Intra- and Extra- cellular Activities of Anti-Infective Agents

Françoise Van Bambeke

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

<www.facm.ucl.ac.be>

UCL Université catholique de Louvain

Anne Sandberg

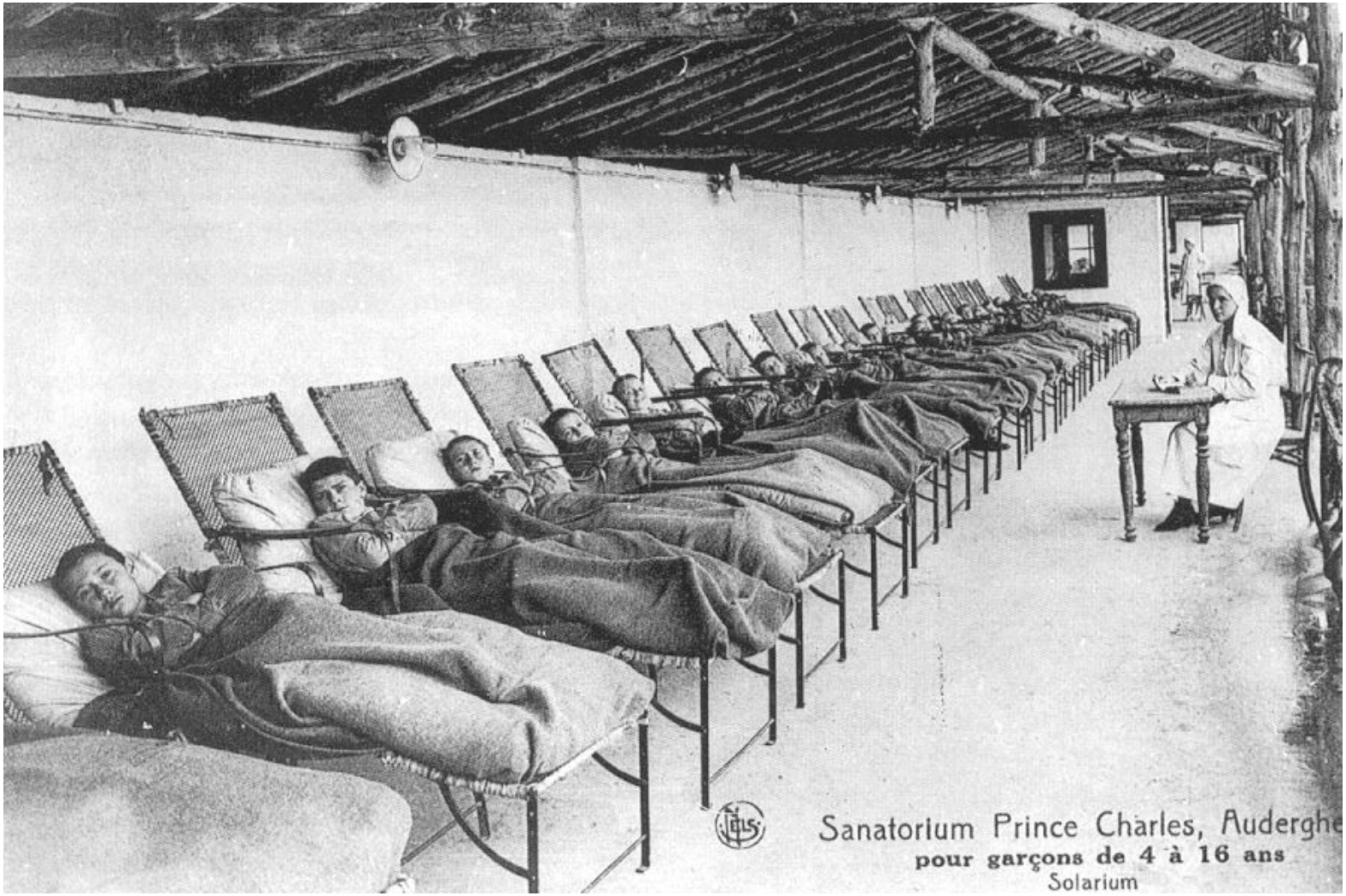
Dept. of Clinical Microbiology
Hvidovre Hospital

Copenhagen, Denmark

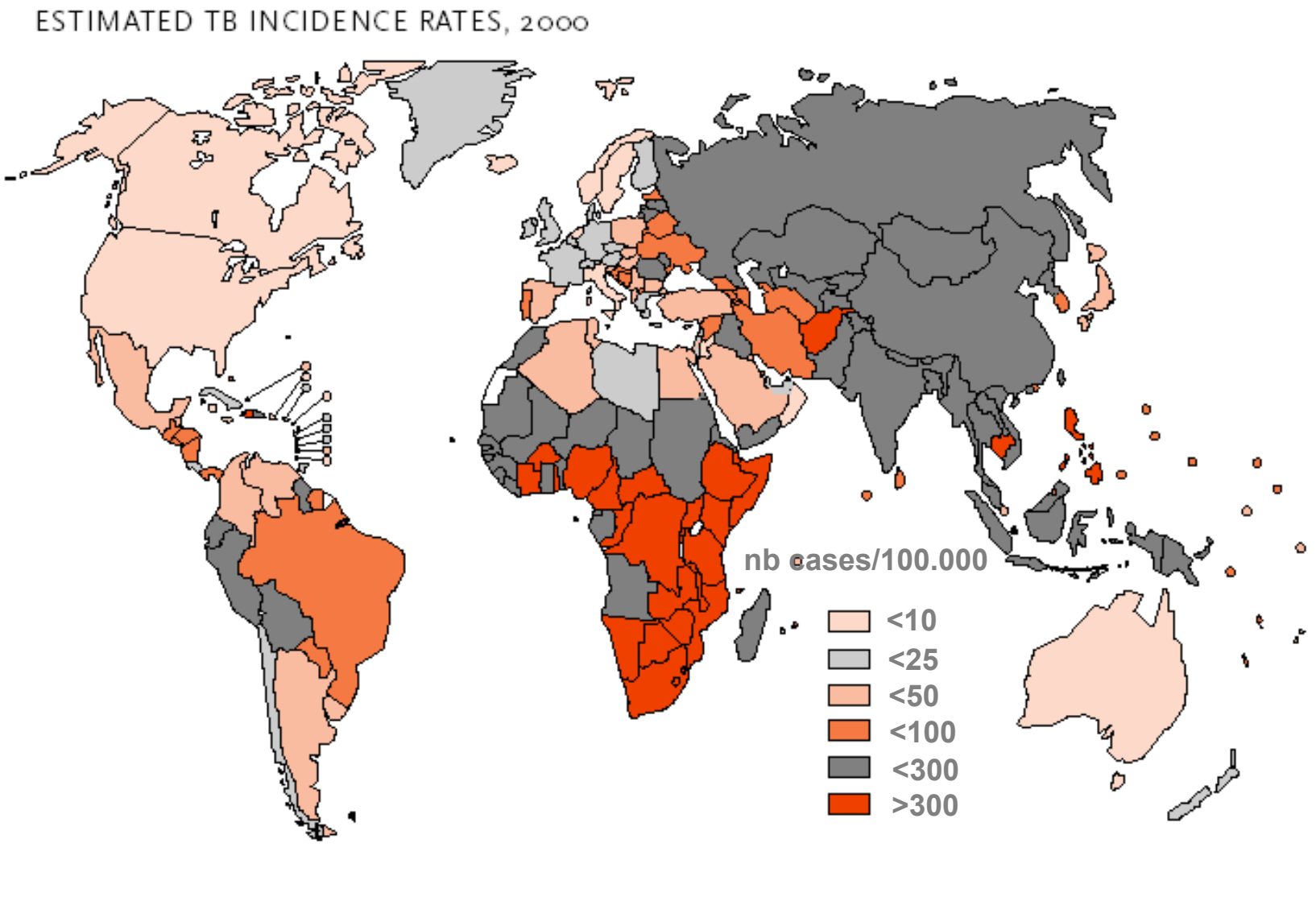
REGION H Hvidovre Hospital

Intracellular infection: do we need to take care of ?

Tuberculosis, an epidemy of the past …



…and of the present

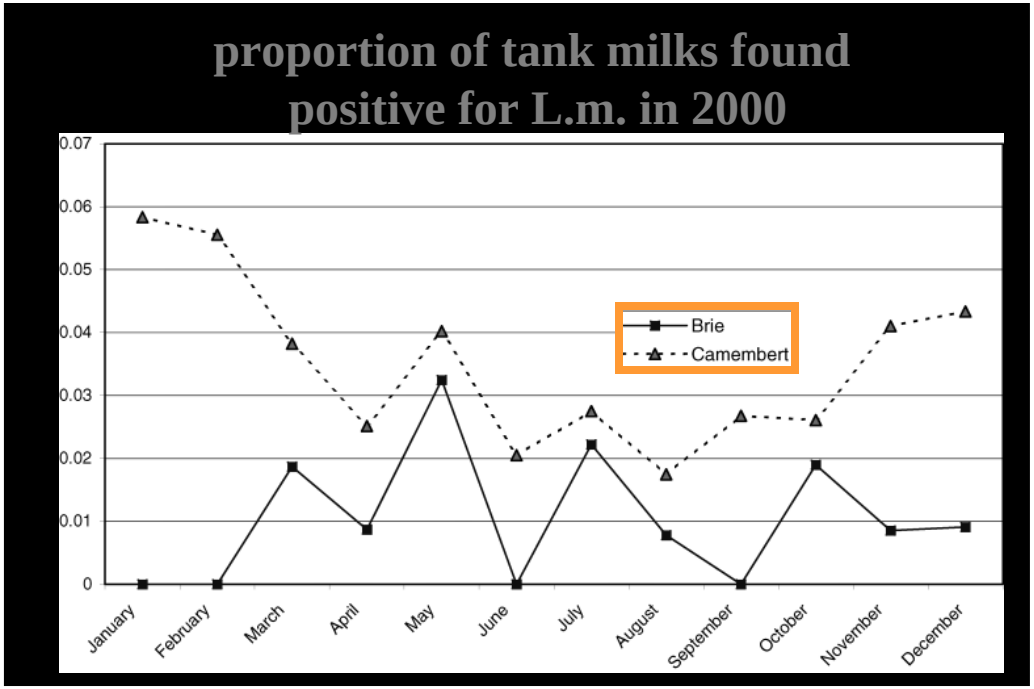


Intracellular infections in our daily life …

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]



Intracellular infections in our daily life …

Eurosurveillance, Volume 5, Issue 11, 01 November 2000

Outbreak report

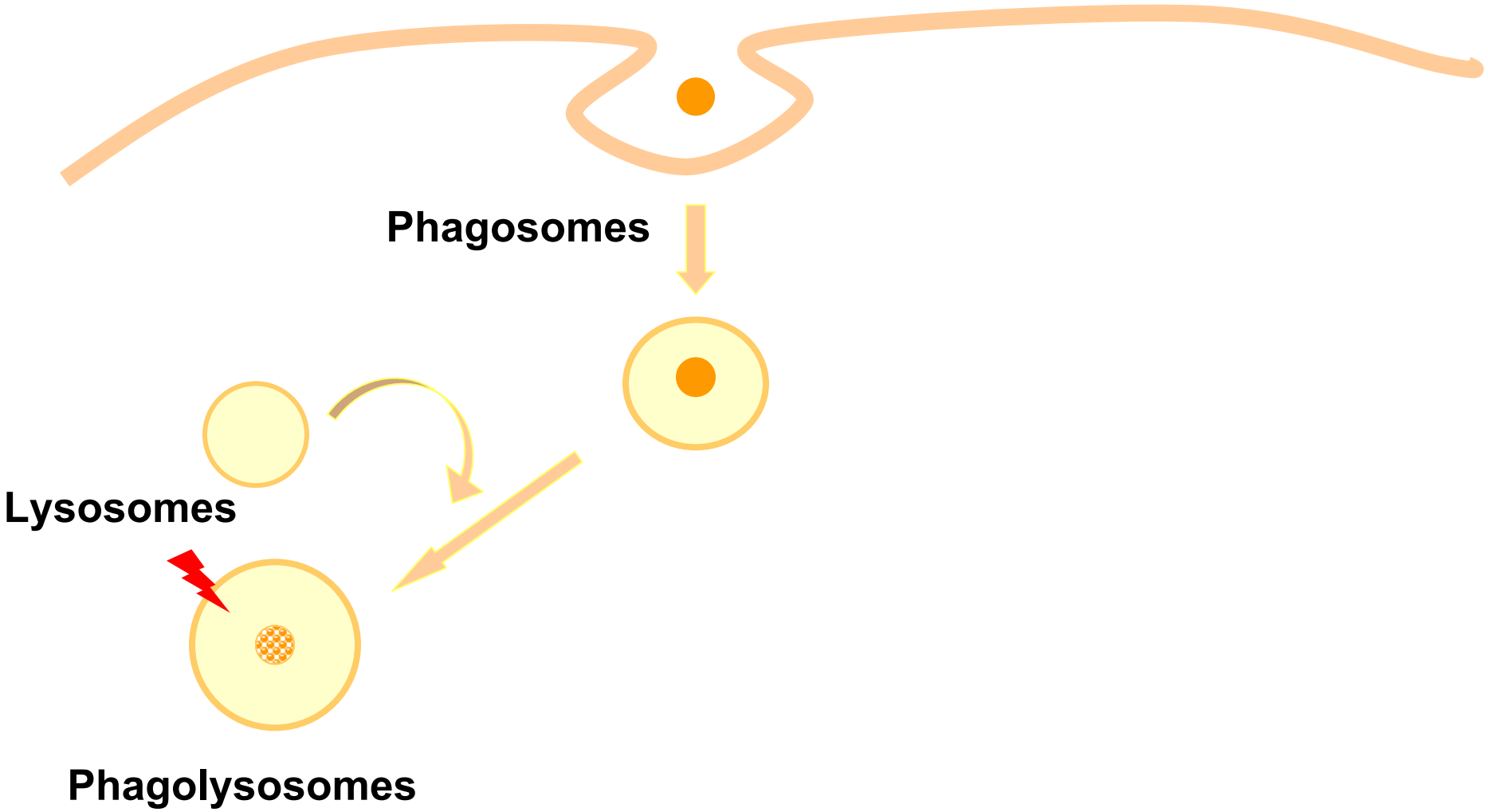
AN OUTBREAK OF LEGIONNAIRE'S DISEASE AMONG VISITORS TO A FAIR IN BELGIUM, 1999

Citation style for this article: De Schrijver K, van Bouwel E, Mortelmans L, van Rossom P, De Beukelaer T, Vael C, Dirven K, Goossens H, Leven M, Ronveaux O. An outbreak of legionnaire's disease among visitors to a fair in Belgium, 1999. Euro Surveill. 2000;5(11):pii=7. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=7

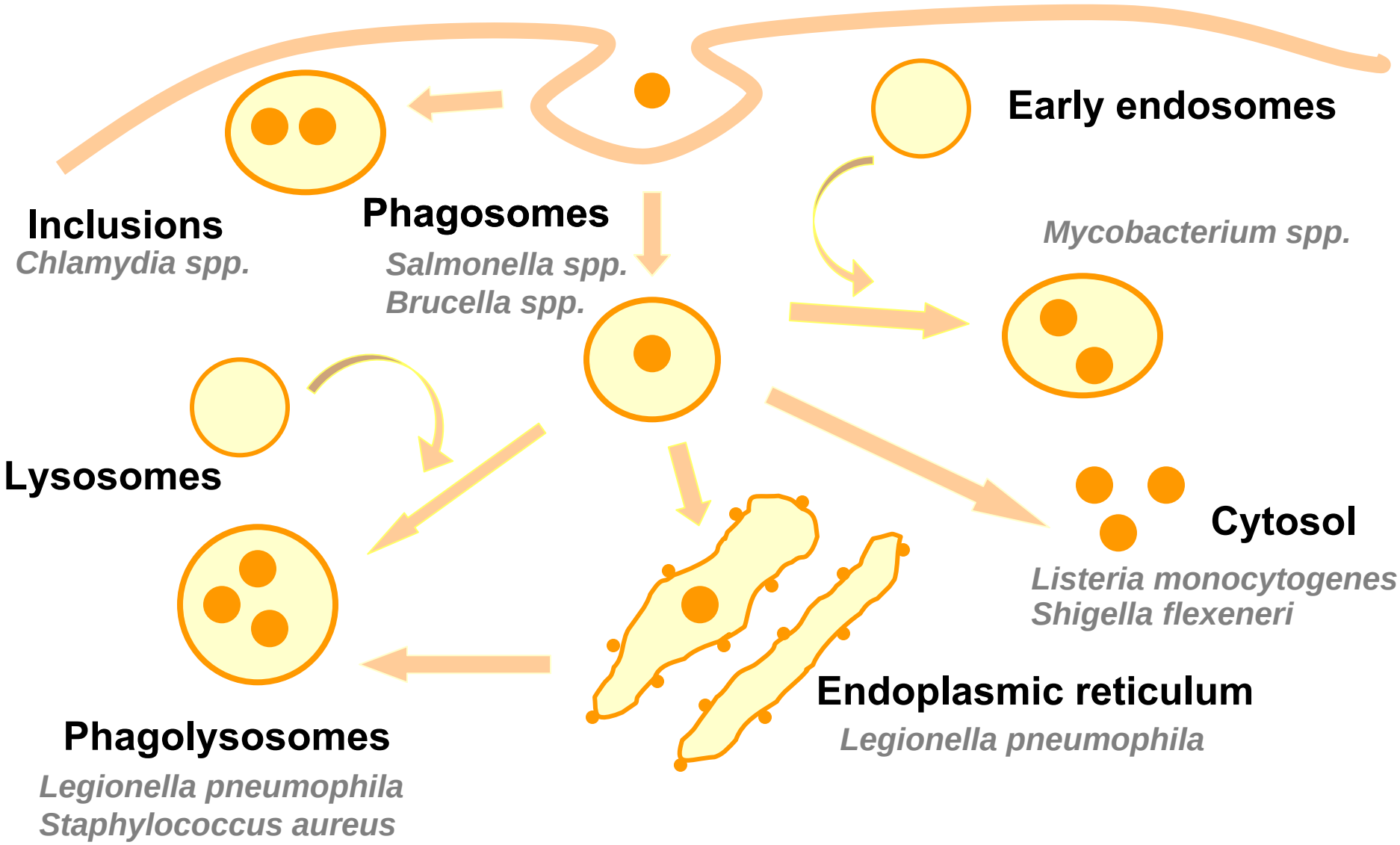
K. De Schrijver[1], E. Van Bouwel[2], L. Mortelmans[3], P. Van Rossom[4], T. De Beukelaer[5], C. Vael[6], K. Dirven[7], H. Goossens[7], M. Leven[7], O. Ronveaux[8]

Ninety-three cases of legionnaires' disease (43 confirmed, 12 presumptive, and 38 possible/clinical) were identified in an outbreak associated with a trade fair in Kapellen, Belgium in November 1999. Five cases died. Epidemiological investigation showed that the length of time spent at the fair and exposure to particular areas of the tent were associated with illness. Polymerase chain reaction tests showed that a whirlpool and a fountain were contaminated with legionella.

