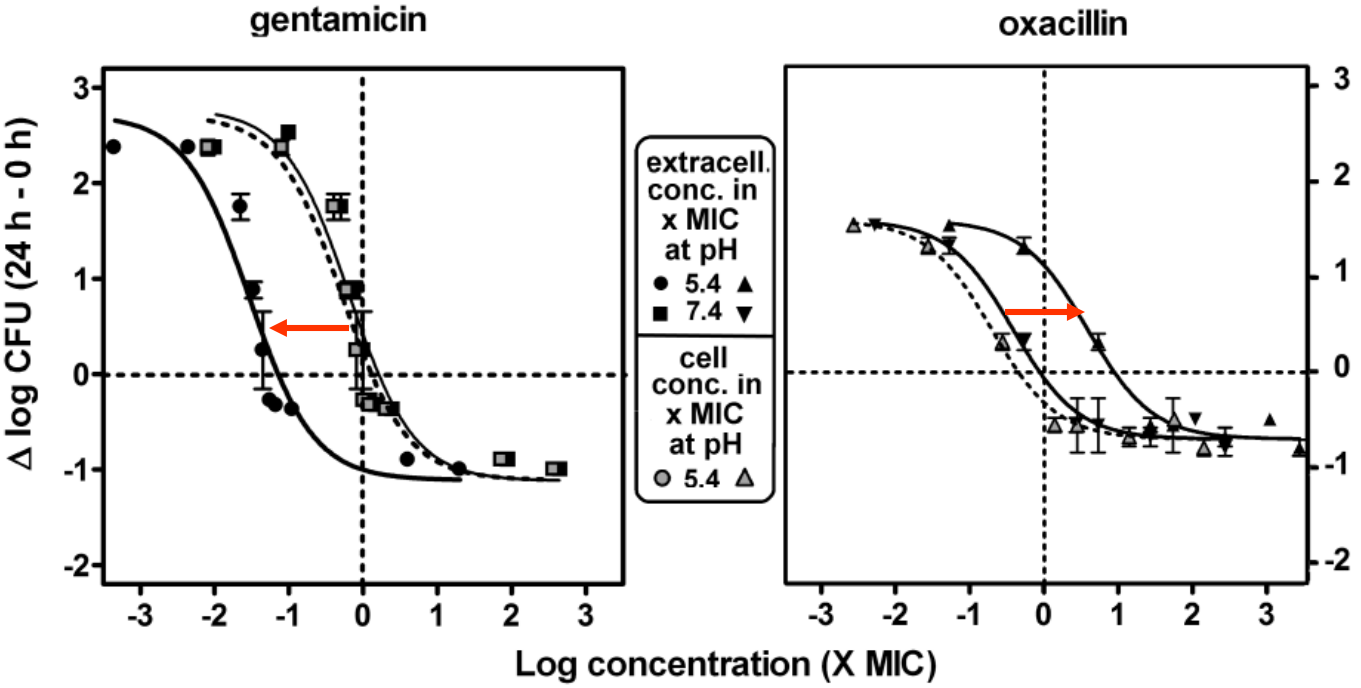


- Cs close or slighthy higher than the MIC for all drugs

How to modulate intracellular potency ?

Change pH!

THP-1 ; phagolysosomal *S. aureus*



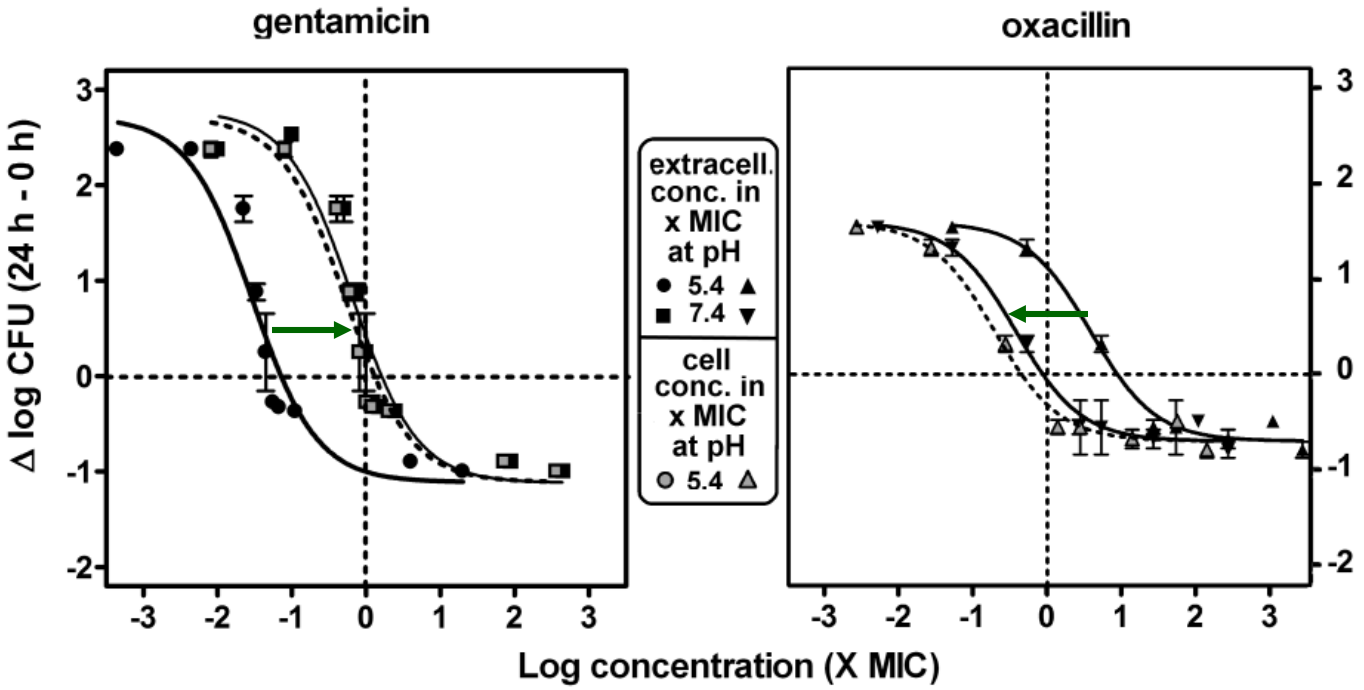
MIC at acidic pH

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

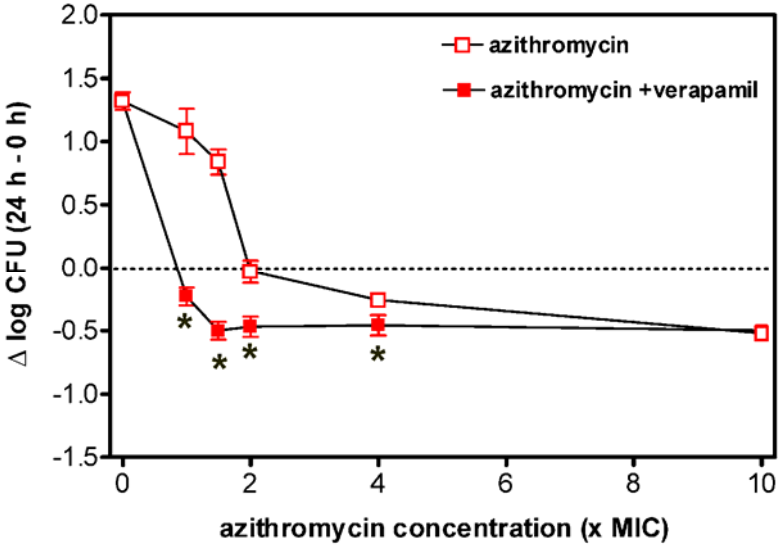
Baudoux et al, JAC (2007) 59:246-53

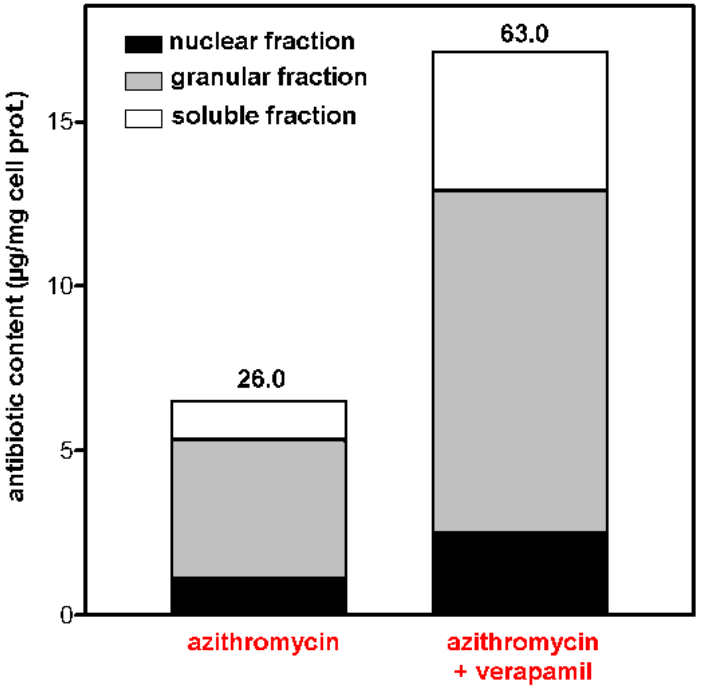
How to modulate intracellular potency ?