Intracellular killing of bacteria by host cell defence mechanisms

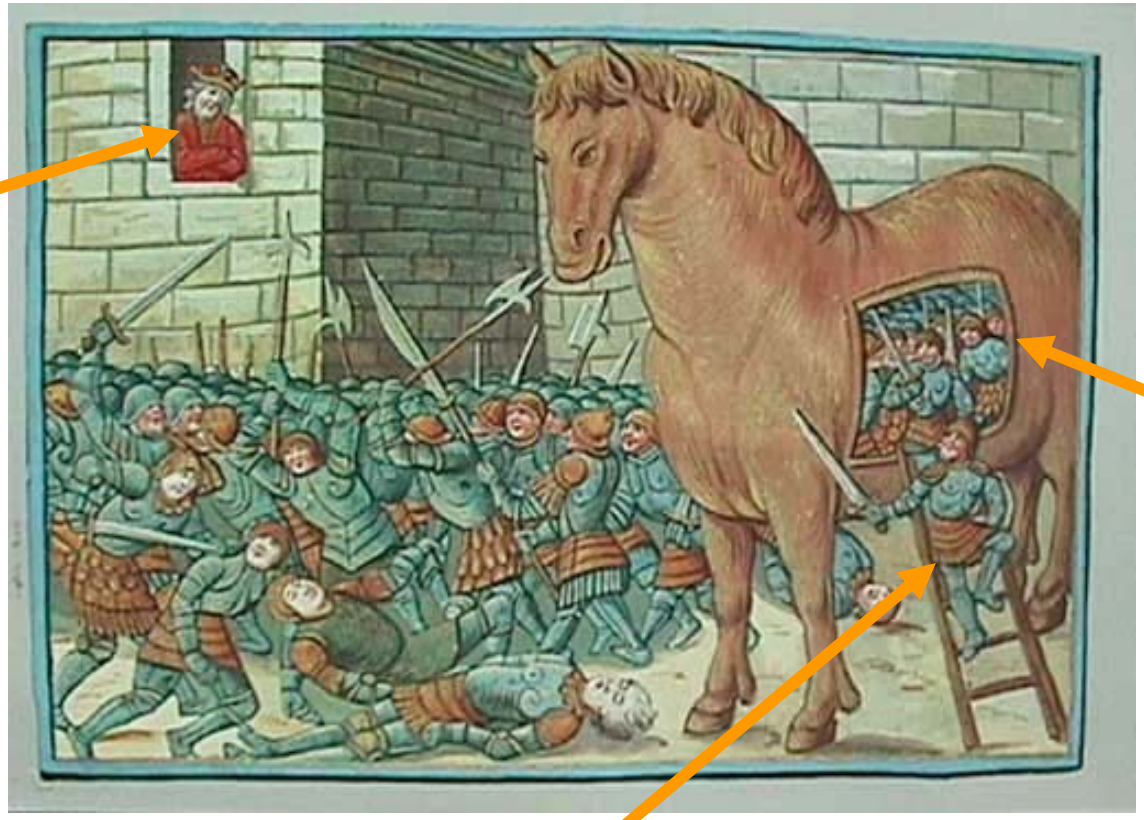


Some bacteria can escape host cell defence mechanisms …



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

Benefits of intracellular life

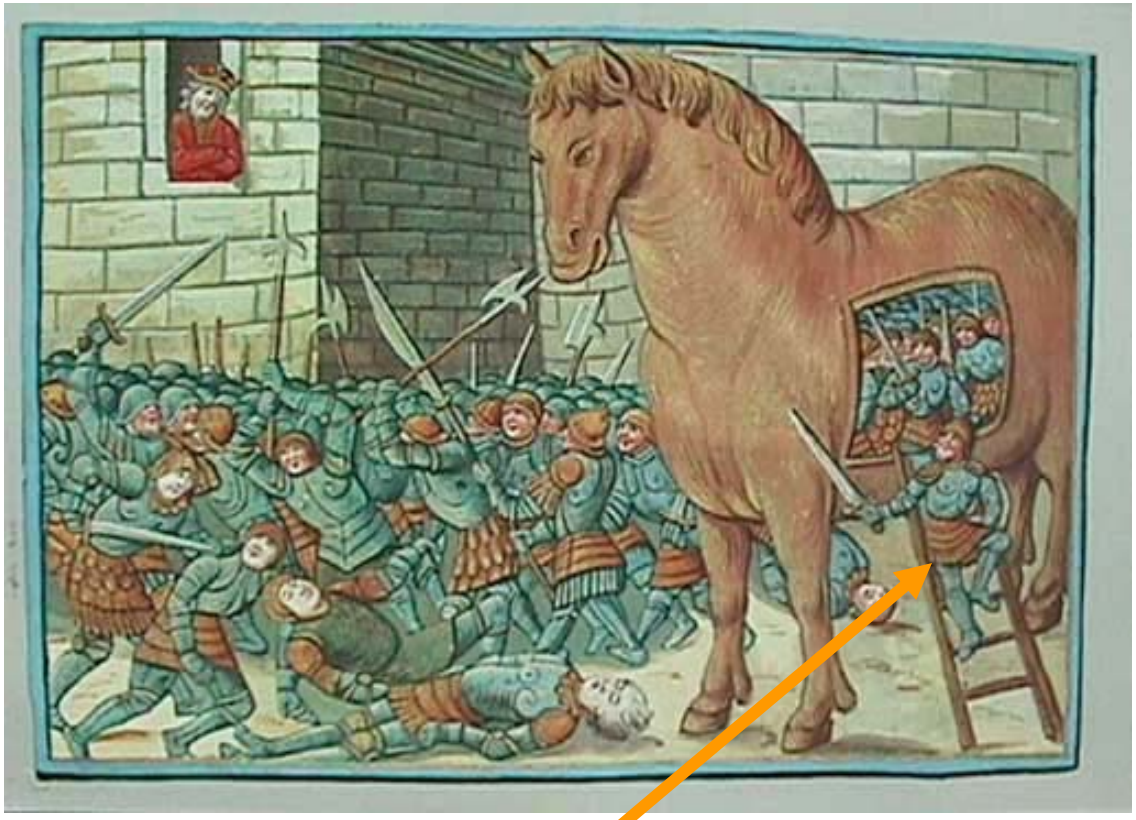


protection

persistence

invasion

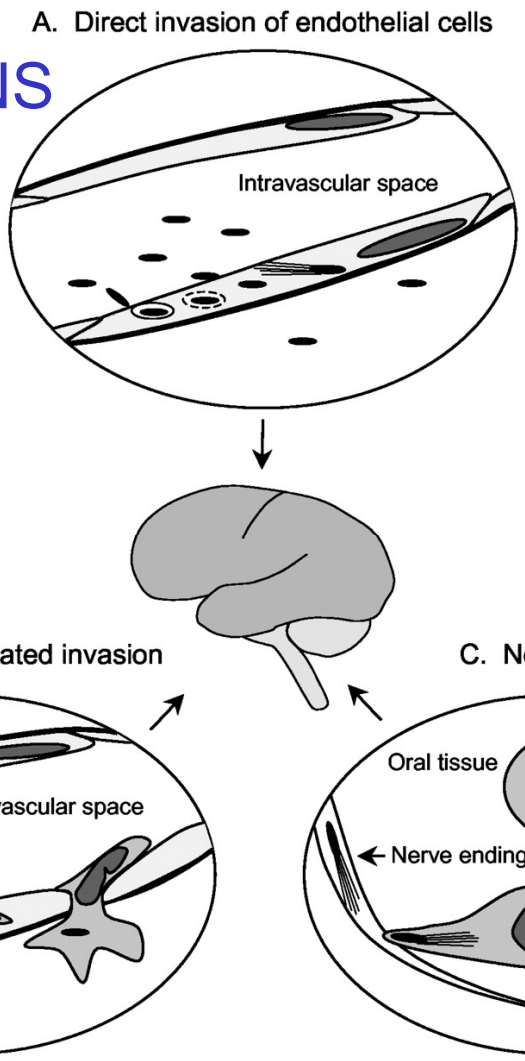
Benefits of intracellular life

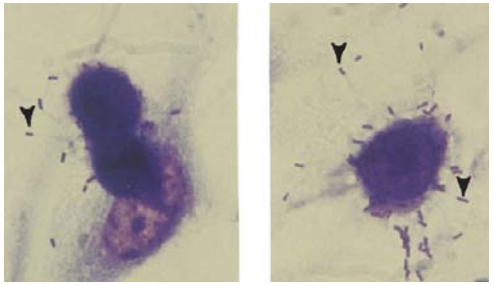


invasion

Migration to the CNS

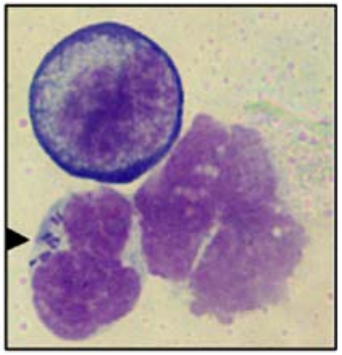
Listeria:
from the gut to the CNS

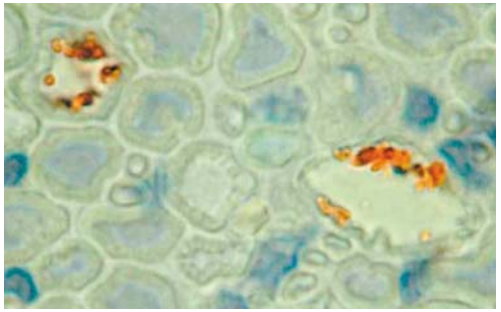




adherence and transfert
from monocytes to endothelial cells

bone-marrow monocyte

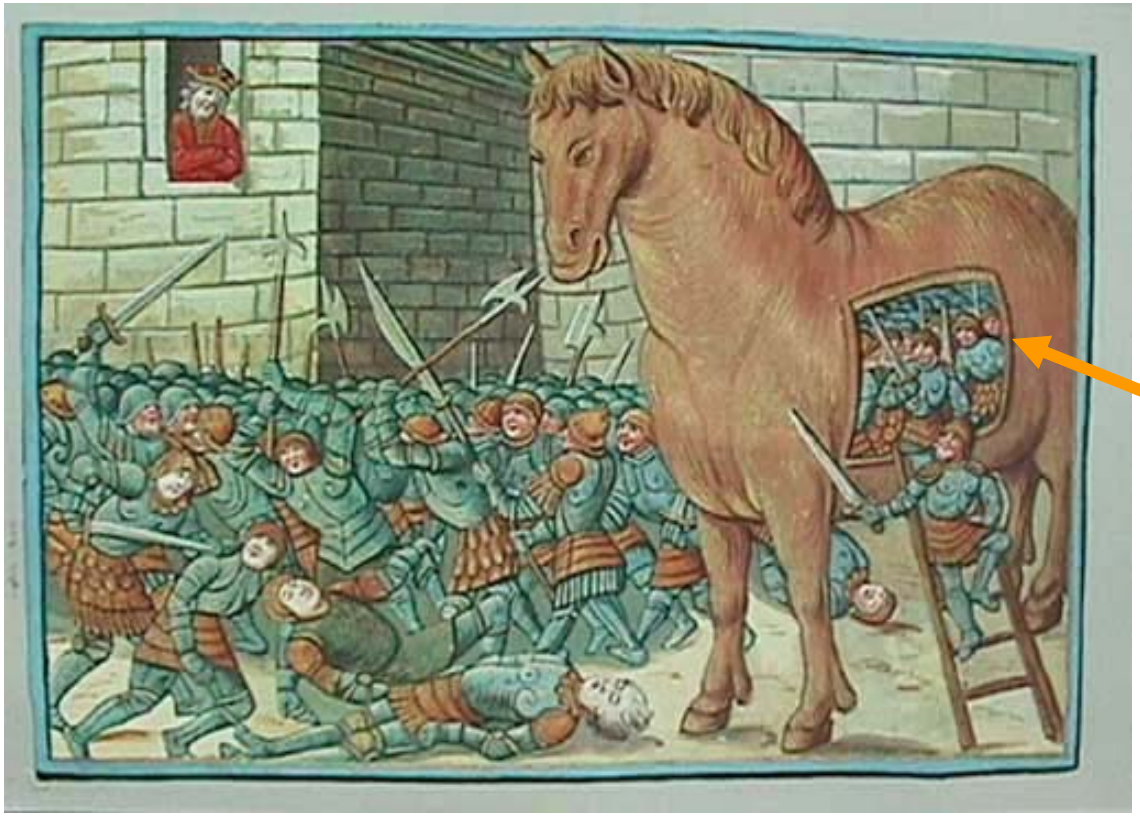




intra-axonal labeling
by anti-listeria antibodies

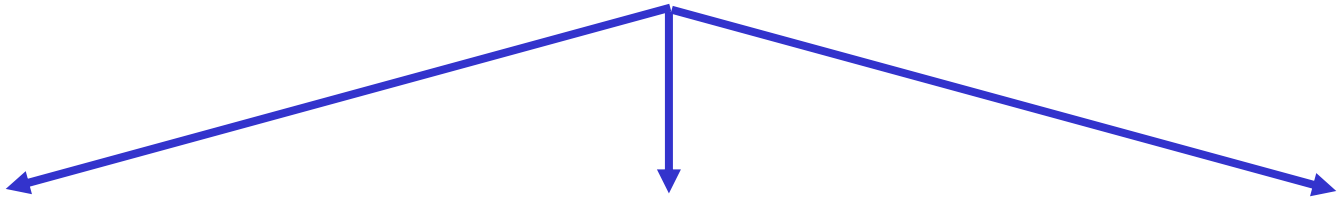
Antal et al., Brain Pathol. (2001) 11:432-8; Drevets & Bronze, FEMS Immunol Med Microbiol. (2008) 53:151-65
Drevets & Leenen, Microbes Infect. (2000) 2:1609-18; Drevets et al., Clin. Microb. Rev. (2004) 17:323-47

Benefits of intracellular life



persistence

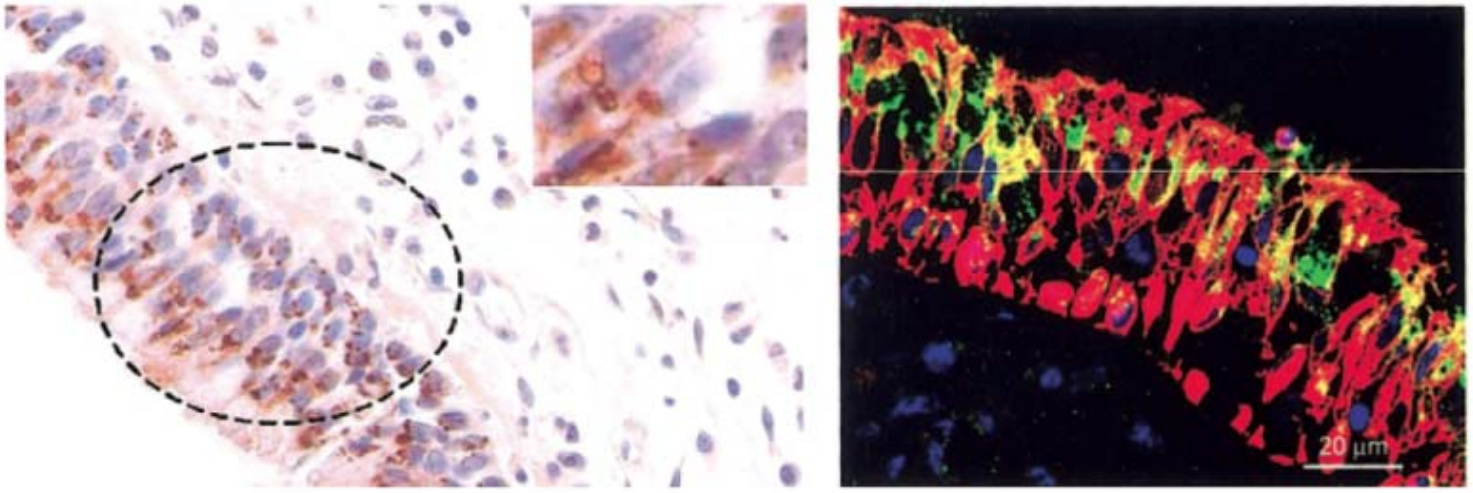
Different types of intracellular bacteria



facultative	obligate	opportunistic
Listeria	*Clamydia*	*Staphylococci*
Legionella	*Rickettsia*	*Streptococci*
Mycobacteria	*Coxiella*	*Pseudomonas*
Salmonella		
Brucella		
Yersinia		
…		

Intracellular survival and persistent infections

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

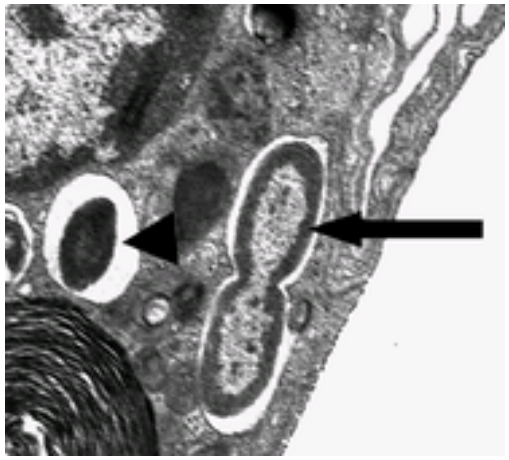


Clement et al., J Infect Dis. (2005) 192:1023-8

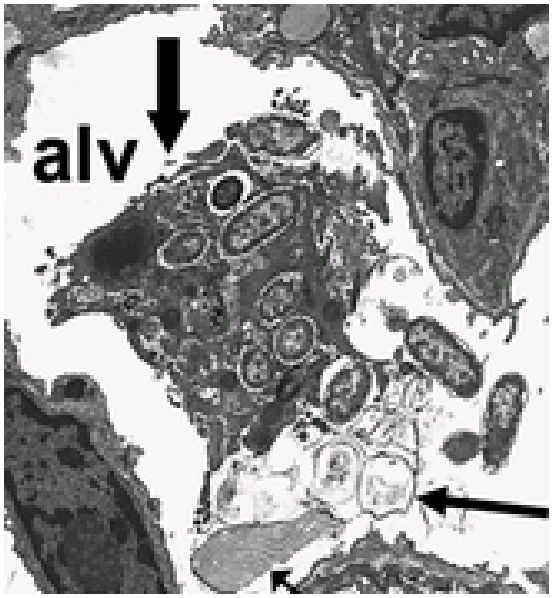
Intracellular survival and persistent infections

Evidence of intracellular *Pseudomonas aeruginosa* in lung parenchyma

Pneumocytes



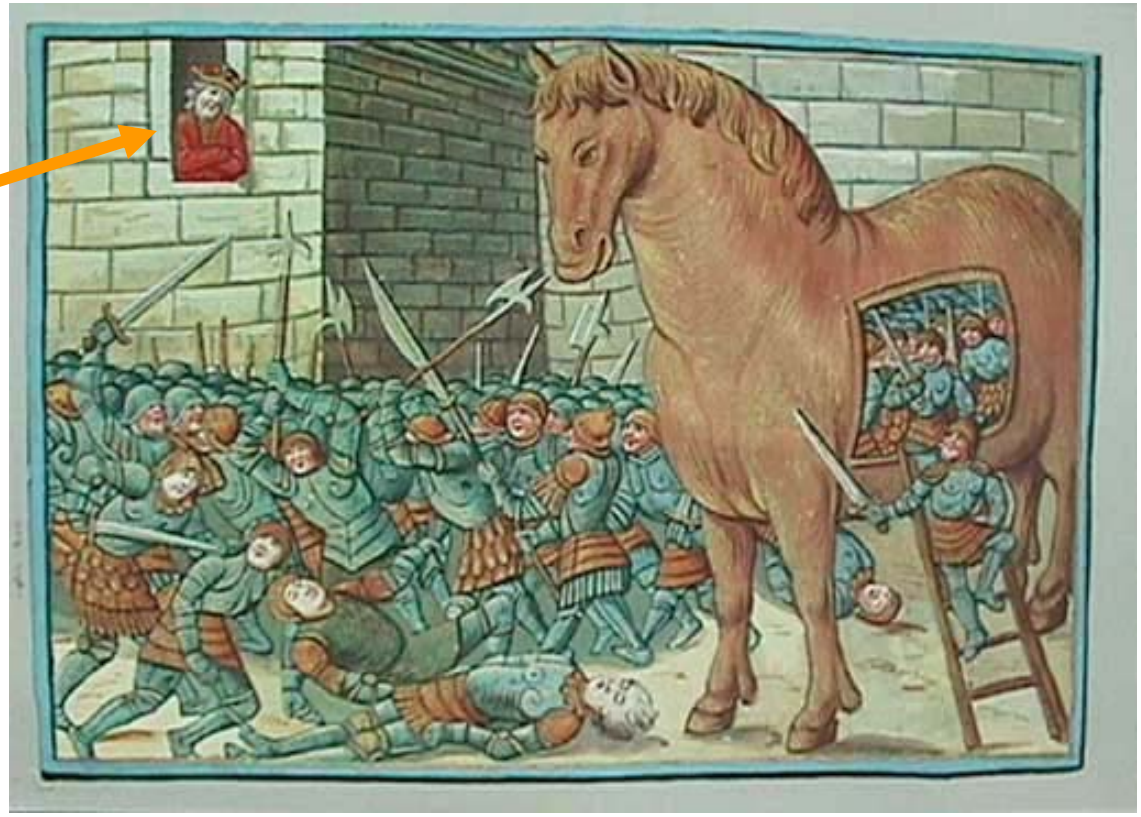
Alveolar macrophages



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

Benefits of intracellular life

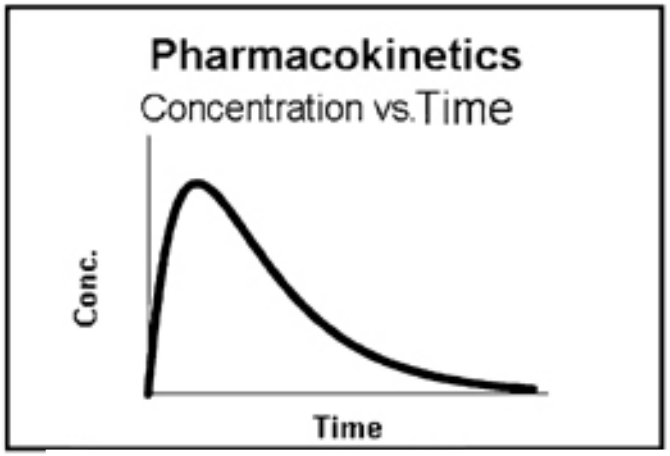
protection

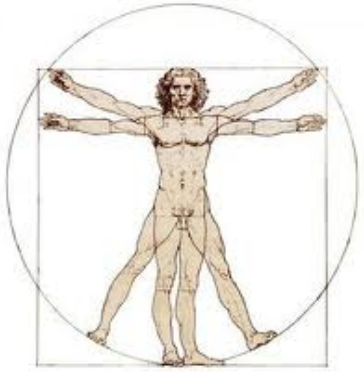


Let's start with pharmacokinetics !

Pharmacokinetics

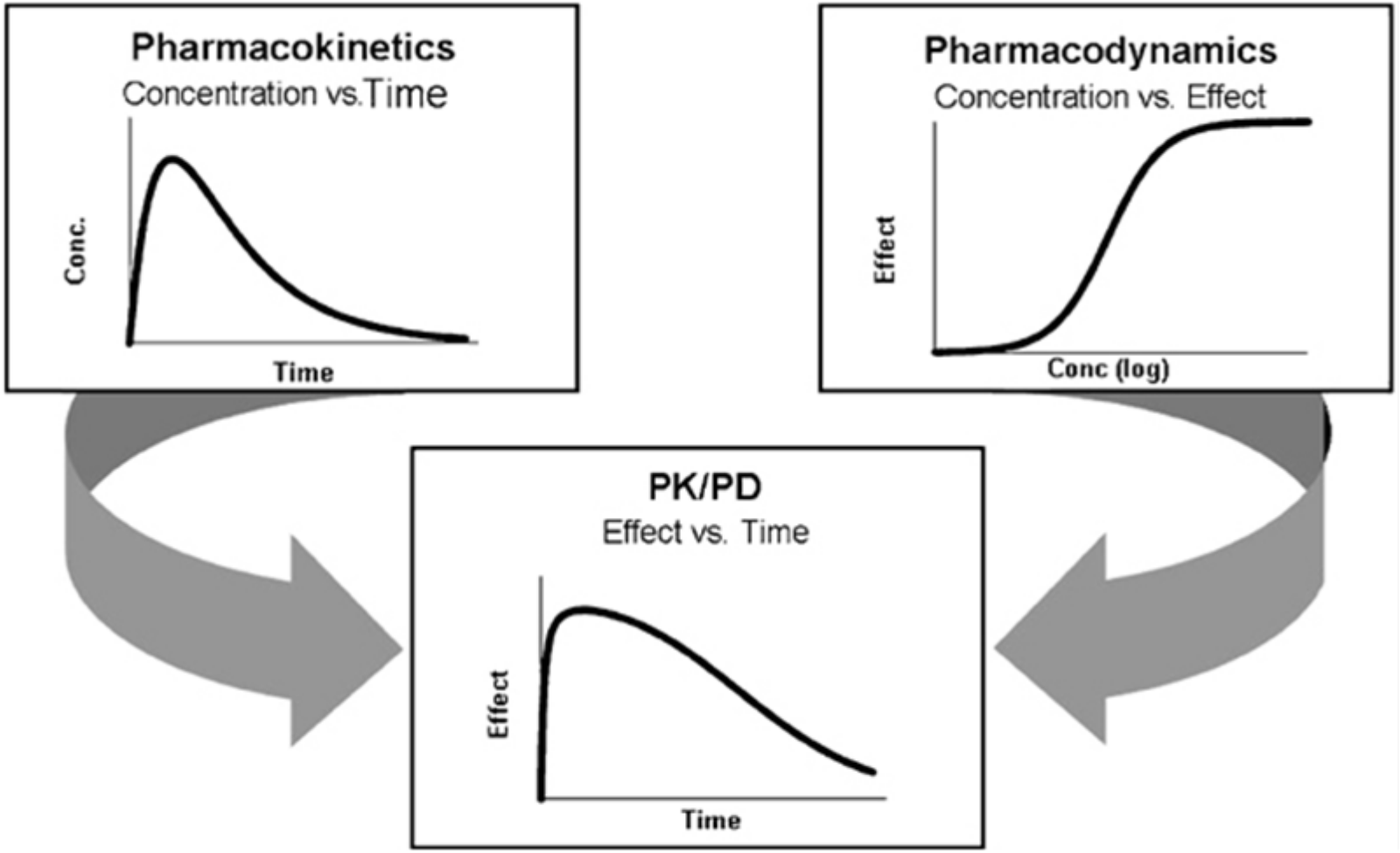
- The general phamacokinetics relate to :
  - Absorption
  - Distribution
  - Metabolism
  - Elimination





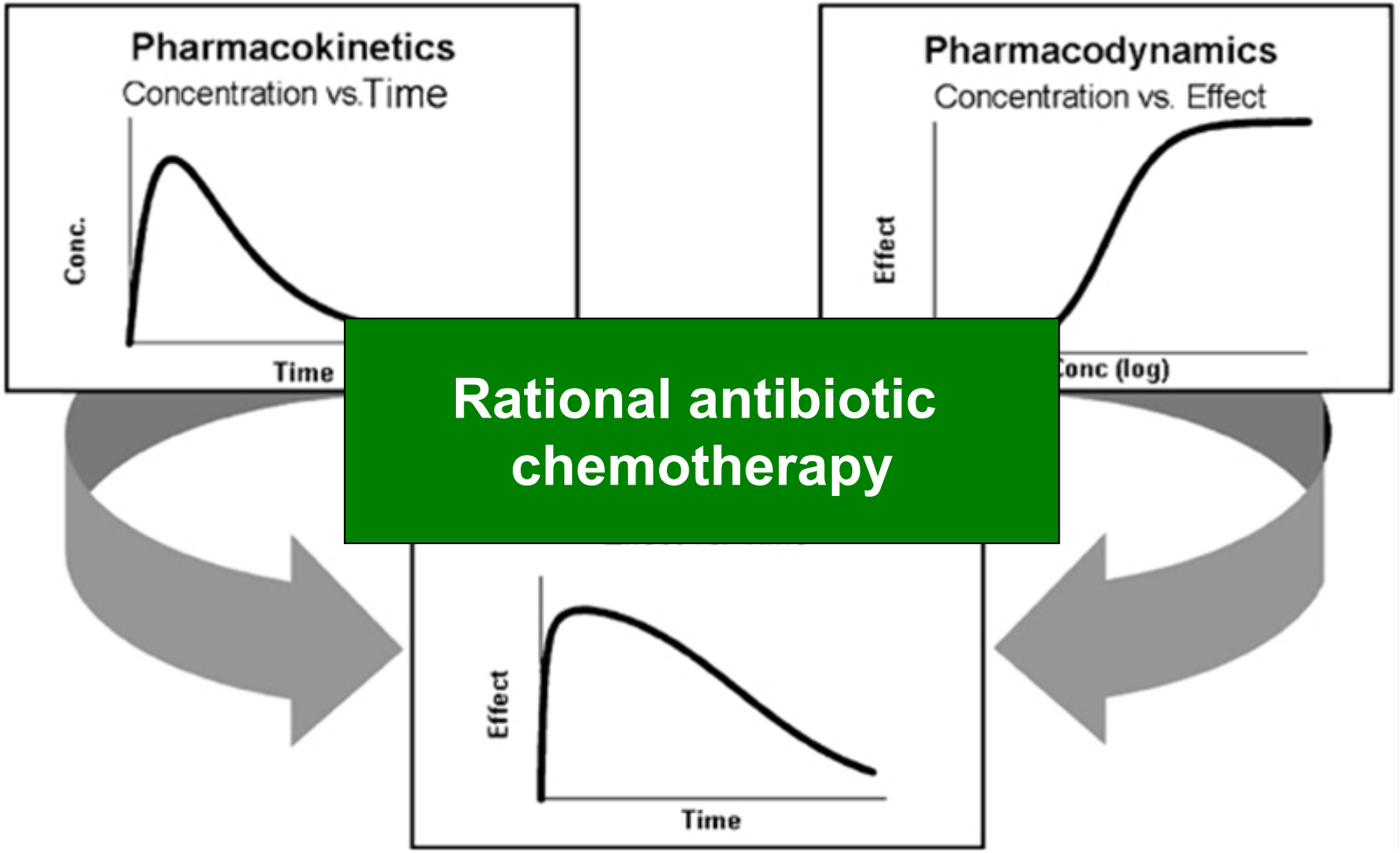
Pharmacokinetics (PK) vs Pharmacodynamics(PD)

- Information of the effect/time course



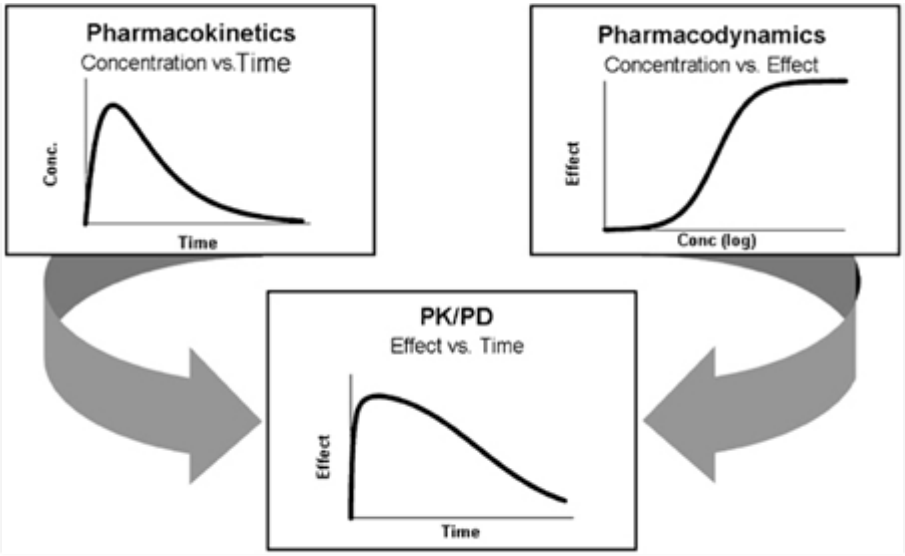
Pharmacokinetics (PK) vs Pharmacodynamics(PD)

- Information of the effect/time course



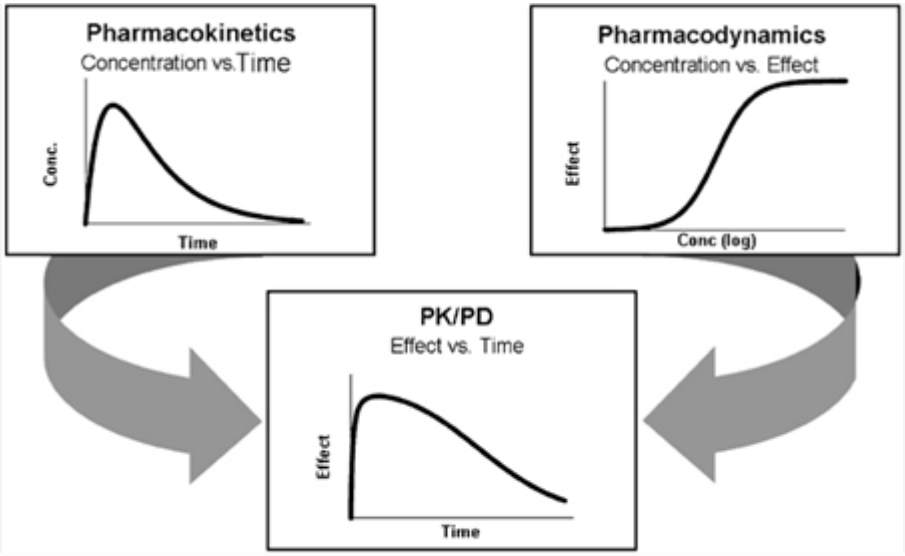
Does this apply for intracellular infections?