Change concentration !

- intracellular potency
- accumulation in lysosomes

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



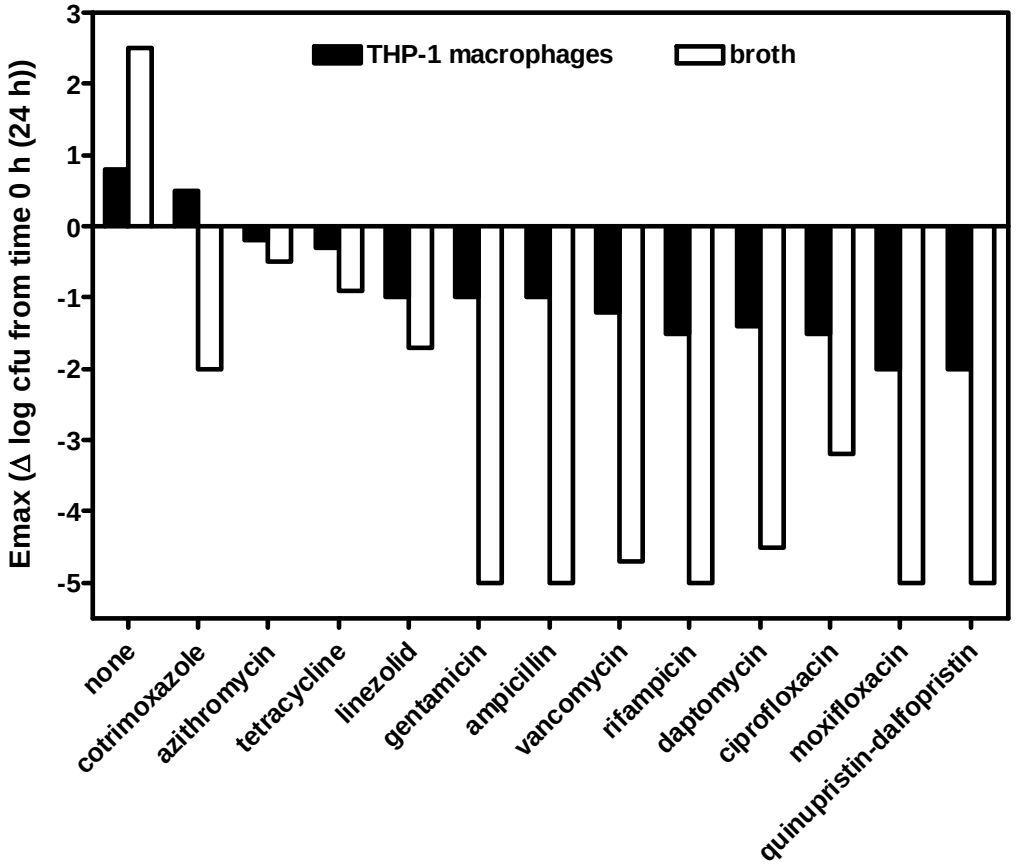


Seral et al., J. Antimicrob. Chemother. (2003) 51:1167-73

What do these models tell us ?

comparison : 1 model ~ different drugs

THP-1 ; *S. aureus*

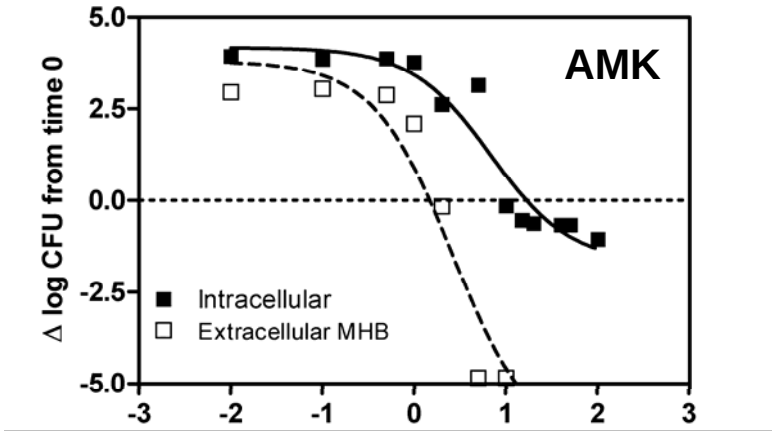


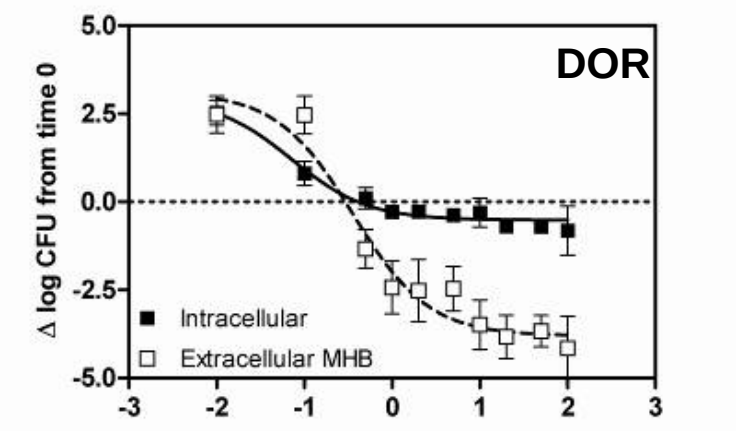
- Emax lower intracellularly
  highly variable depending on the drug

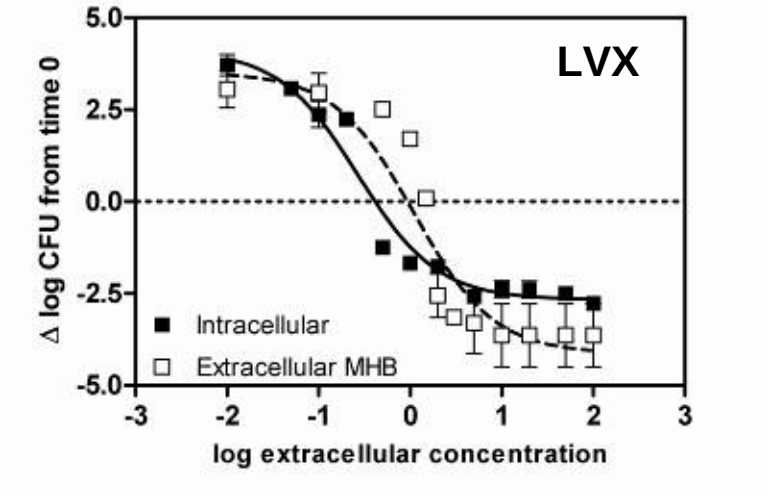
How to modulate intracellular efficacy ?

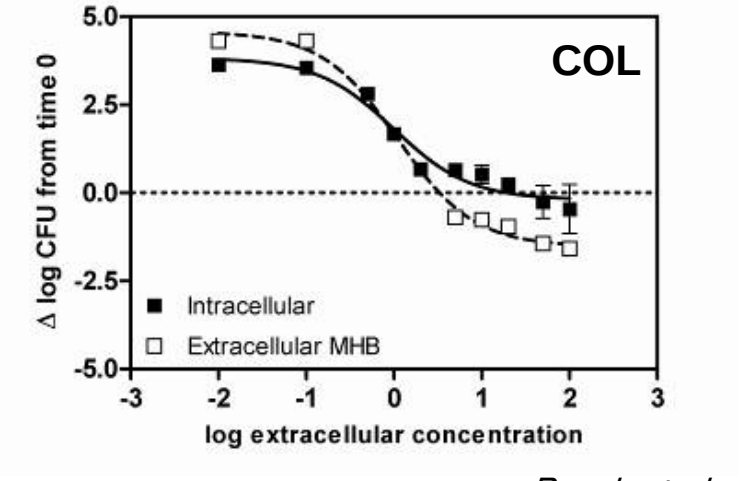
comparison : 1 model ~ different drugs : does this apply to other bugs ?