- **Yes** ☐
- **No** ☐



Does this apply for intracellular infections?

- **Yes** ☐
- **No** ☒



PK/PD is based on serum concentrations and applies only for extracellular infections in well-vascularized tissue

More complex situation in less accessible compartments e.g. intracellular infections

Intracellular activity of antibiotics depends on:

Cellular influx:

- Passive diffusion

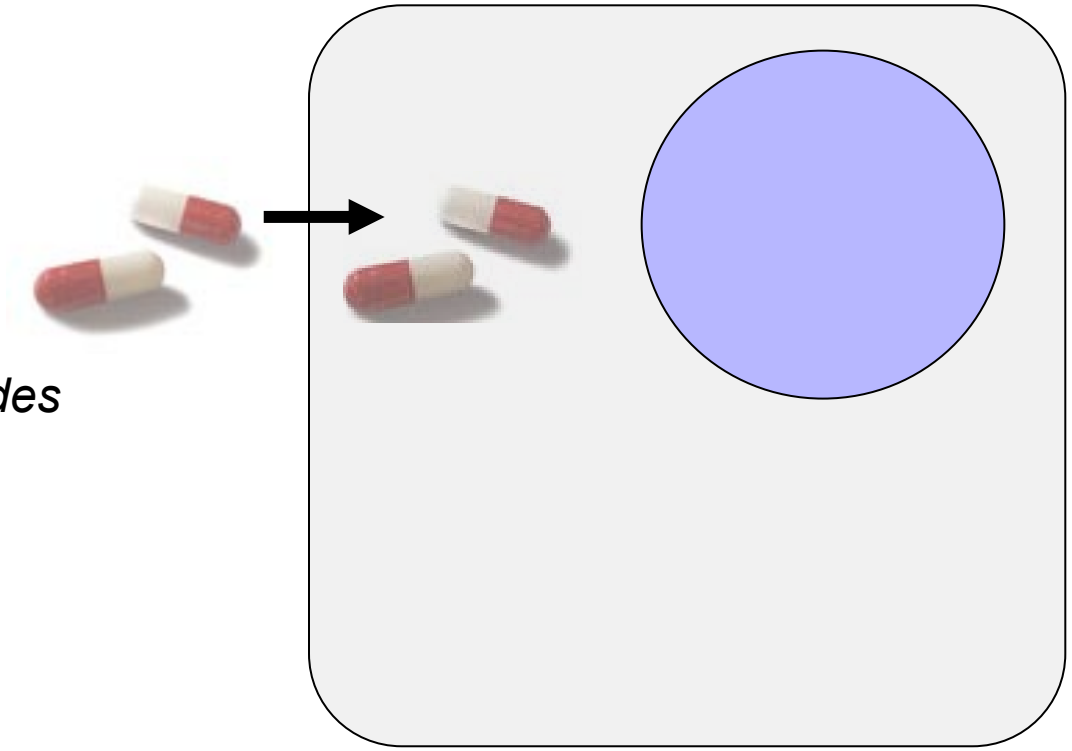
β-lactams, fluoroquinolones and macrolides

- Endocytosis

Aminoglycosides and glycopeptides

- Active transport

β-lactams, fluoroquinolones and clindamycin



Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

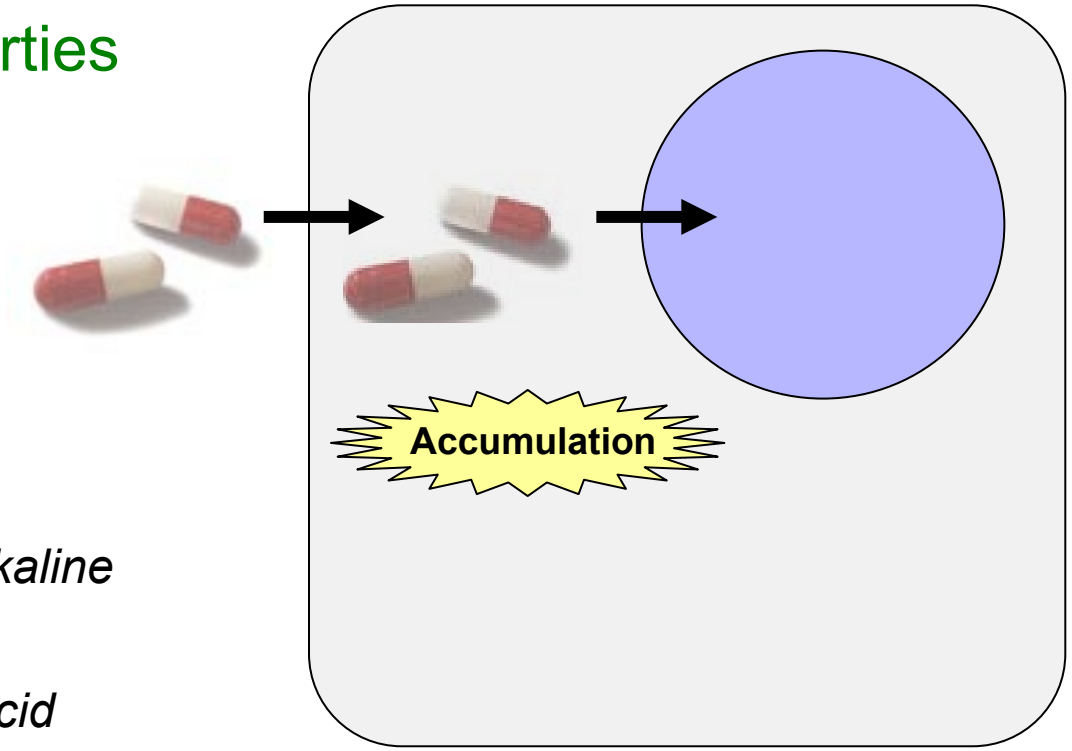
- Physicochemical properties of the drug molecule
- Environmental pH

↓

- Ion-trapping

Weak acids will accumulate in alkaline compartments

Weak bases will accumulate in acid compartments



Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

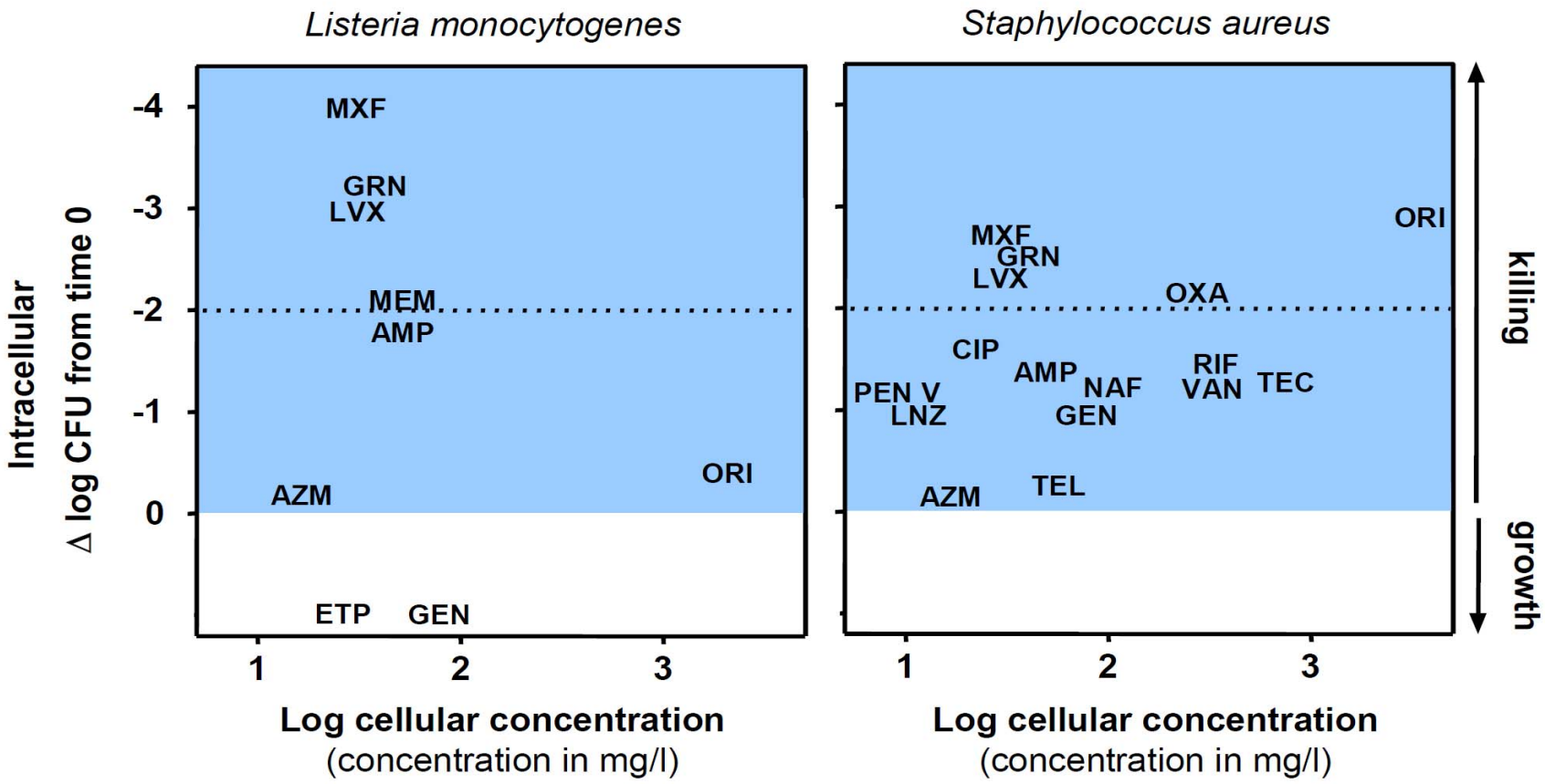
Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

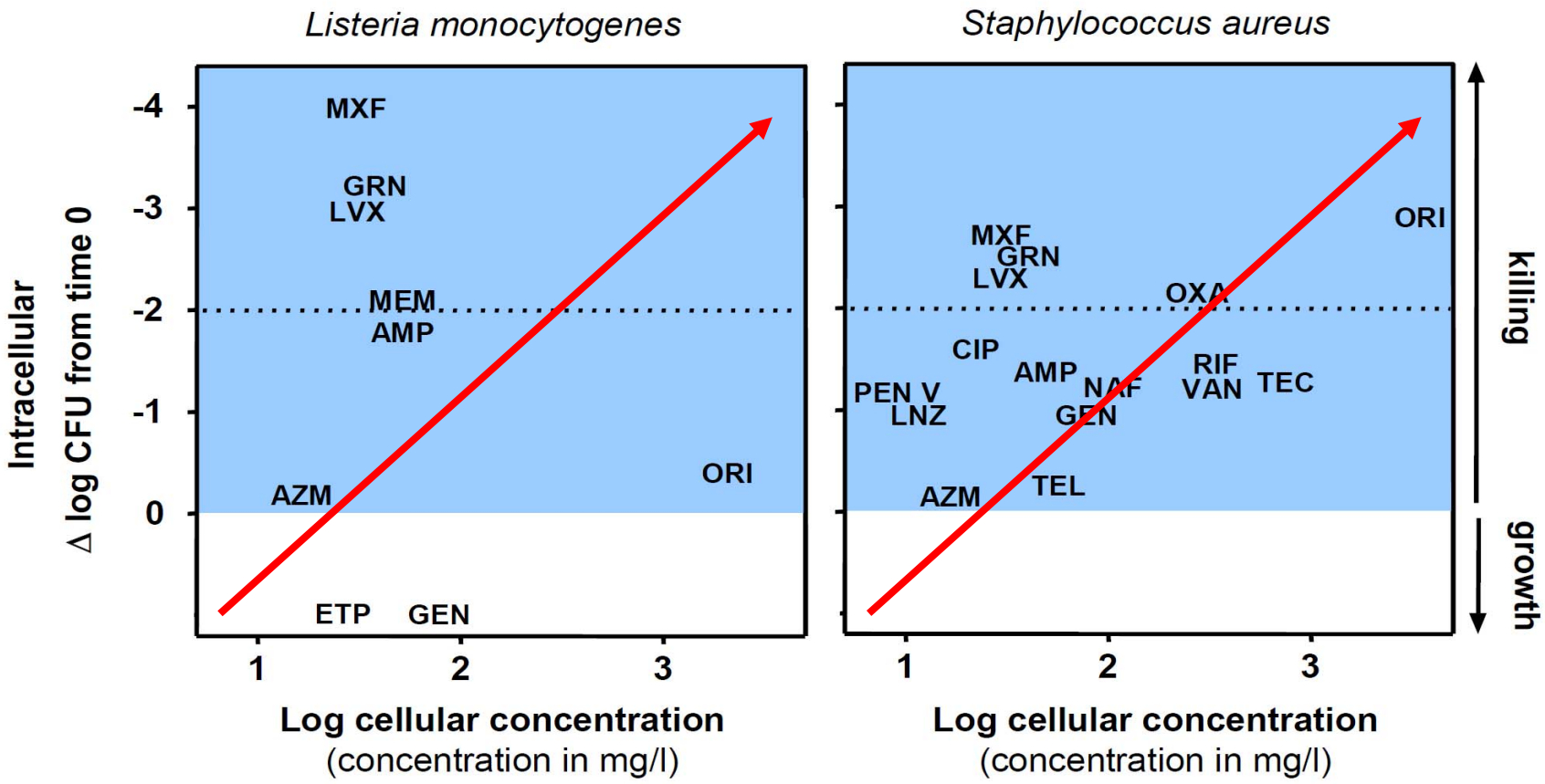
Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Can we predict the intracellular activity based on intracellular concentrations?



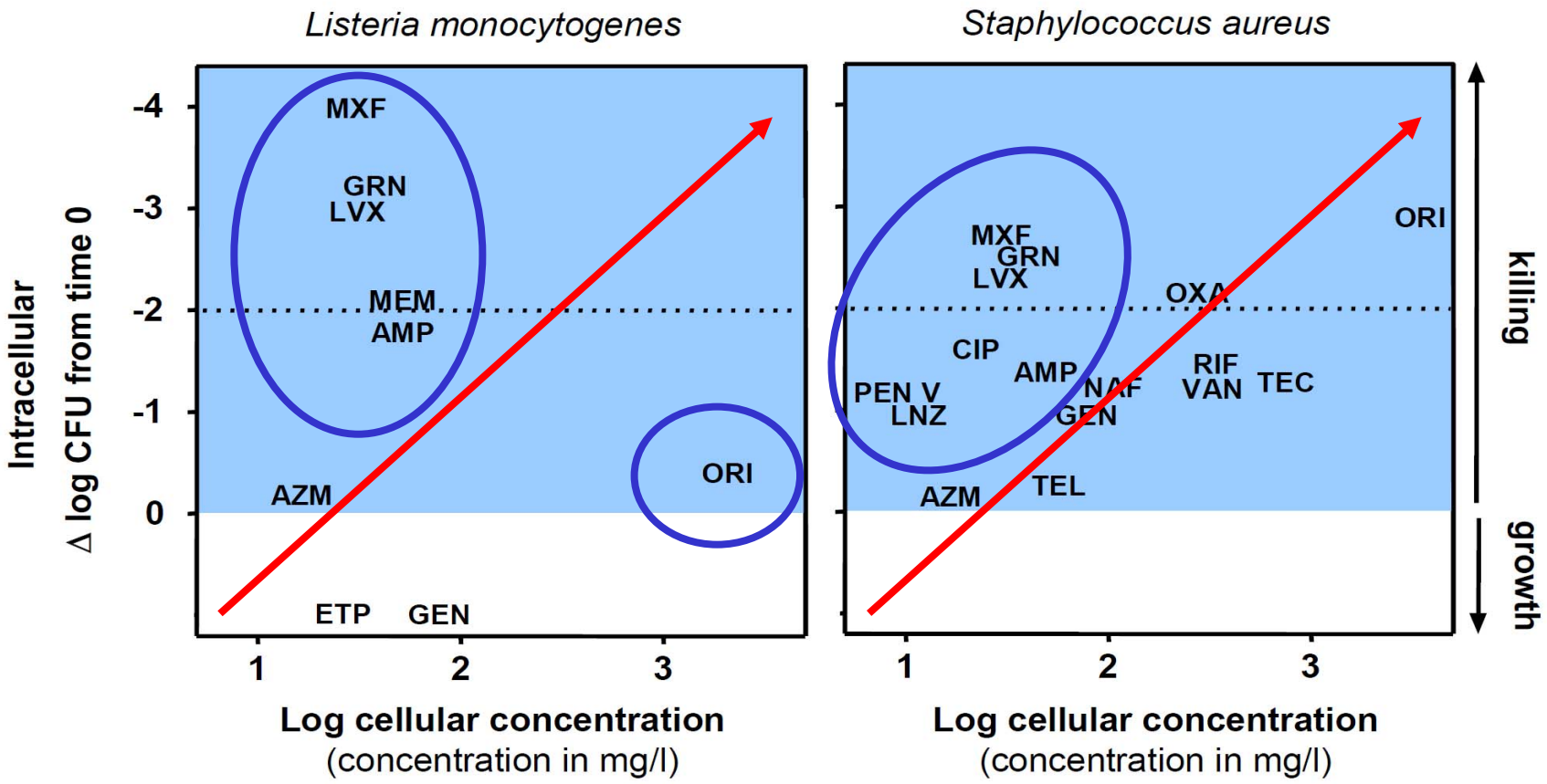
Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Can we predict the intracellular activity based on intracellular concentrations?



Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Can we predict the intracellular activity based on intracellular concentrations?



No correlation between intracellular concentration and intracellular activity

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

- Cellular bioavailability?

↓

Where is the drug and where is the bug ?

Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Cellular accumulation and sub-cellular distribution

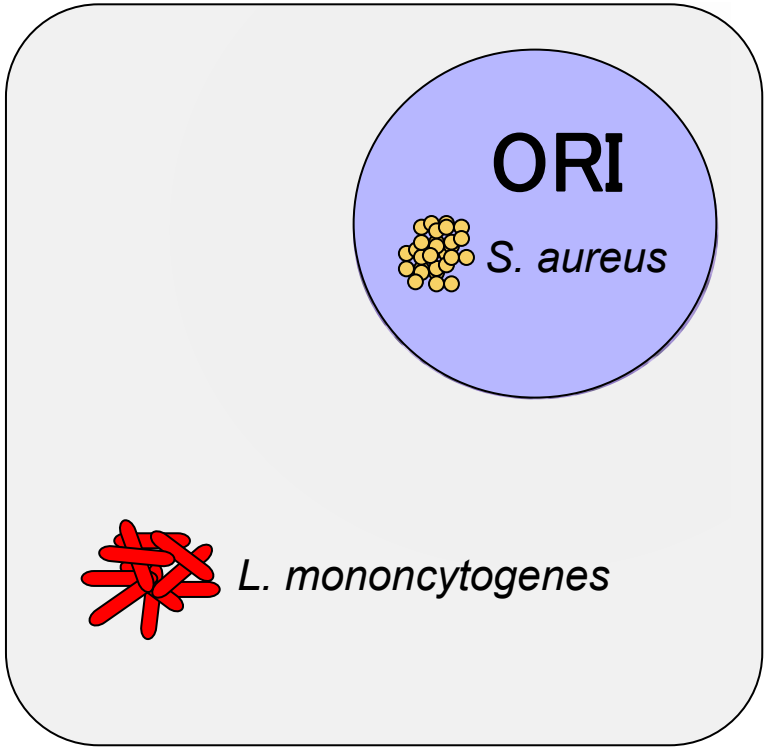
- Cellular bioavailability?

ORITAVANCIN as an example:

- Intracellular accumulation (x 150-300)
- Highly accumulated in the lysosomes

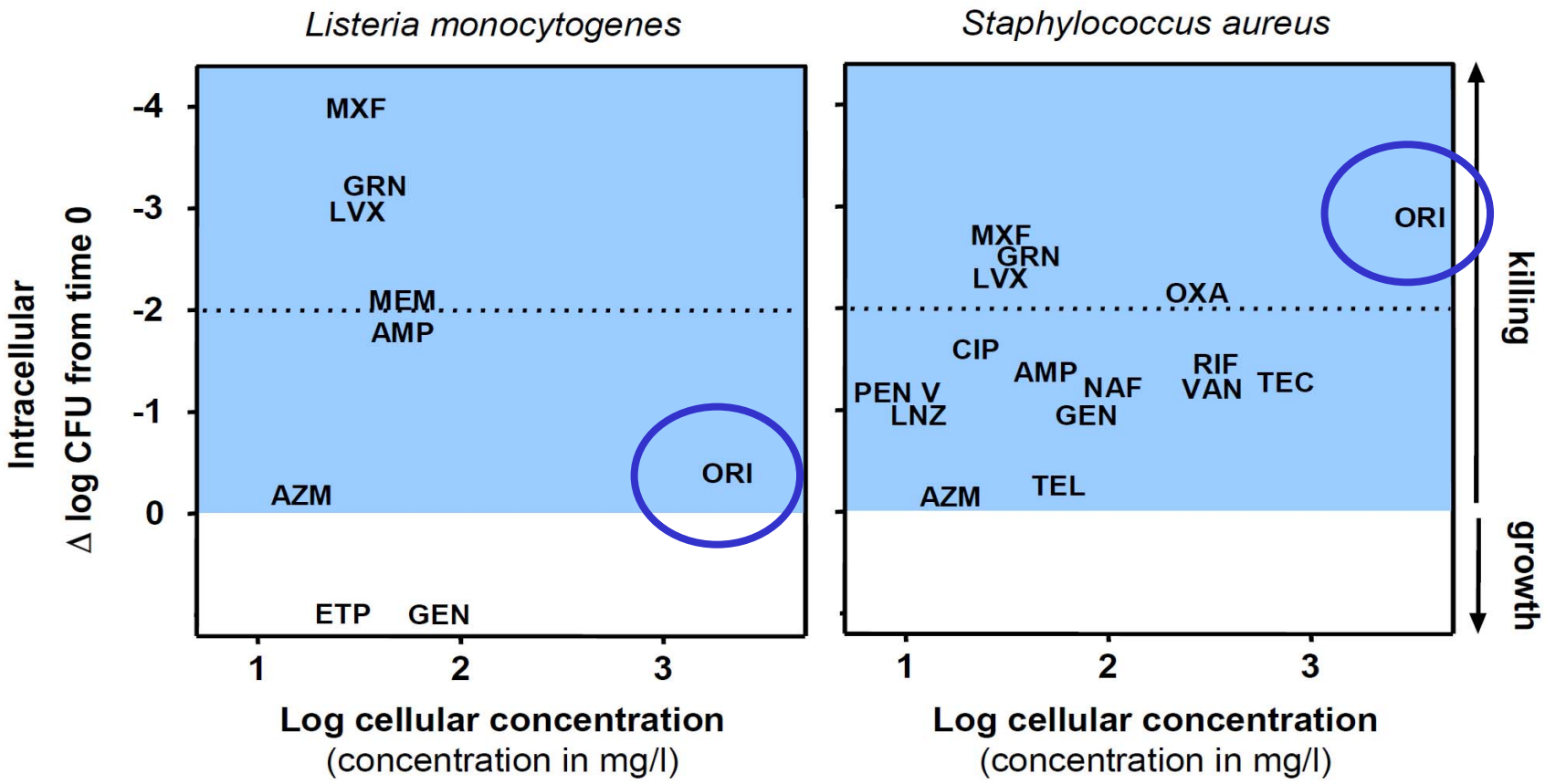
↓

- Inactive against *L. monocytogenes*
- Active against *S. aureus*



Van Bambeke et al., Antimicrob Agents Chemother (2004) 48:2853-2860

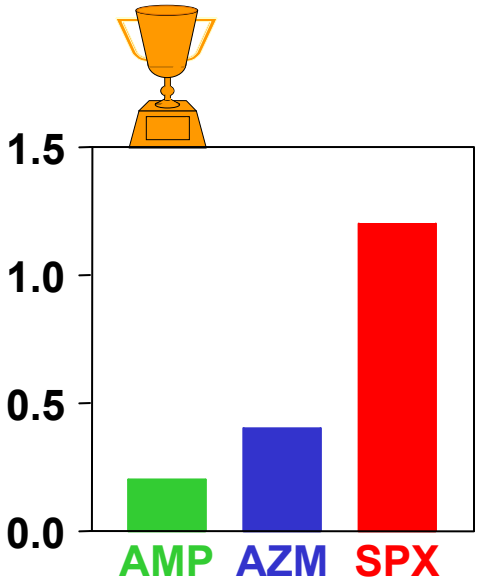
Can we predict the intracellular activity based on intracellular concentrations?



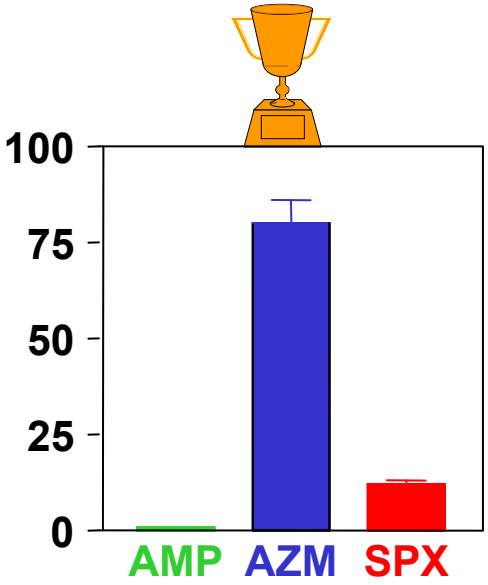
Adapted from Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Another example.....

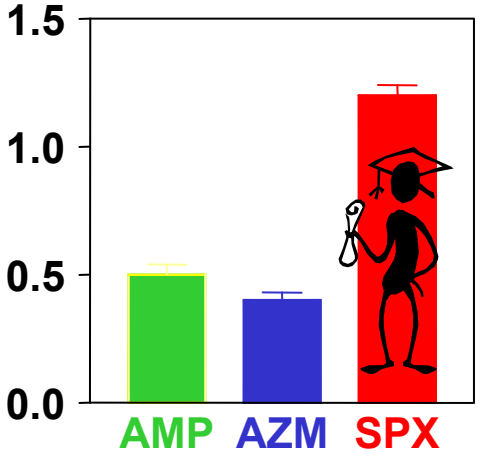
MIC



Antibiotic accumulation



Intracellular activity

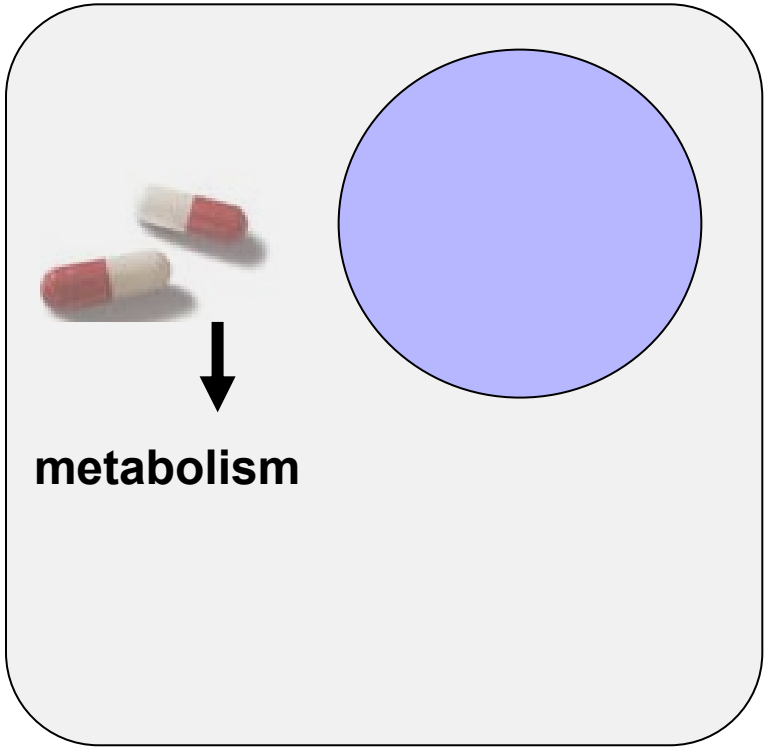


Ouadrhiri et al, Antimicrob. Ag. Chemother. (1999) 43:1242-51

Intracellular activity of antibiotics depends on:

Other parameters

- Intracellular metabolism

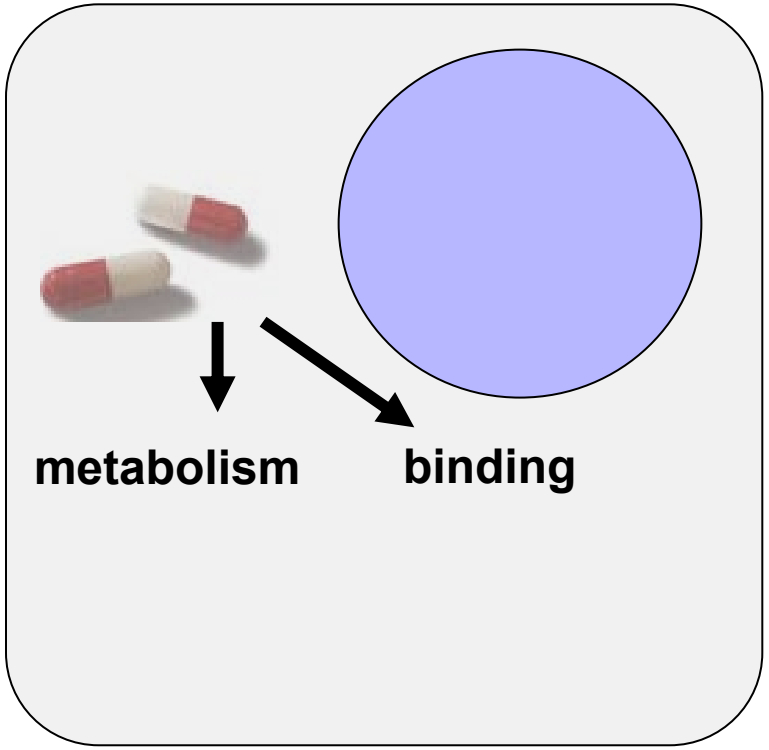


Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Other parameters

- Intracellular metabolism
- Intracellular protein binding

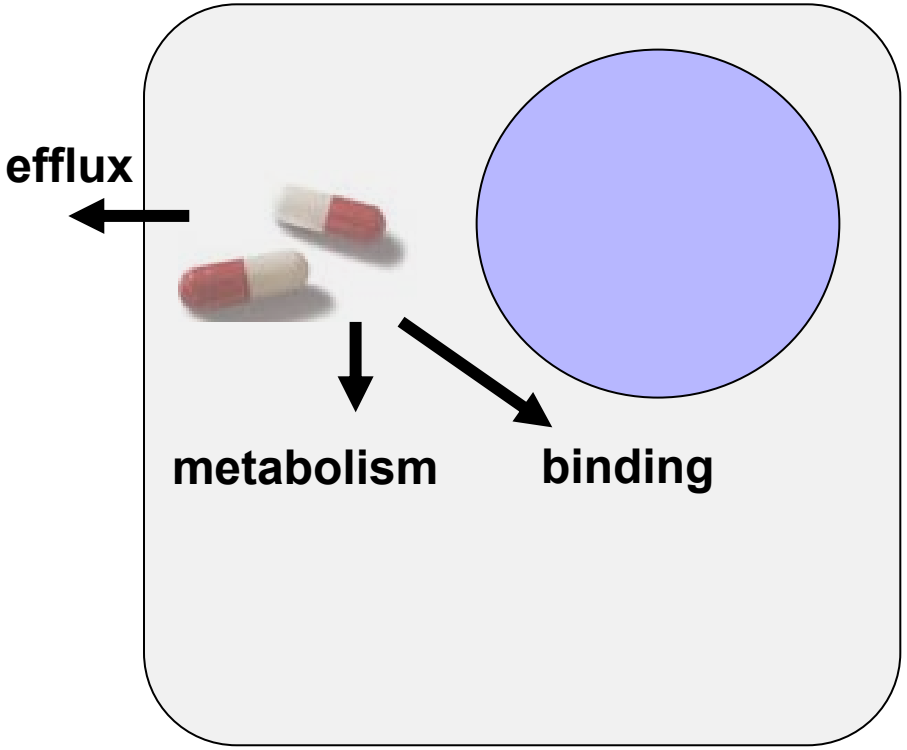


Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Other parameters

- Intracellular metabolism
- Intracellular protein binding
- Efflux



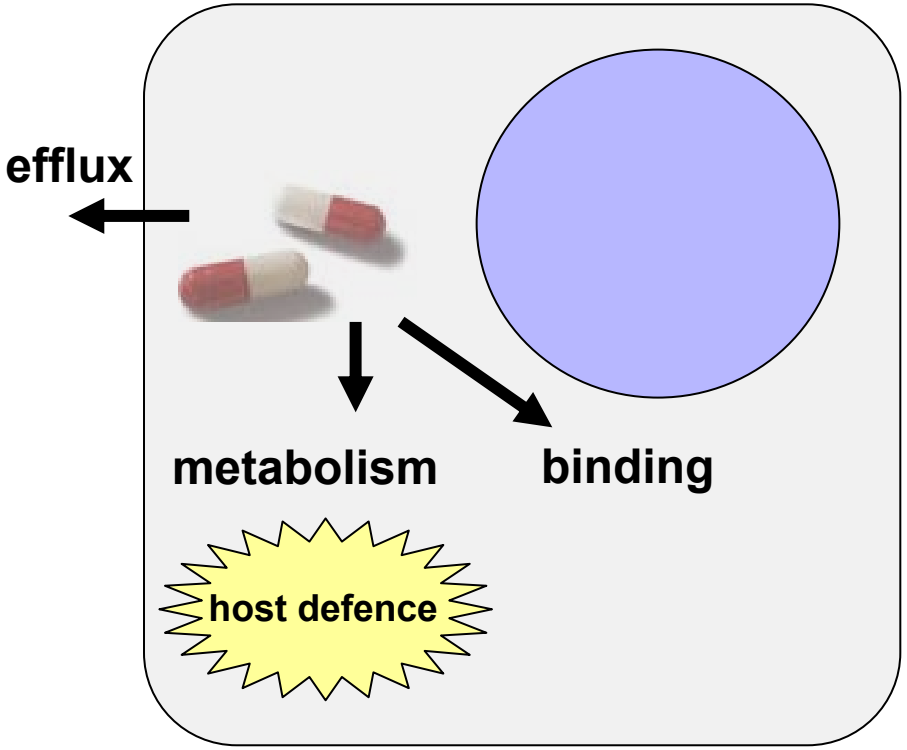
Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