THP-1 ; *P.aeruginosa*









Buyck et al, unpublished

How to modulate intracellular efficacy ?

comparison : 1 model ~ different drugs

THP-1 ; *P.aeruginosa*

	MIC	Extracellular			Intracellular		
	(mg/L)	E_{max}	C_{static} (x MIC)	R^2	E_{max}	C_{static} (X MIC)	R^2
Amikacin	1	-6.81 ± 0.55	1.49	0.96	-1.7 ± 0.58	16.65	0.93
Doripenem	1	-3.80 ± 0.31	0.30	0.81	-0.51 ± 0.13	0.41	0.87
Levofloxacin	0.5	-4.13 ± 0.43	1.82	0.85	-2.67 ± 0.16	0.8	0.97
Colistin	2	-1.52 ± 0.18	1.64	0.99	-0.20 ± 0.17	9.48	0.93

- intracellular Cs close or higher than the MIC depending on the drug
- Emax much lower intracellularly than extracellularly
  highly variable among drugs

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

- intracellular drug efficacy

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

Use of these models for drug discovery/development

"First of all, I'd like to thank the bacteria..."

Use of these models for drug discovery/development

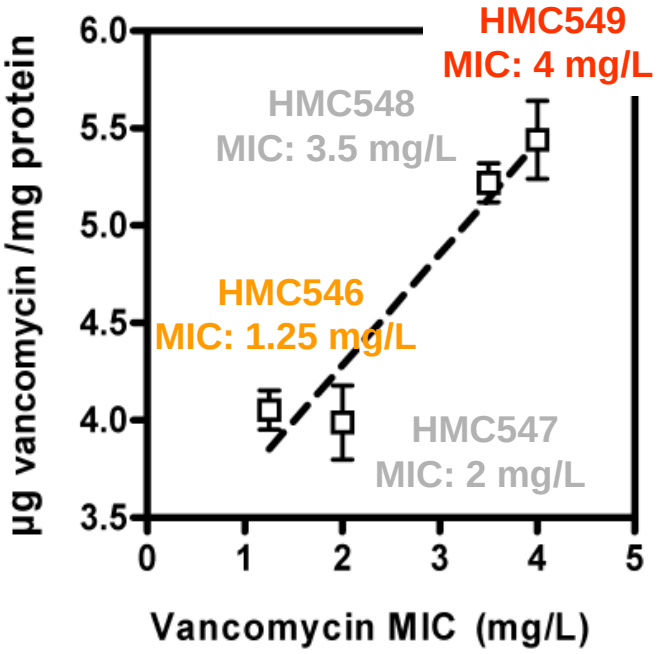
- to explore reasons for therapeutic failures
- to suggest new therapeutic alternatives
- to position new molecules
- to evaluate new strategies

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

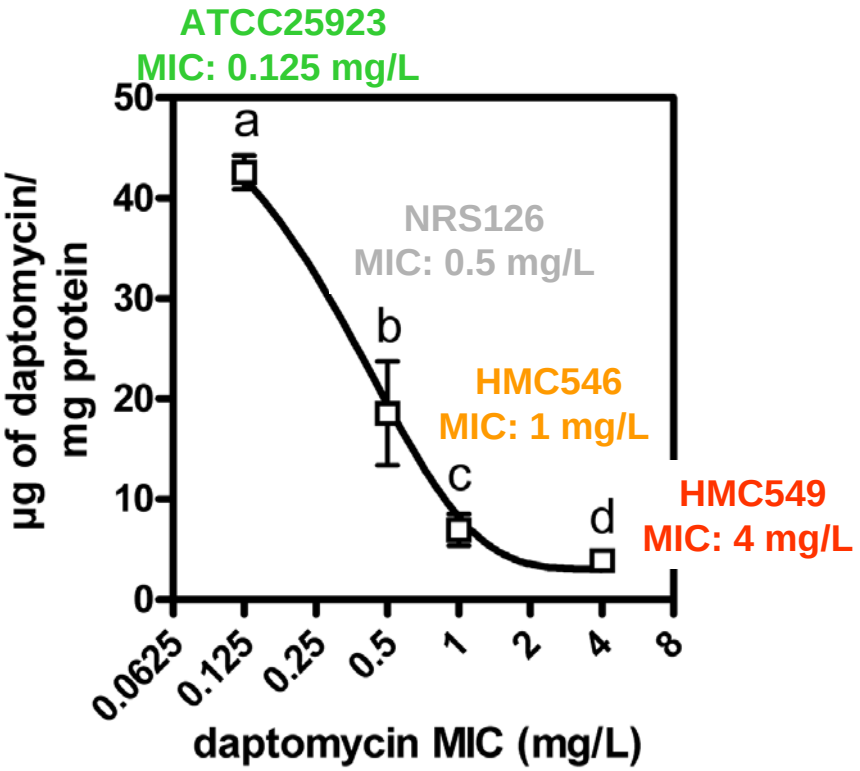
Julian et al. AAC (2007) 51:3445-8.

Reduced susceptibility associated with

increased amount of bound vancomycin



decreased amount of bound daptomycin

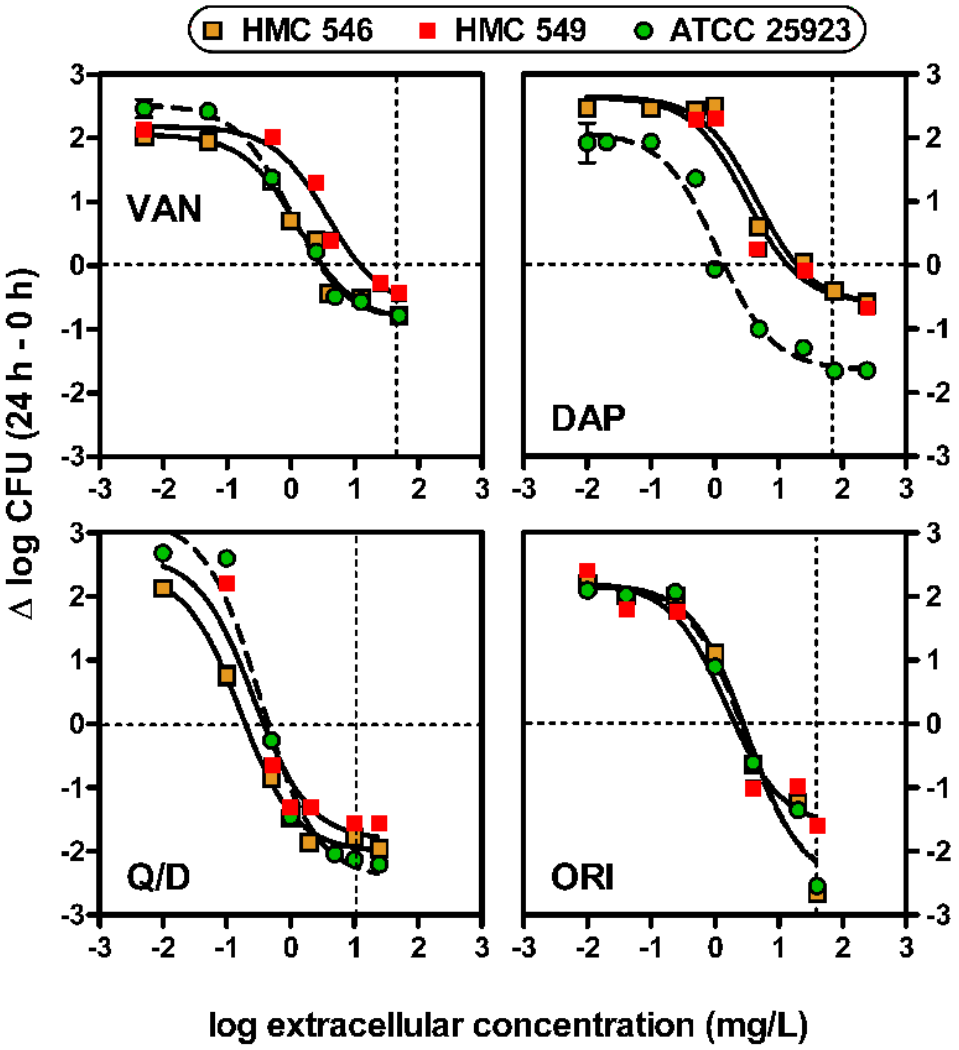


Lemaire et al., CMI (2008) 14:766-77

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

higher intracellular EC_{50}

no effect of resistance phenotype



higher intracellular EC_{50}

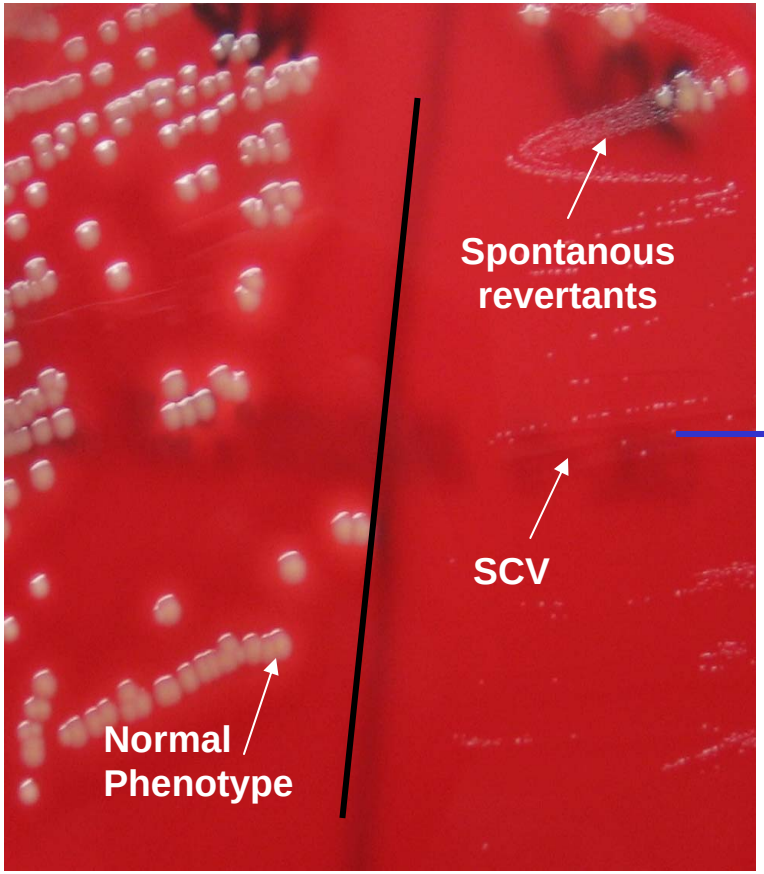
lower intracellular Emax

no effect of resistance phenotype

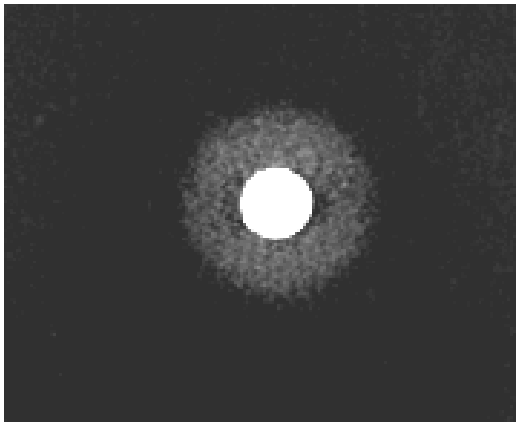
Lemaire et al., CMI (2008) 14:766-77

SCV isolated from a cystic fibrosis patient

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

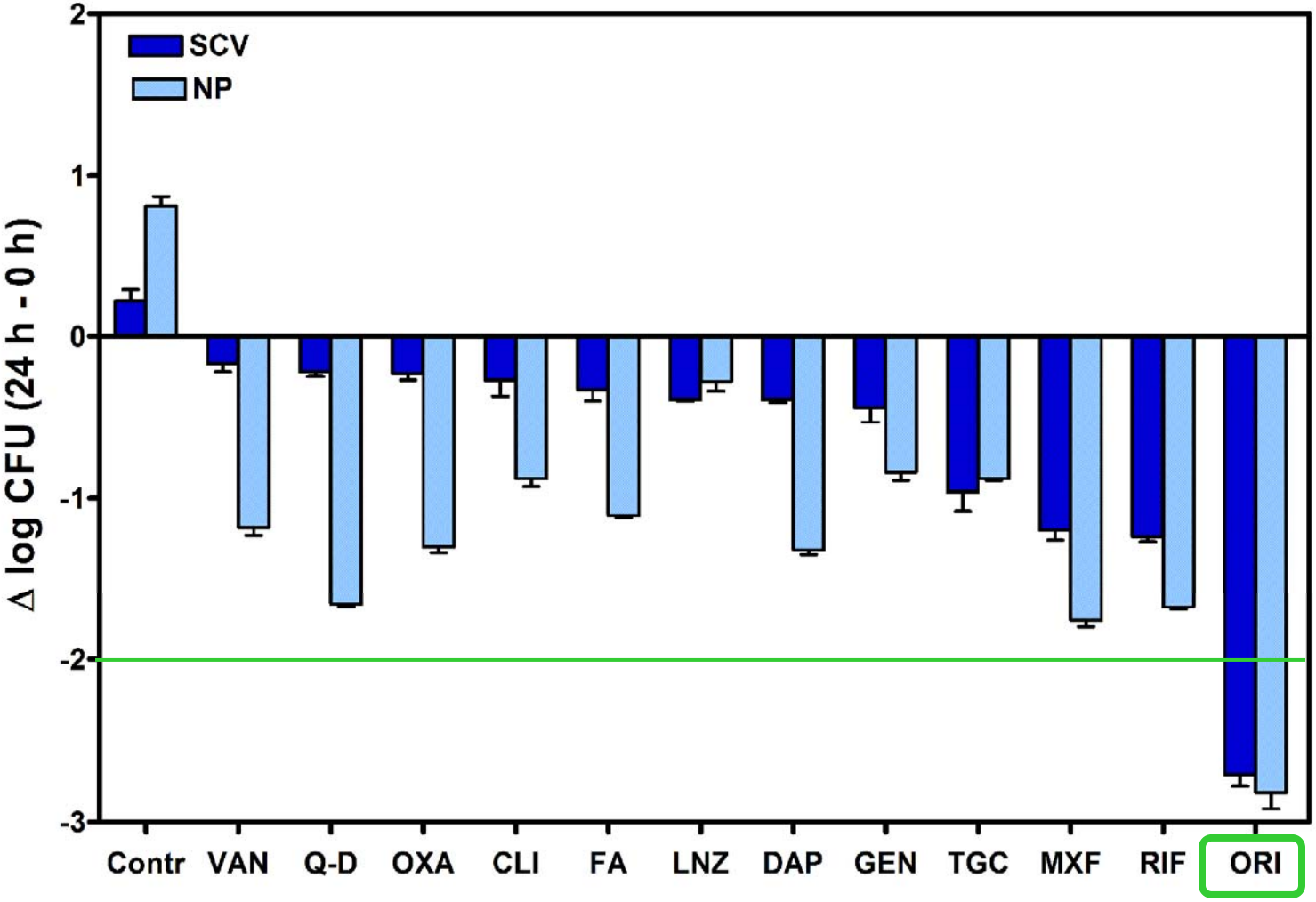


Thymidine dependent



Intracellular activity, SCV *vs*. normal phenotype

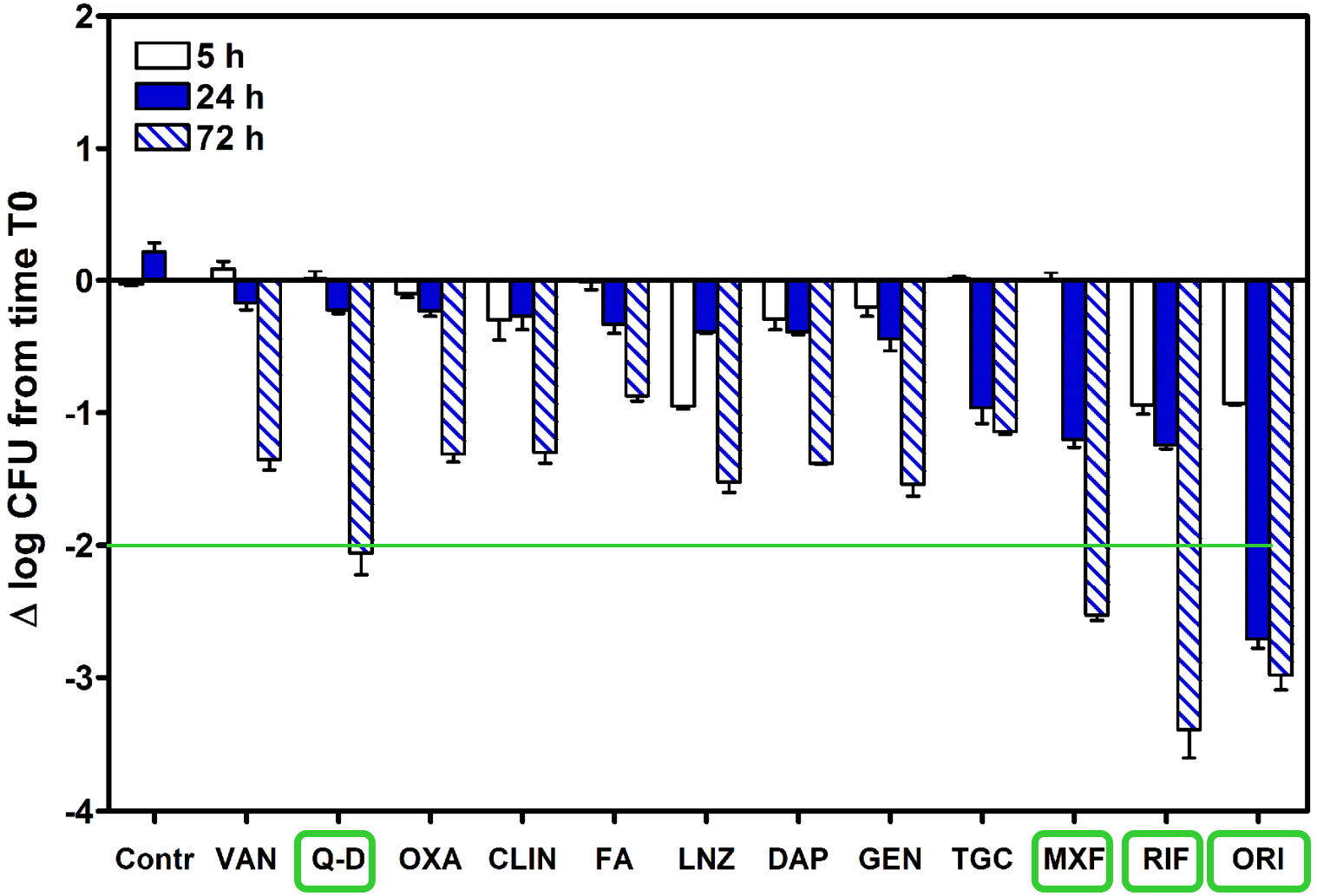
THP-1; 24 h, antibiotics at Cmax



Nguyen et al, AAC (2009) 53:1434–42

Intracellular activity, SCV over time

THP-1; SCV, antibiotics at Cmax for up to 3 days



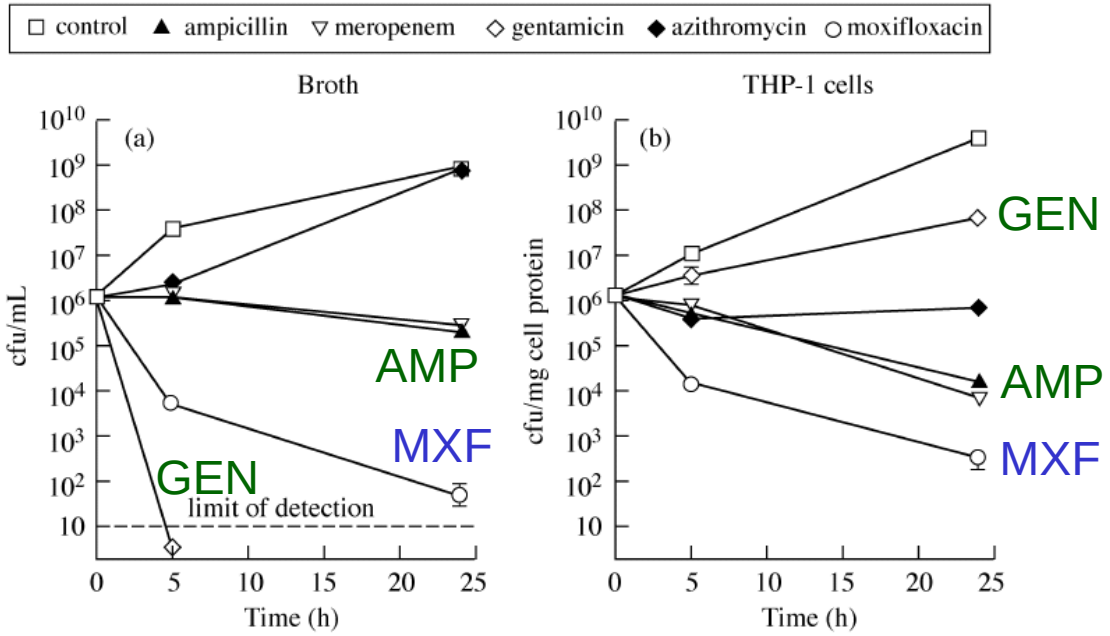