Other parameters

- Intracellular metabolism
- Intracellular protein binding
- Efflux
- Interaction with host defense

  e.g. increase or decrease of the oxidative burst



Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics depends on:

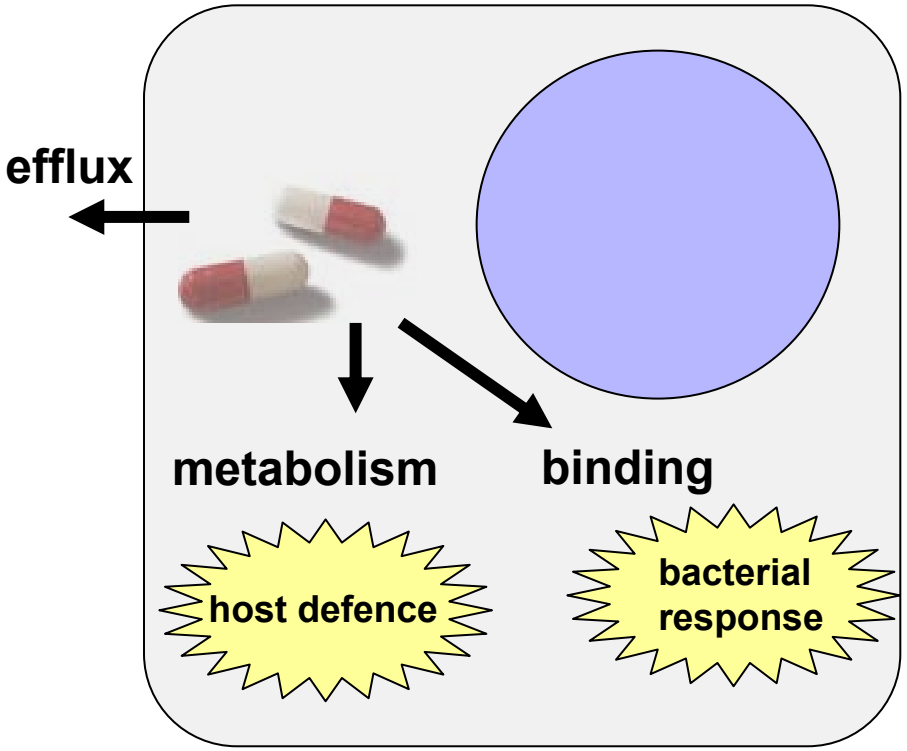
Other parameters

- Intracellular metabolism
- Intracellular protein binding
- Efflux
- Interaction with host defense

  e.g. increase or decrease of the oxidative burst

- Bacterial responsiveness

  e.g. decreased bacterial growth rate



Van Bambeke et al., Curr. Opin. Drug Discov. Devel (2006) 9:218-230

Intracellular activity of antibiotics

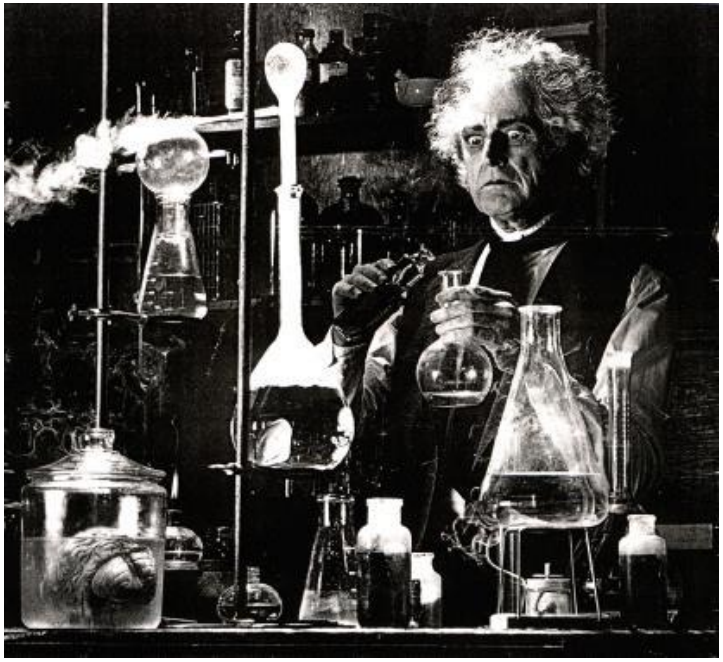
Summery:

Intracellular activity depends highly on the cellular pharmacokinetic of the drug and is difficult to predict

Generally a poor correlation between accumulation and activity

↓

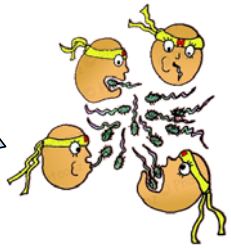
Intracellular activity should be estimated experimentally in appropriate models



In vitro, an easy model to begin with !

In vitro model of intracellular infection

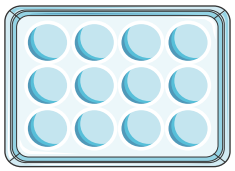
Opsonization
Culture medium +
human serum



Phagocytosis

Extracellular Wash
(Gentamicin 100 X MIC; ~1 h)

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

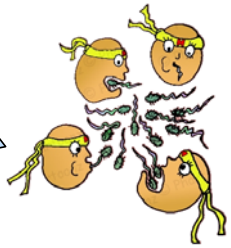


Incubation (with antibiotics)
For up to 24 h
(control: Gentamicin 0.5 X MIC)

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

In vitro model of intracellular infection

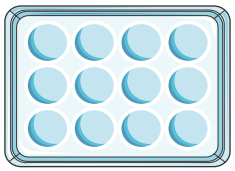
Opsonization
Culture medium +
human serum



Phagocytosis

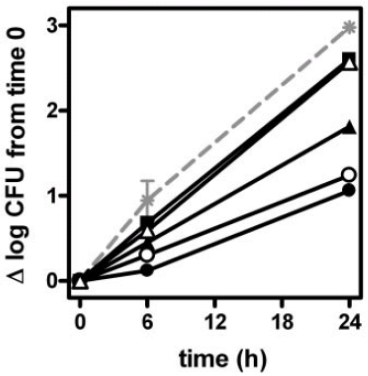
Extracellular Wash
(Gentamicin 100 X MIC; ~1 h)

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



Incubation (with antibiotics)
For up to 24 h
(control: Gentamicin 0.5 X MIC)

S. aureus as an example



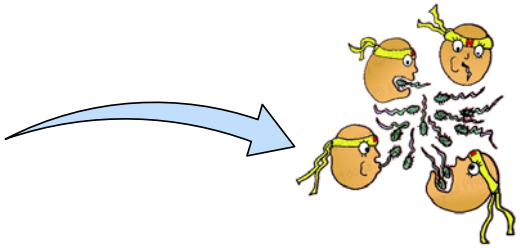
extracell. [GEN] *	extracell. contamin.**
0	17.2 ± 1.9
0.001	16.0 ± 1.0
0.01	0.013 ± 0.001
0.1	0.0026 ± 0.0003
1	< 0.001

* x MIC
** % of total bacteria in culture

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

In vitro model of intracellular infection

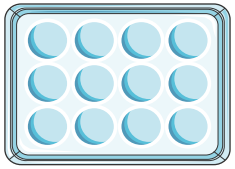
Opsonization
Culture medium +
human serum



Phagocytosis

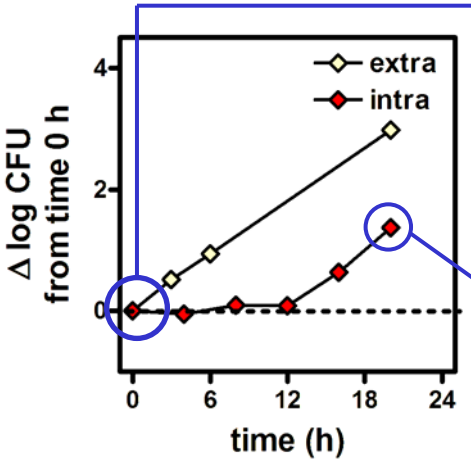
Extracellular Wash
(Gentamicin 100 X MIC; ~1 h)

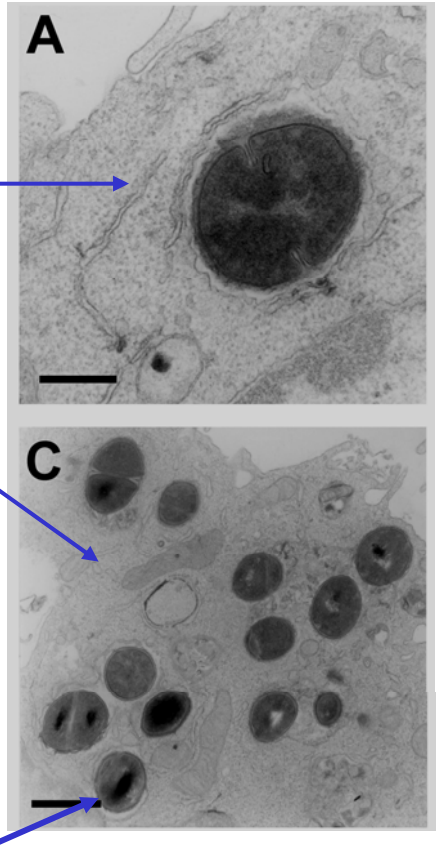
5 -10 x10^5 CFU/mg prot.
Time 0



Incubation (with antibiotics)
For up to 24 h

S. aureus as an example



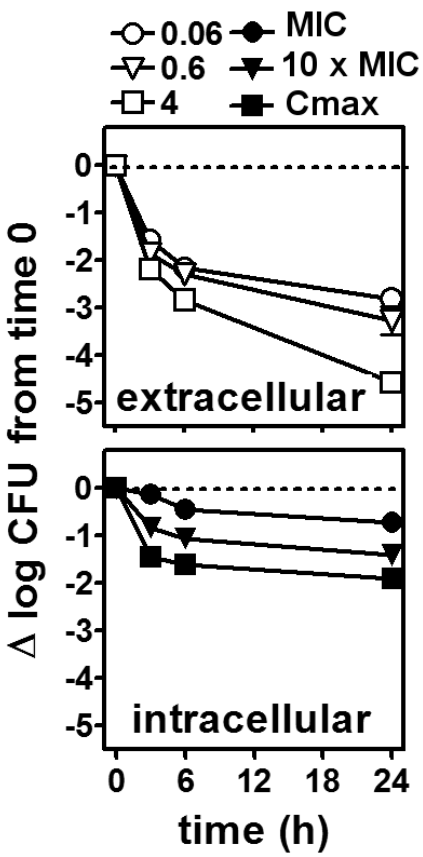


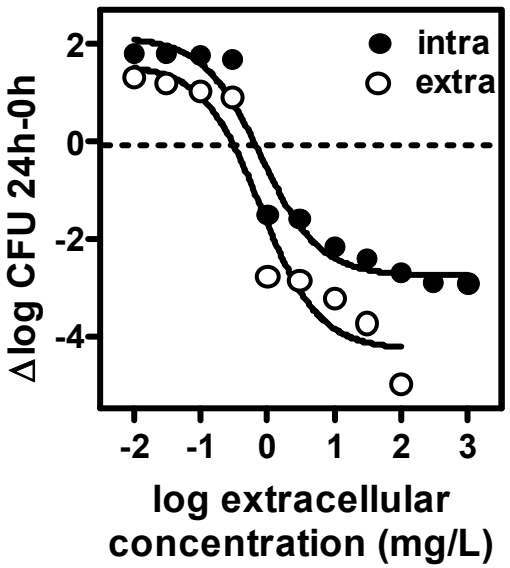
remains in vacuoles

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*

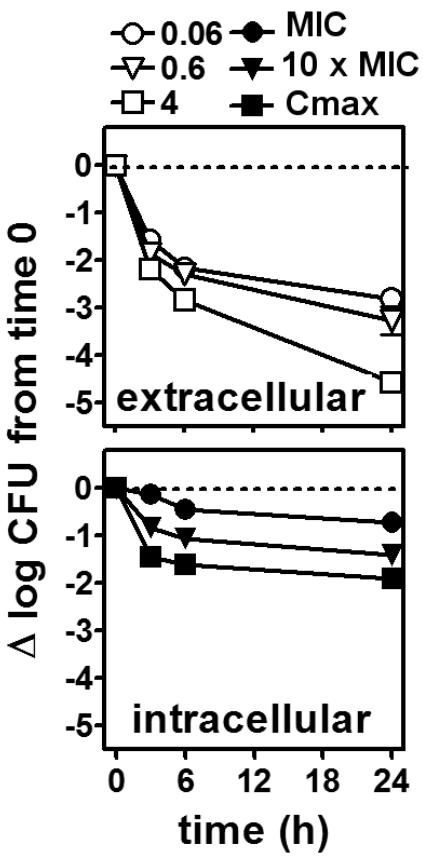


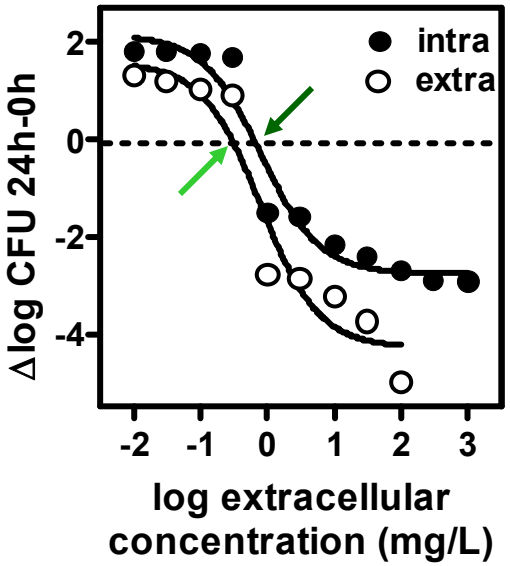


Barcia-Macay et al, Antimicrob. Ag. Chemother. (2006) 50:841-51

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

moxifloxacin & *S. aureus*





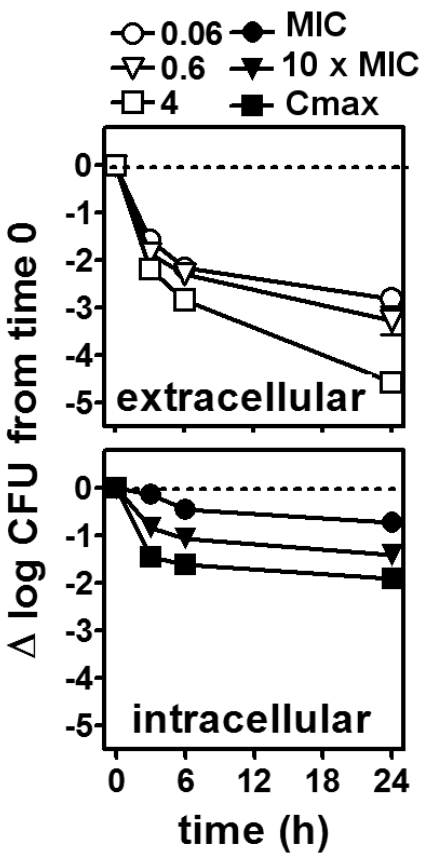
model	C_{stat} (x MIC)
extra	0.27
intra	0.63