Nguyen et al, AAC (2009) 53:1434–42

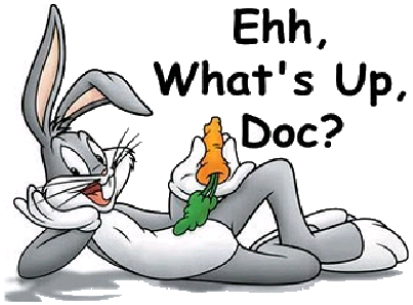
Use of these models for drug discovery/development

- to explore reasons for therapeutic failures
- to suggest new therapeutic alternatives
- to position new molecules
- to evaluate new strategies

Use of these models for drug discovery/development

Listeria in vitro





Listeria in vivo

MXF
GEN+AMP

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.

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

Use of these models for drug discovery/development

- to explore reasons for therapeutic failures
- to suggest new therapeutic alternatives
- to position new molecules
- to evaluate new strategies

Use of these models to position new molecules

Guidance for Industry

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

DRAFT GUIDANCE

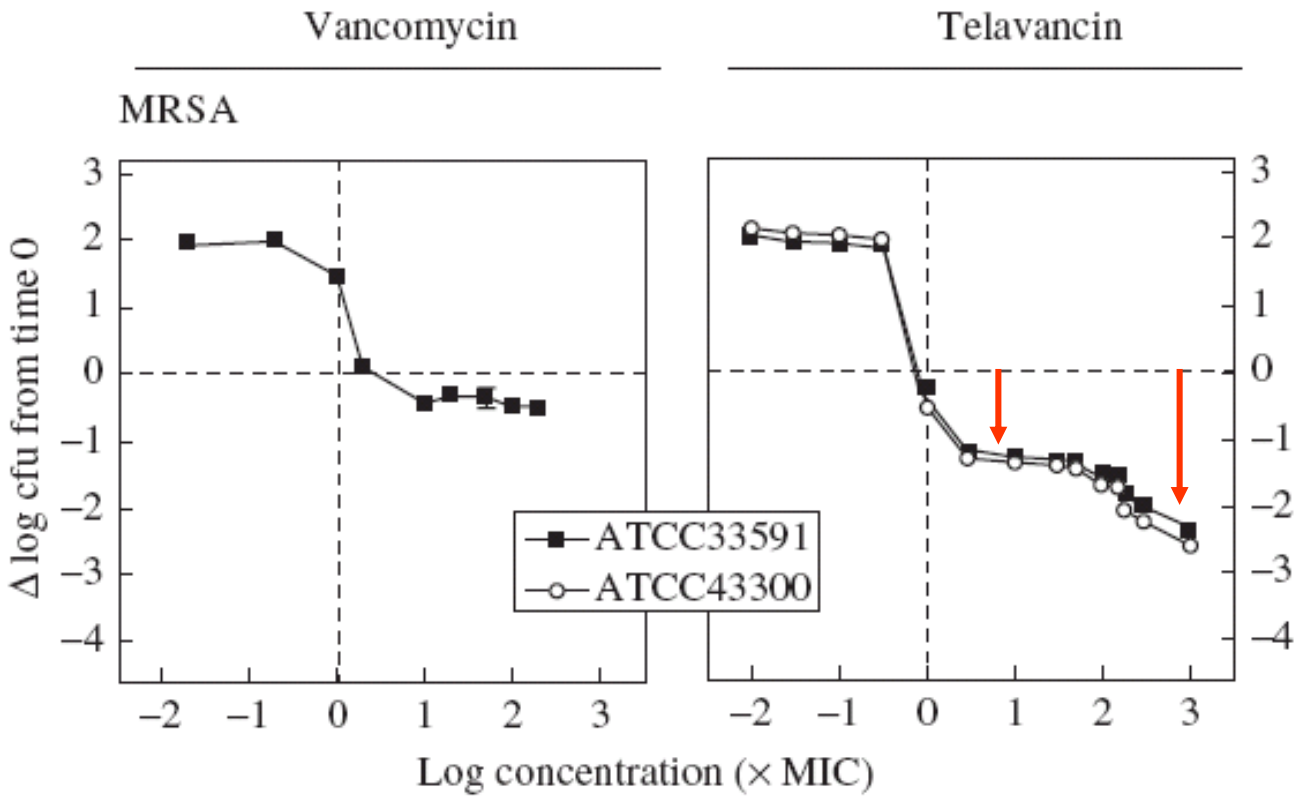
U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

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.