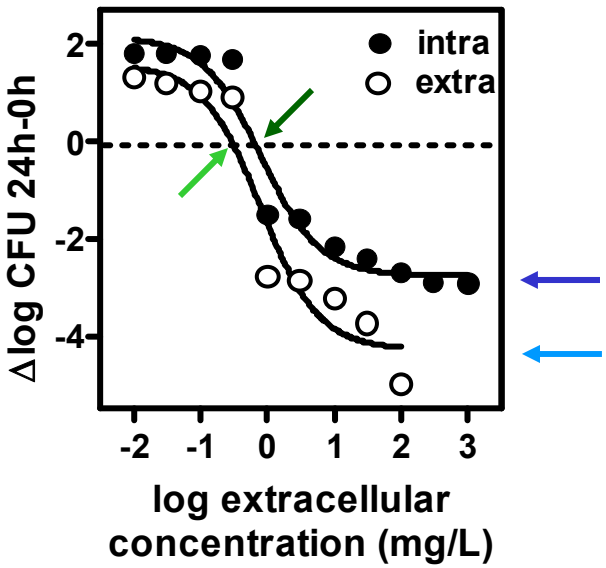
relative potency

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

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

moxifloxacin & *S. aureus*





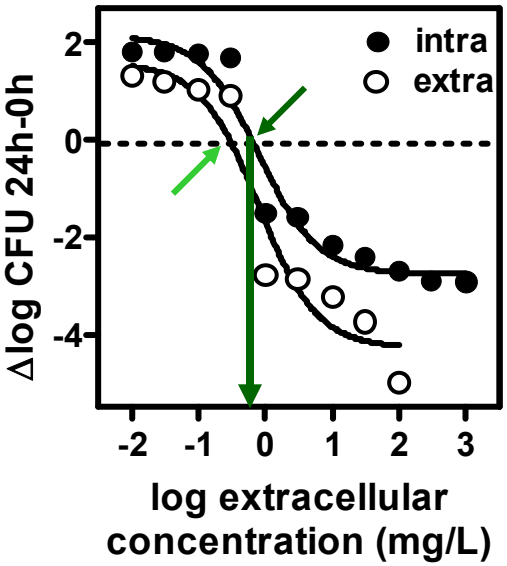
model	C_{stat} (x MIC)	E_{max}
extra	0.27	-3.86 (5.22 to 2.51)
intra	0.63	-2.77 (3.31 to 2.22)

relative potency

maximal efficacy

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

What do these parameters tell you ?



relative potency

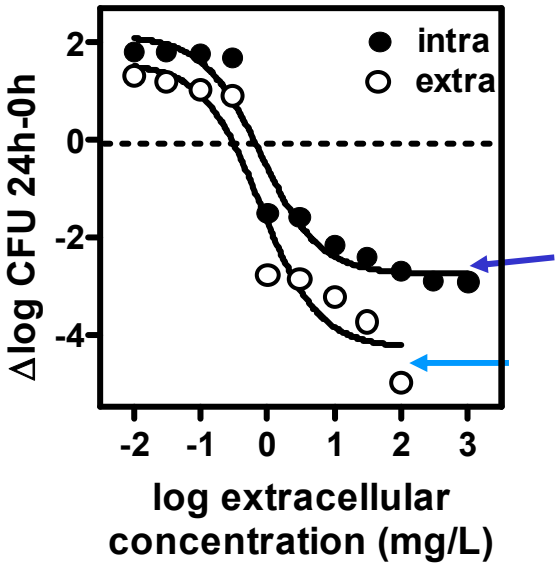
- Estimation of the concentration needed to reach a specified effect
- Measure of the « intracellular MIC »

⇨ « PK-related » parameter:
- accumulation in the infected compartment
- intracellular bioavailability

⇨ influence of local environment on intrinsic activity
- pH
- oxidant species

In most cases
Cs intra ≥ Cs extra

What do these parameters tell you ?



maximal efficacy

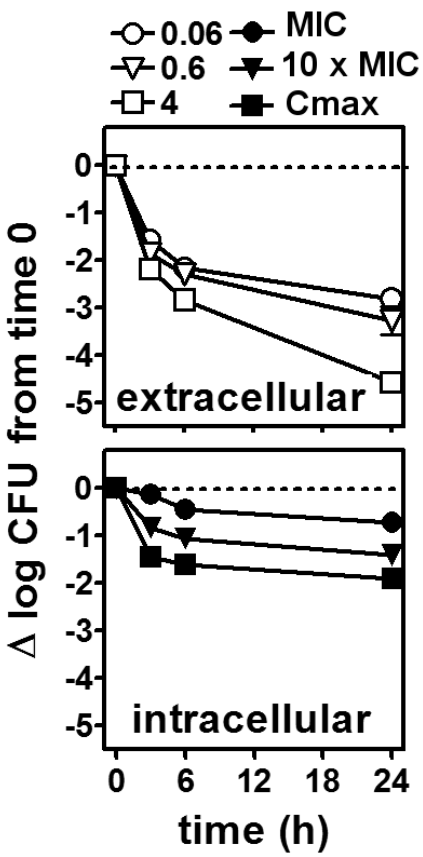
- Estimation of the maximal reduction in inoculum for an infinitely large concentration
- Measure of the killing capacity

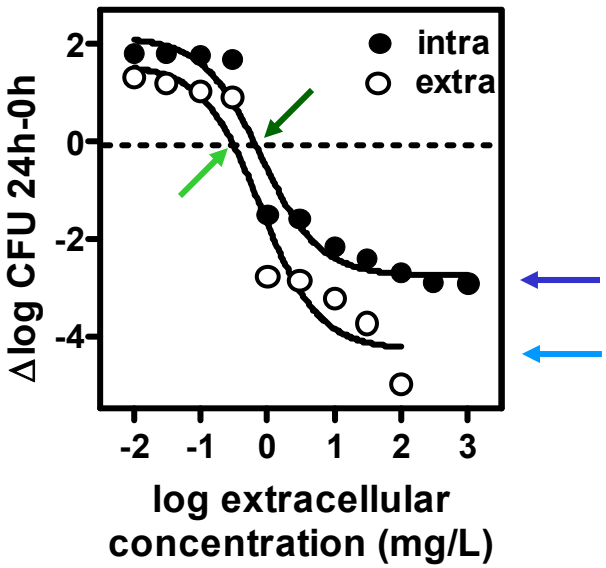
⇨ « PD-related » parameter
- mode of action of the drug
- bacterial responsiveness
- cooperation with host defenses

In most cases
Emax intra <<< Emax extra

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)

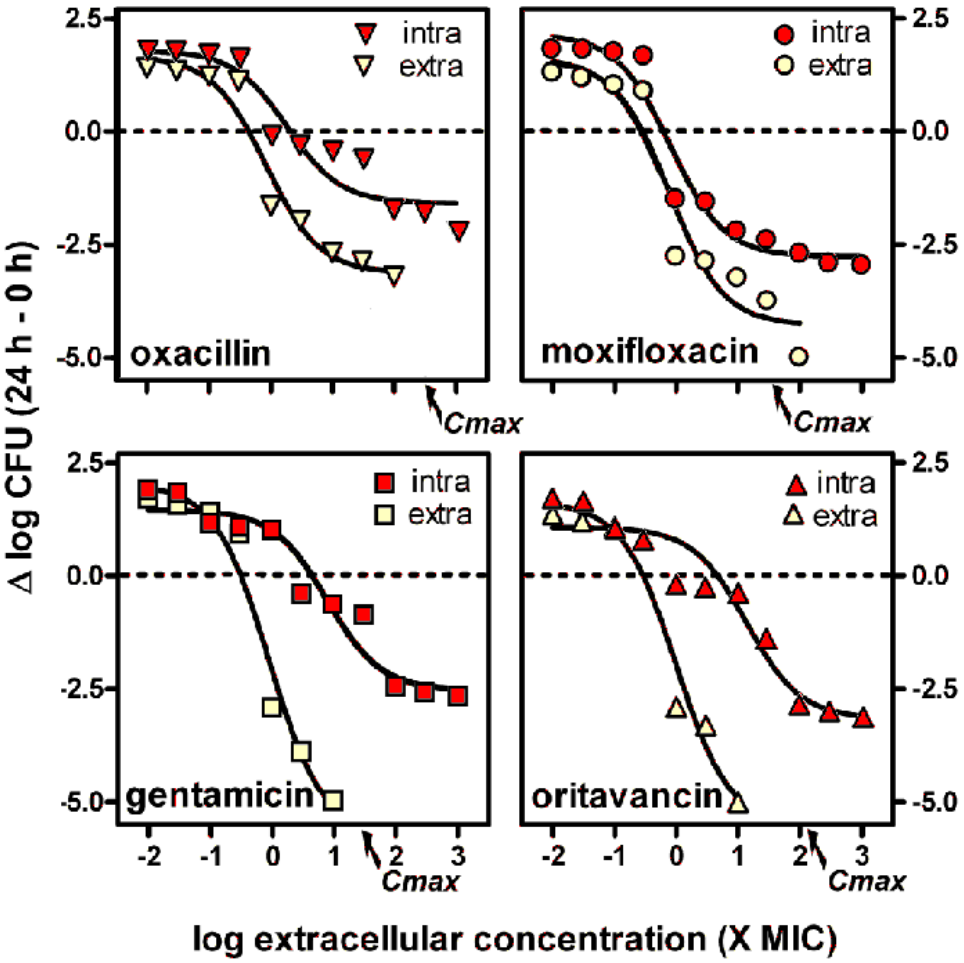
Quantitative comparison
~ models
~ drugs

relative potency

maximal efficacy

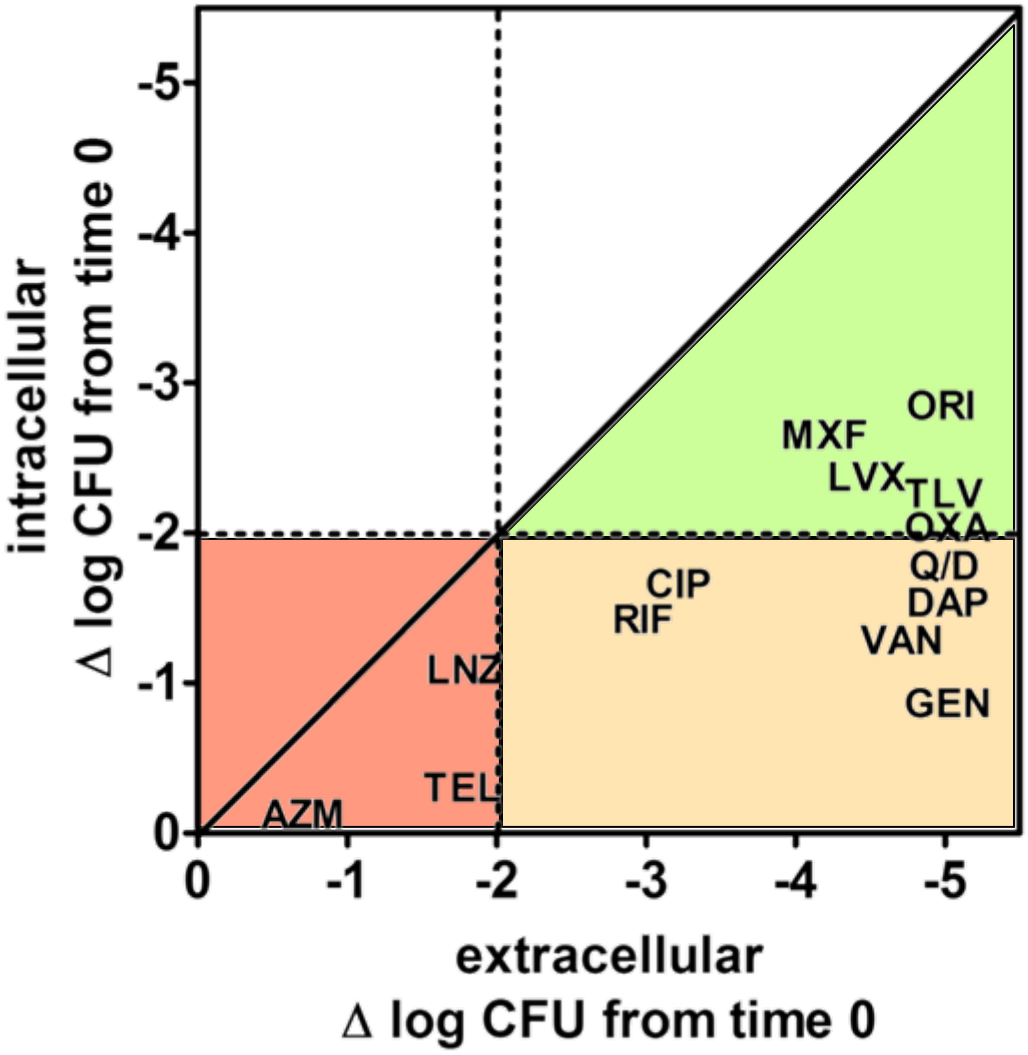
Comparing different drugs against a single species

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



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

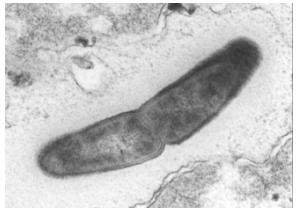
Balance of extra-and intracellular activity of antibiotics against *S. aureus*

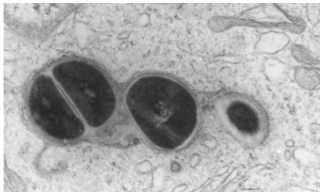


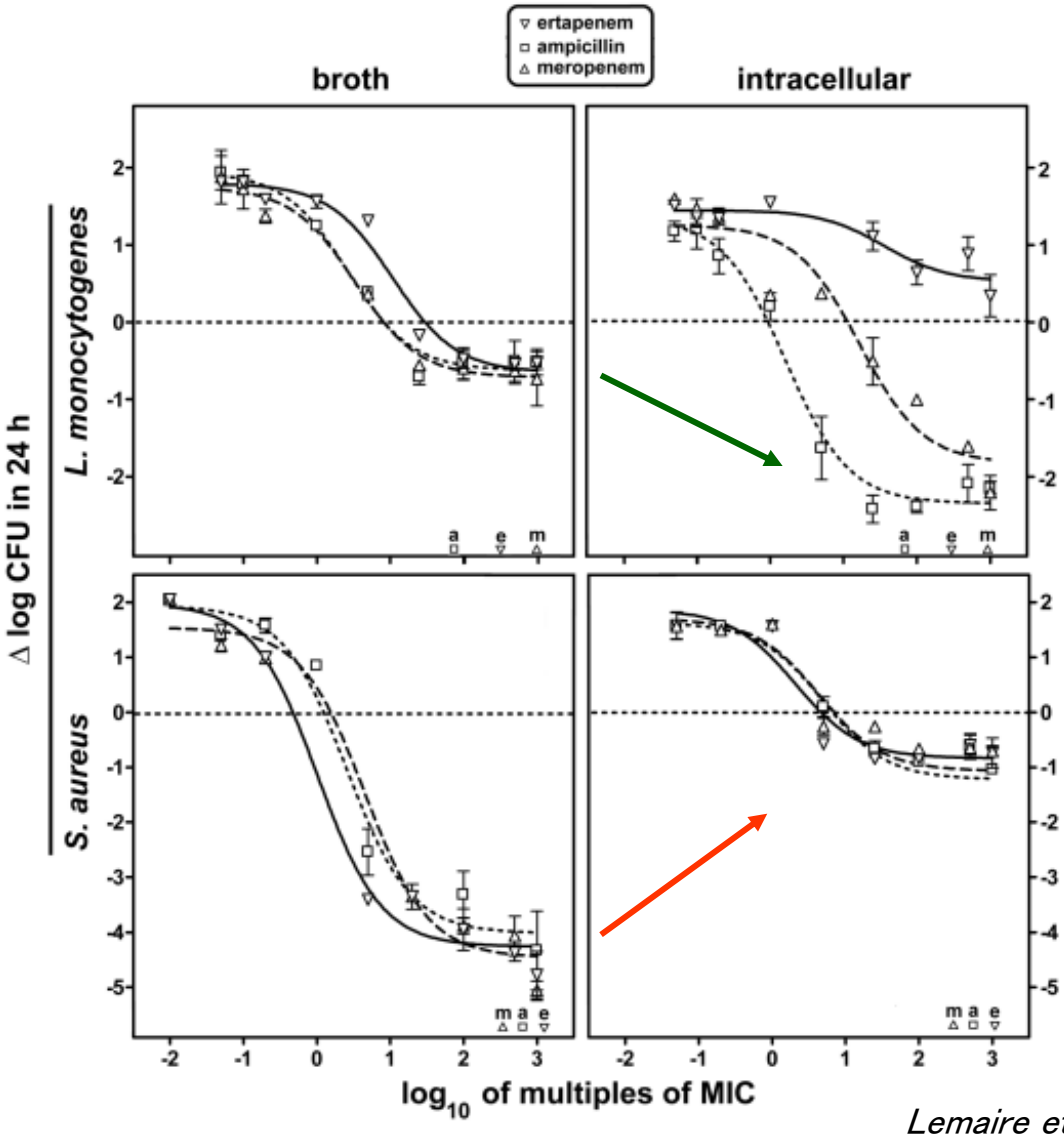
Adapted from Van Bambeke et al., Curr Opin Drug Discov Devel. (2006) 9:218-30

Comparing a class of drugs against different bacteria

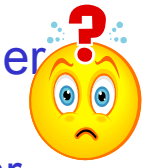
Beta-lactams vs. *L. moncytogenes* and *S. aureus*







Emax higher against intracellular bacteria ?



Lemaire et al. JAC (2005) 55:897–904

Comparing a class of drugs against different bacteria

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

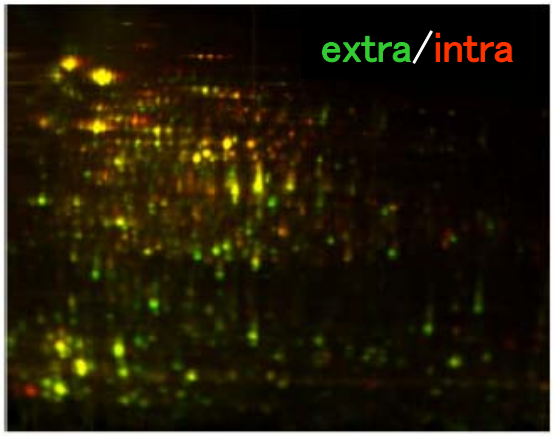
⇨« PD-related » parameter

- mode of action of the drug ?
- bacterial responsiveness ?
- cooperation with host defenses ?