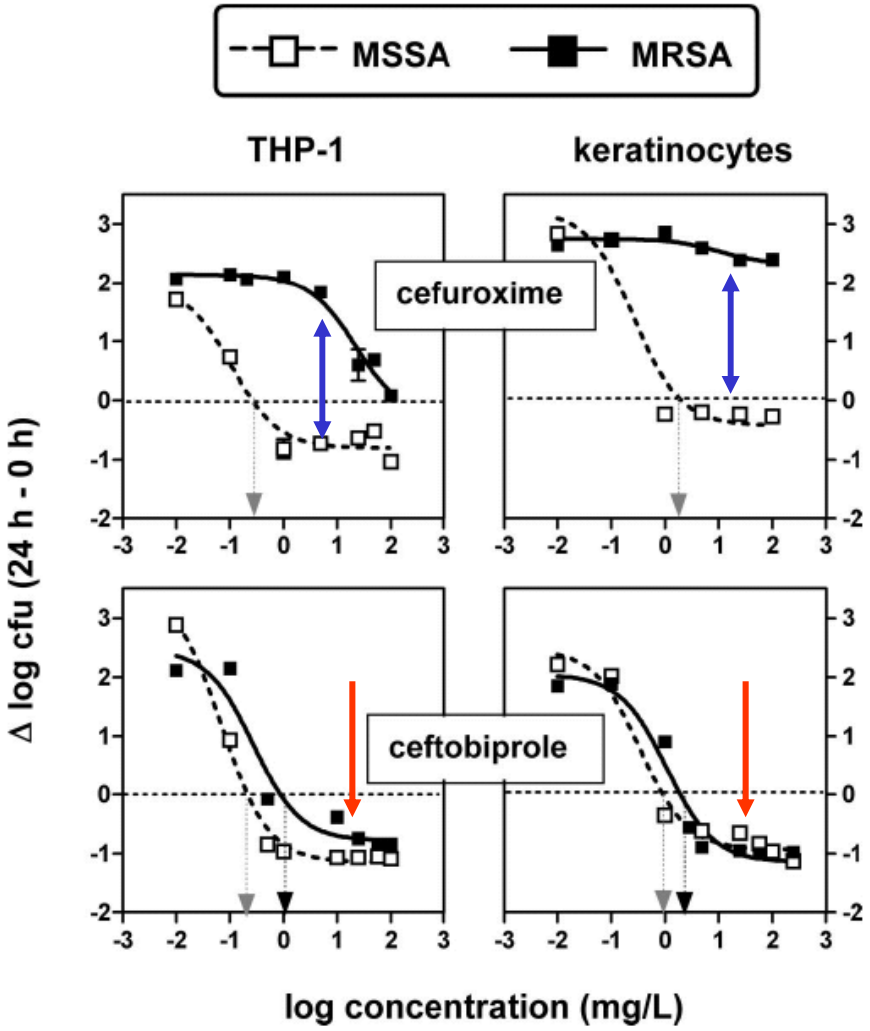
Use of these models to position new molecules



Telavancin shows bimodal effects and improved efficacy as compared to vancomycin probably related to dual mode of action

Barcia-Macay et al, JAC (2006) 58:1177–84

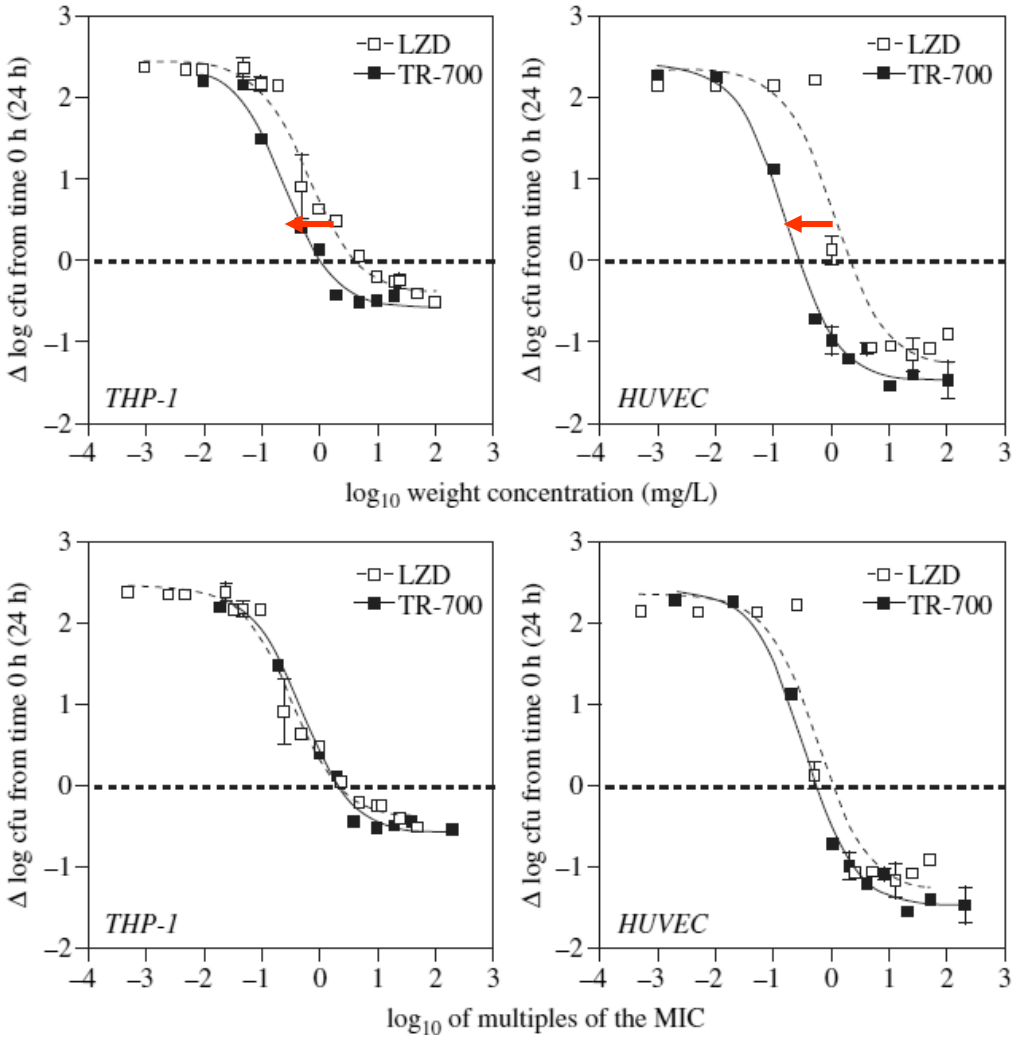
Use of these models to position new molecules

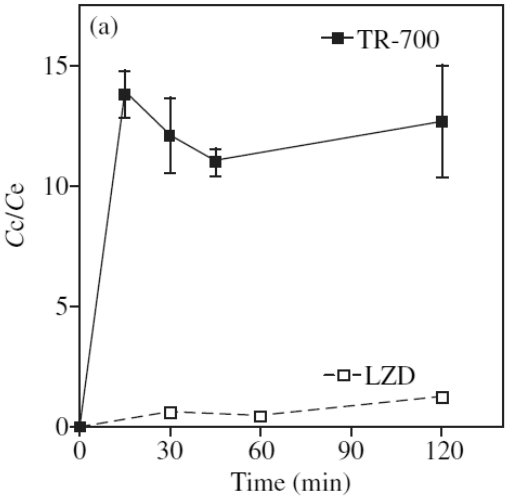


Ceftobiprole proves as efficient against MSSA than MRSA
due to improved interaction with PBP2a

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

Use of these models to position new molecules

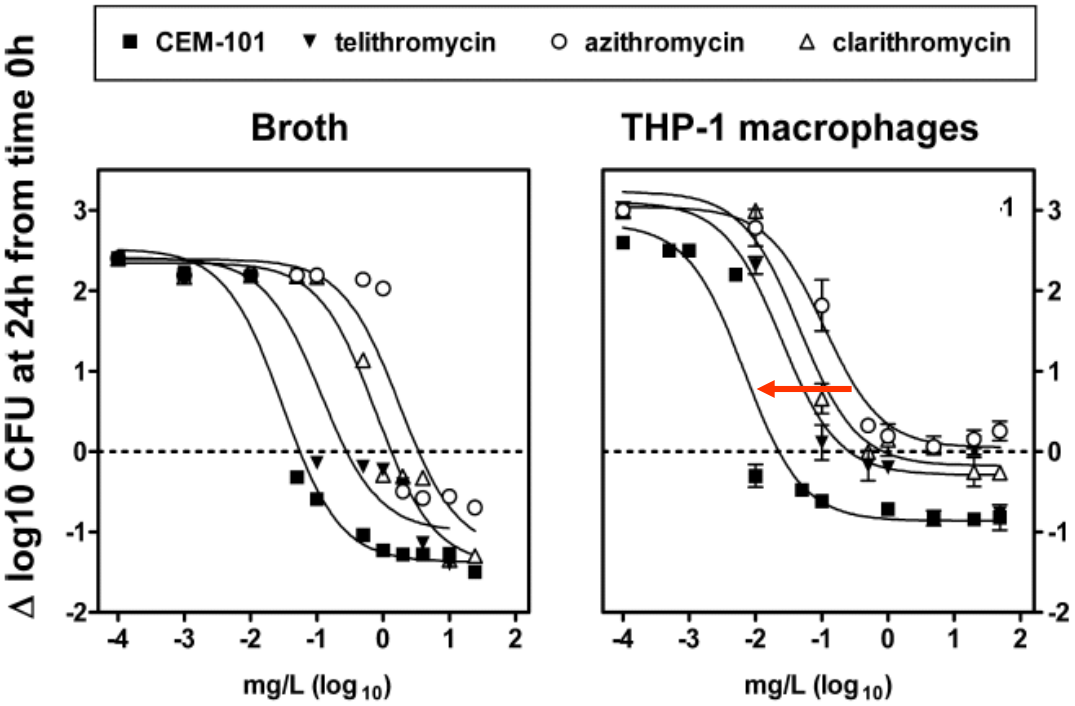


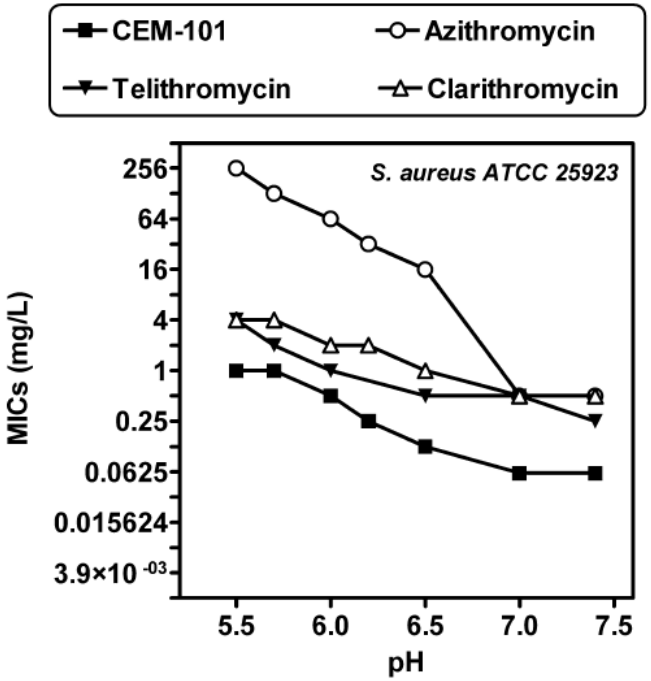


Torezolid shows improved potency relative to linezolid
probably rather related to lower MIC than to higher accumulation

Lemaire et al, JAC (2009) 64:1035-43

Use of these models to position new molecules

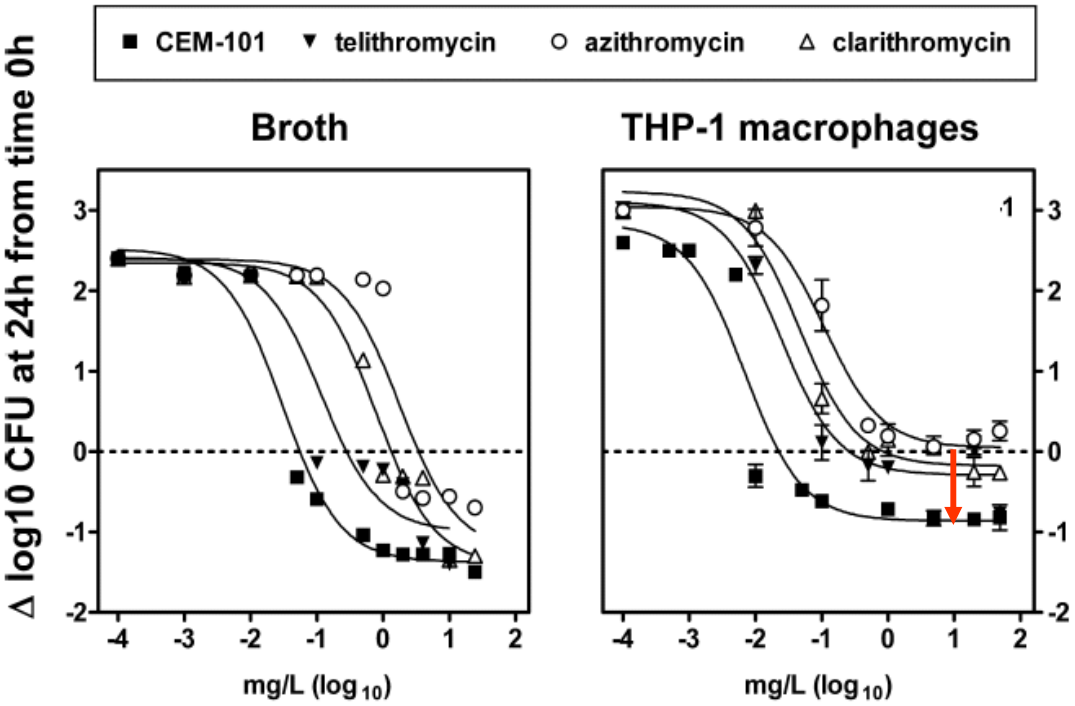


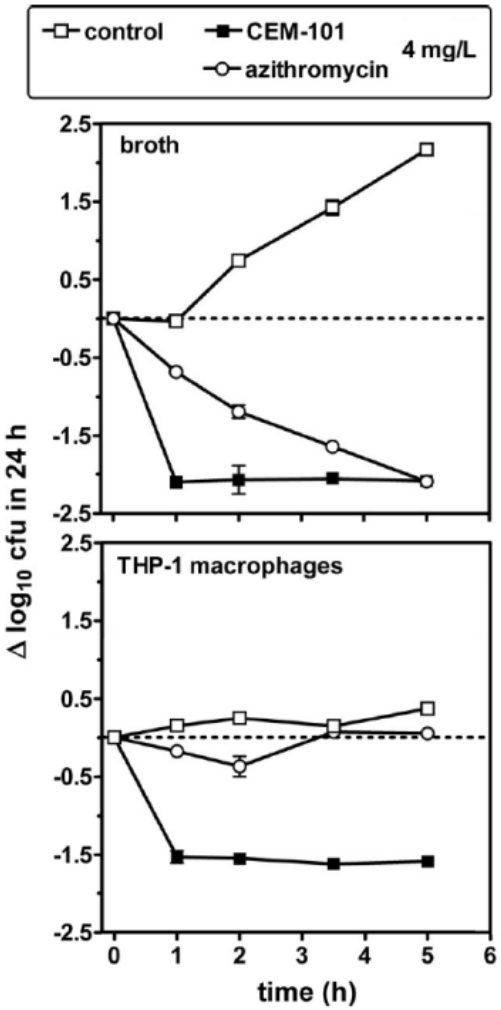


CEM-101 shows higher potency relative to azithromycin, as it keeps activity in acidic environments

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

Use of these models to position new molecules





CEM-101 shows higher efficacy relative to azithromycin, as it causes much rapid decrease in bacterial load even in broth

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

Use of these models for drug discovery/development

- to explore reasons for therapeutic failures
- to suggest new therapeutic alternatives
- to position new molecules
- to evaluate new strategies

Activity of combinations against intracellular SCV

Fractional maximal effect (FME) approach

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

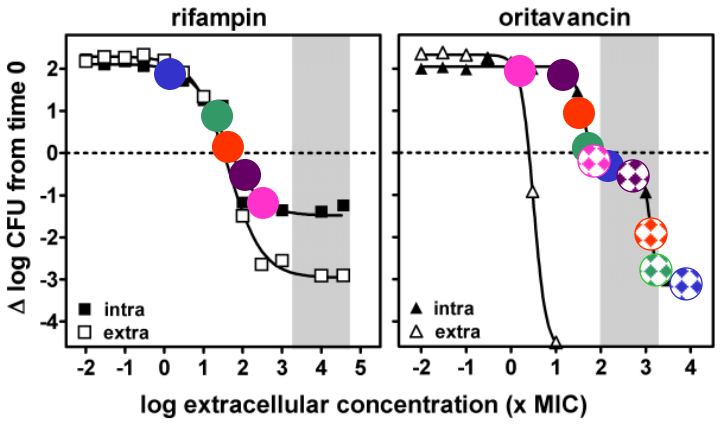
Effect (E): decrease of inoculum after 24 h. Sigmoid E_{max} model $\Rightarrow$ E_{max}, EC_{50}

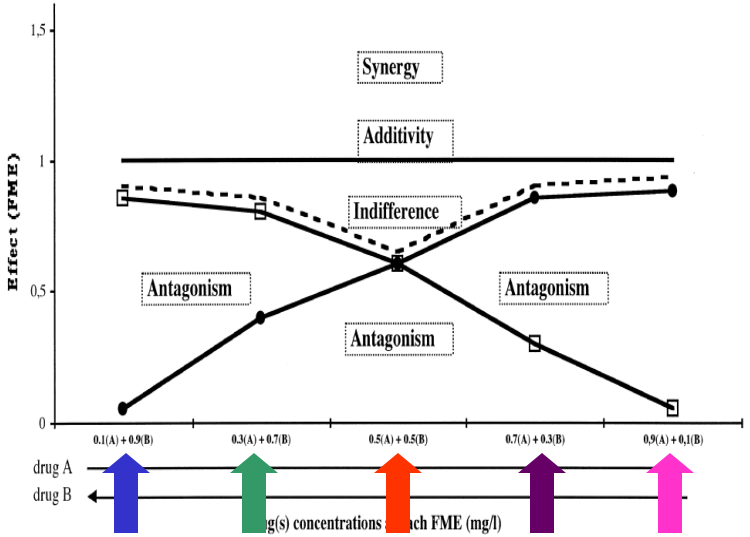
$$E = \frac{E_{max} \cdot C^n}{EC_{50}^n + C^n}$$

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:

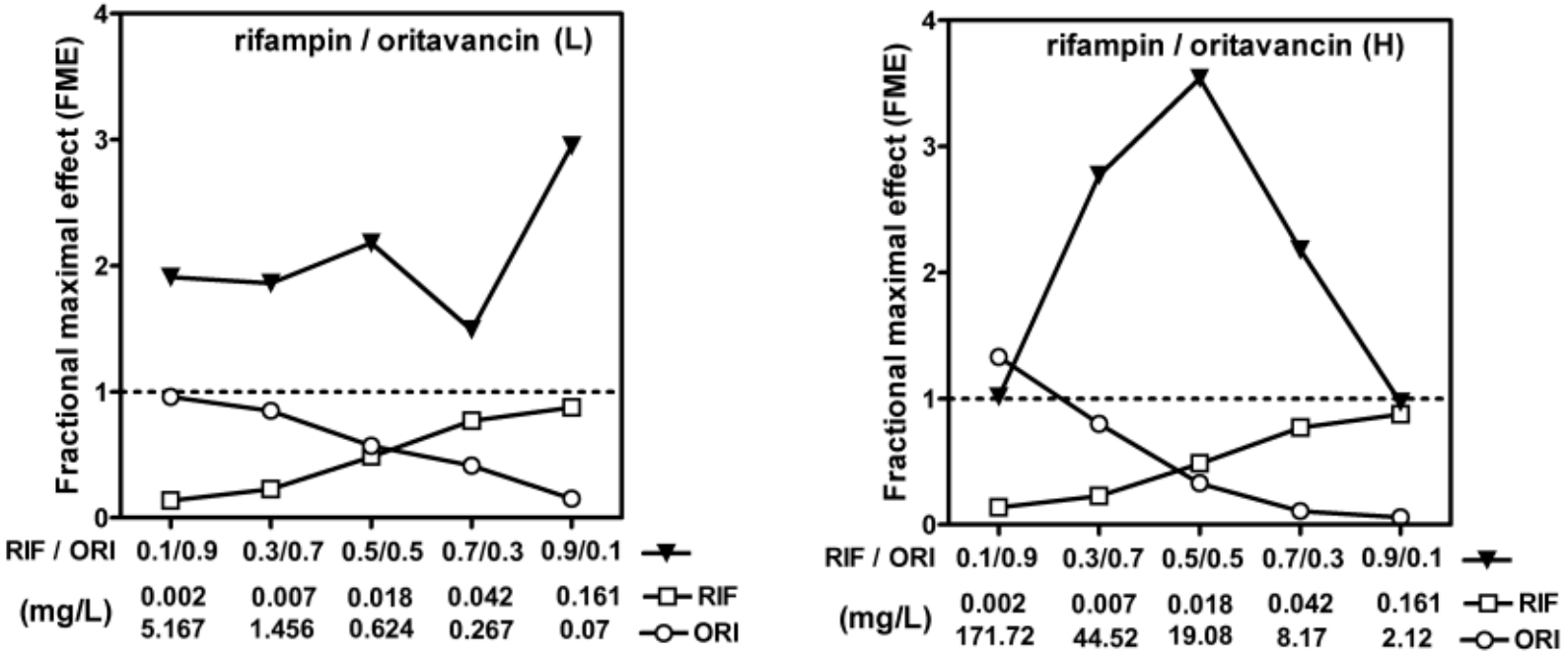




Desbiolles et al, Antimicrob. Agents Chemother. (2001) 45: 3328-33

Activity of RIF-ORI combination against intracellular SCV

Fractional maximal effect (FME) approach



FME > 1 : synergistic; = 1: additive

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

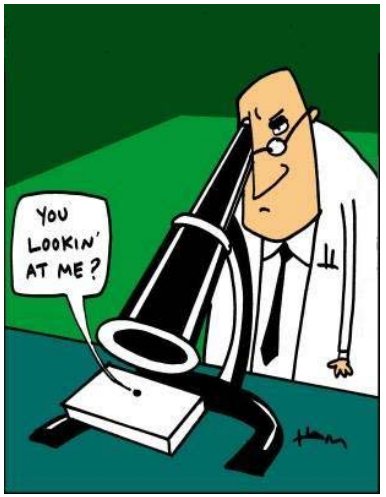
Nguyen et al, AAC (2009) 53:1443-49

Take home message

Where is the drug ?

doesn't matter !

How is the drug working inside the cell ?



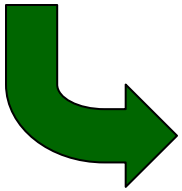
Where is the bug ?

doesn't matter !

How is the bug feeling inside the cell ?

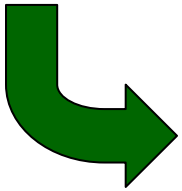
Take home message

Where is the drug ?



How is the drug working inside the cell ?

Where is the bug ?



How is the bug feeling inside the cell ?

How is the cell influencing PK/PD of antibiotics & bacterial response ?

Our intracellular PK/PD team over the years …


C. Seral


M. Barcia-Macay


S. Lemaire


H.A. Nguyen


A. Olivier


P. Baudoux

S. aureus


L. Garcia


Y. Ouadhriri

L. monocytogenes


S. Carryn


S. Vandevelde


A. Lismond


J. Buyck


G. De Laminne

P. aeruginosa

Have a safe trip back home …