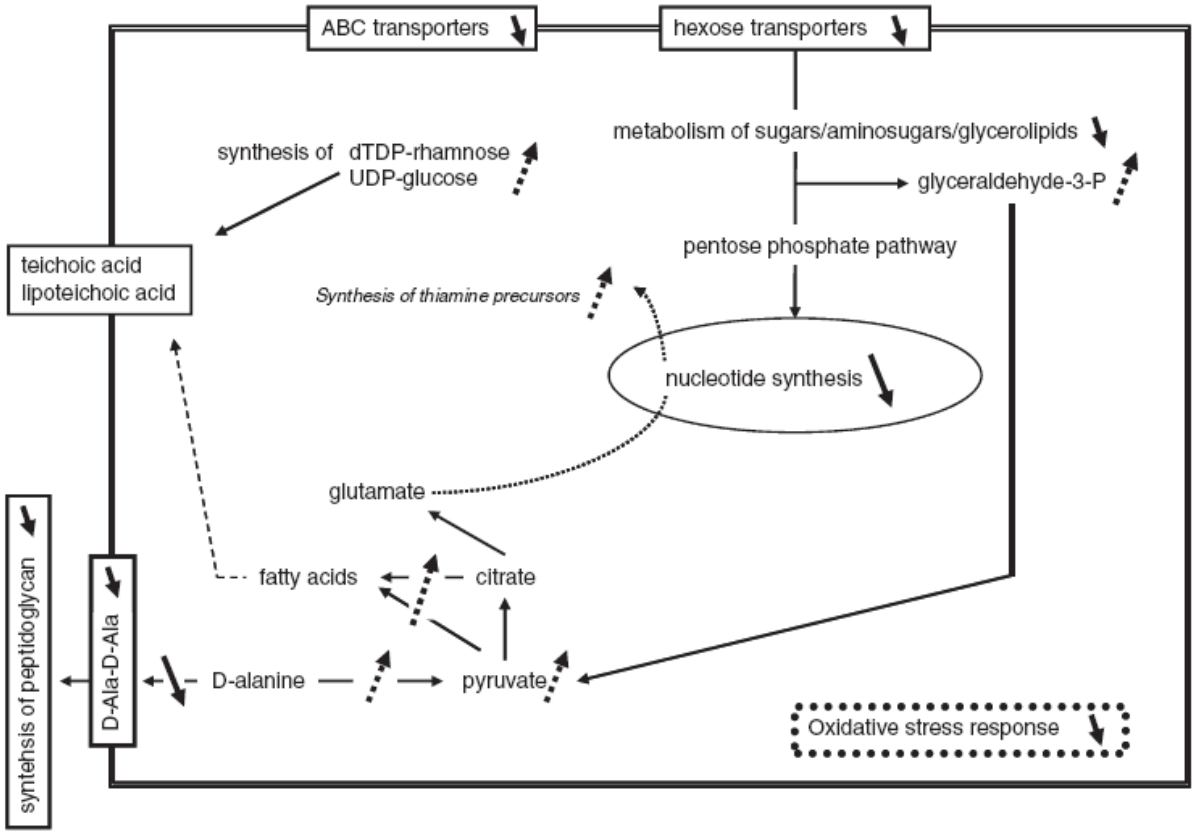
Emax higher against intracellular bacteria ?

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

Proteome analysis of intra- vs extra- *Listeria*



Reduced cell wall synthesis

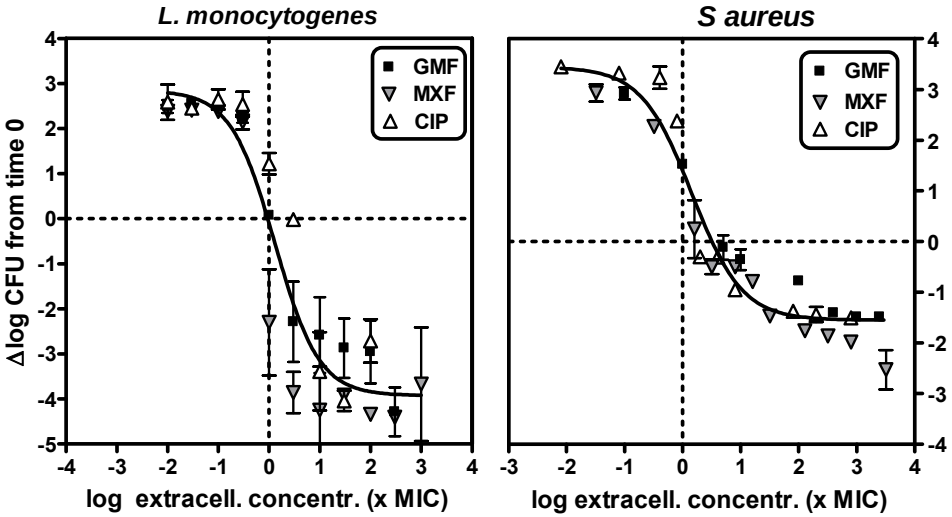


Van de Velde et al. Proteomics (2009) 9:5484-5496

Comparing a class of drugs against different bacteria

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

They all look the same …

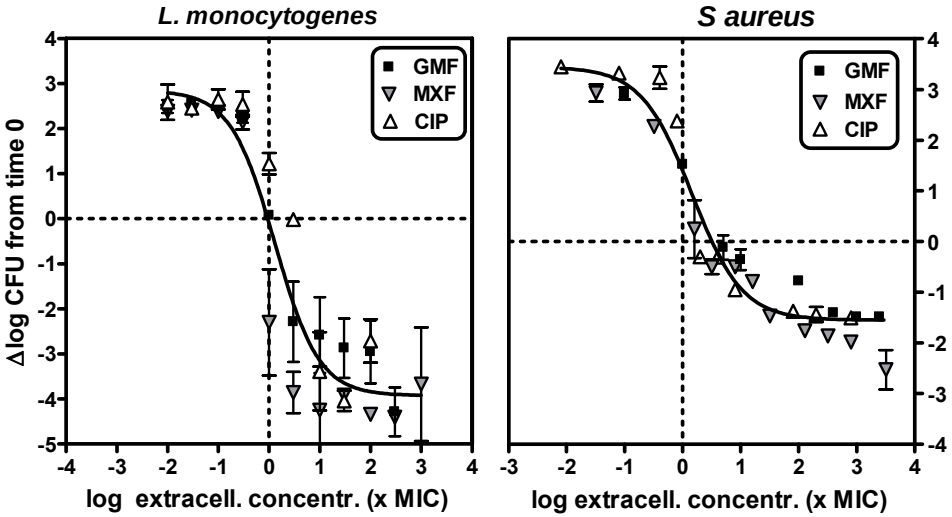


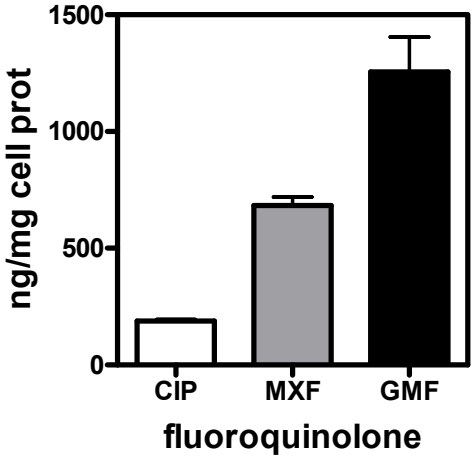
Vallet et al. Int. J. Antimicrob. Ag. (2011) 38:249-256.

Comparing a class of drugs against different bacteria

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

…eventhough they accumulate to variable levels

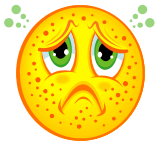




Vallet et al. Int. J. Antimicrob. Ag. (2011) 38:249-256.

Comparing a class of drugs against different bacteria

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



…eventhough they accumulate to variable levels

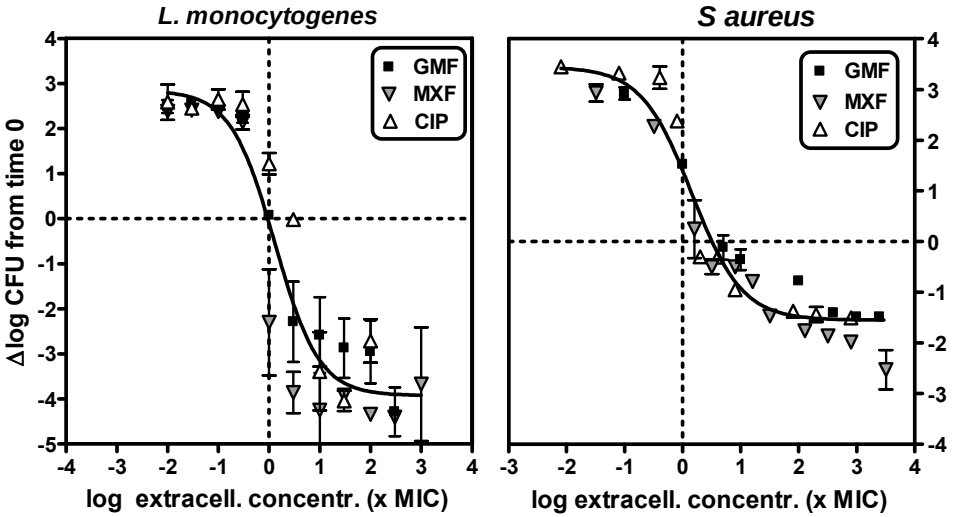
⇨« PK-related » parameter

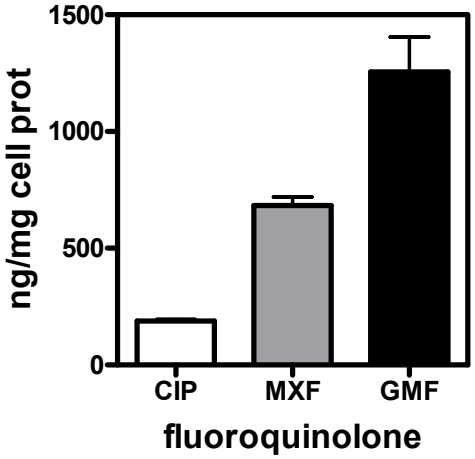
- accumulation ?
- bioavailability ?
- local environment ?

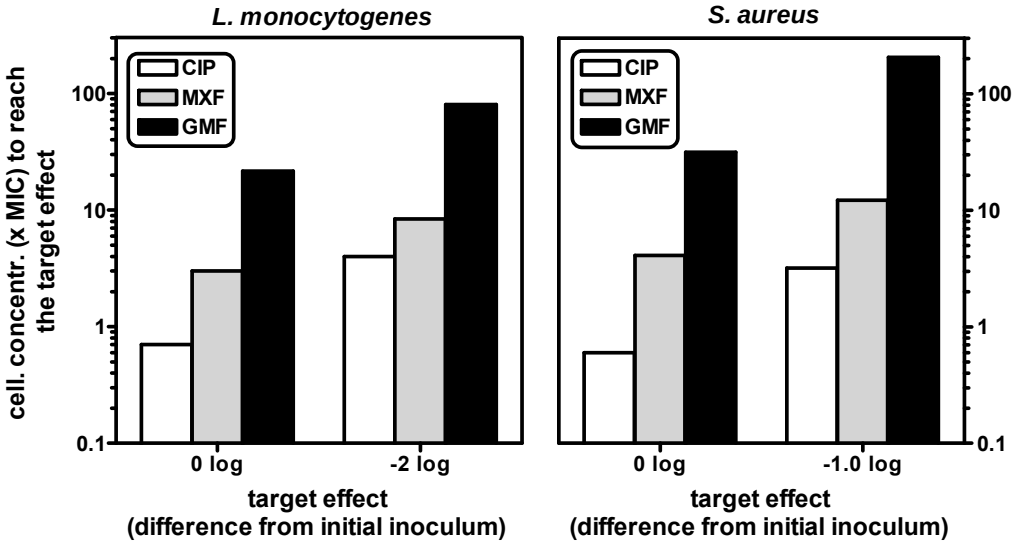
Comparing a class of drugs against different bacteria

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

intracellular bioavailability ?







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

How can we modulate efficacy ?

⇨ « PD-related » parameter

- mode of action of the drug
- bacterial responsiveness
- cooperation with host defenses

How can we modulate efficacy ?

⇨ « PD-related » parameter

- mode of action of the drug
- bacterial responsiveness
- cooperation with host defenses

⇨ **Try combinations ?**

⇨ **Restore growth ?**

⇨ **Add cytokines ?**

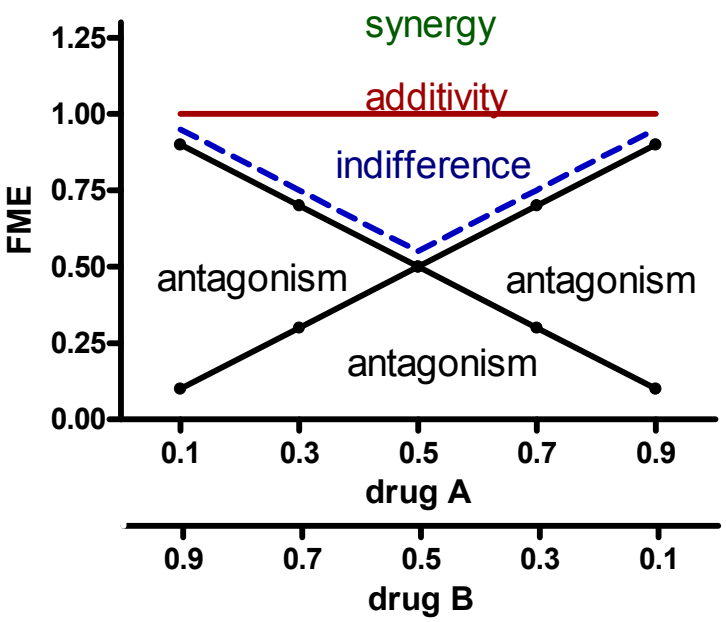
How can we modulate efficacy ?

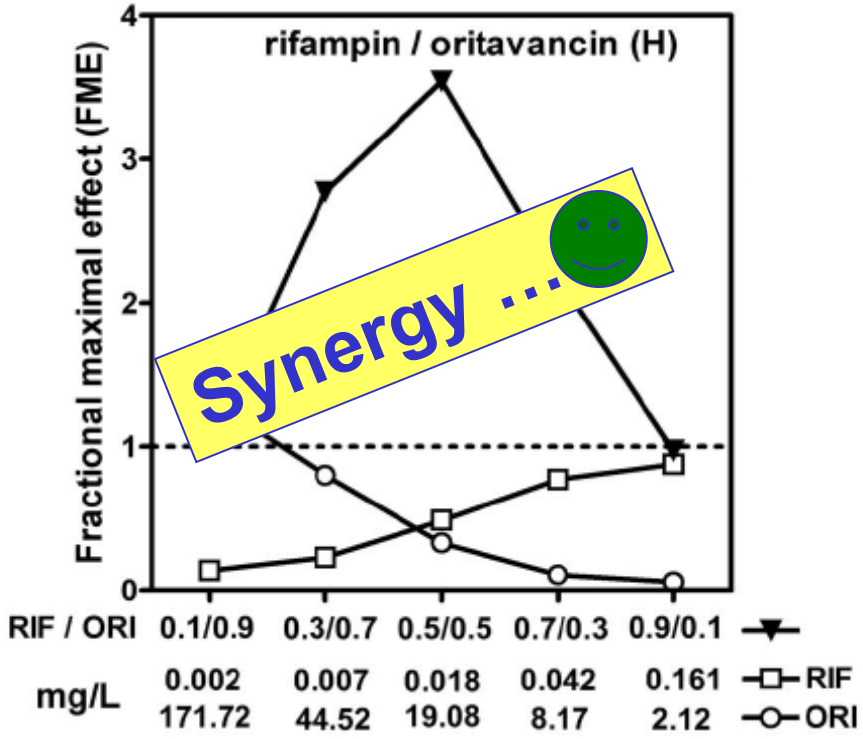
⇨ « PD-related » parameter

- mode of action of the drug
- bacterial responsiveness
- cooperation with host defenses

⇨ **Try combinations ?**

Antibiotic combinations





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

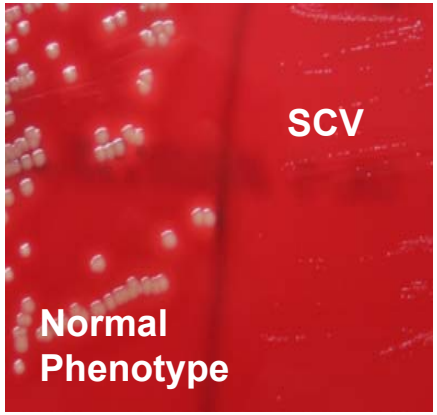
How can we modulate efficacy ?

⇨ « PD-related » parameter

- mode of action of the drug
- bacterial responsiveness
- cooperation with host defenses

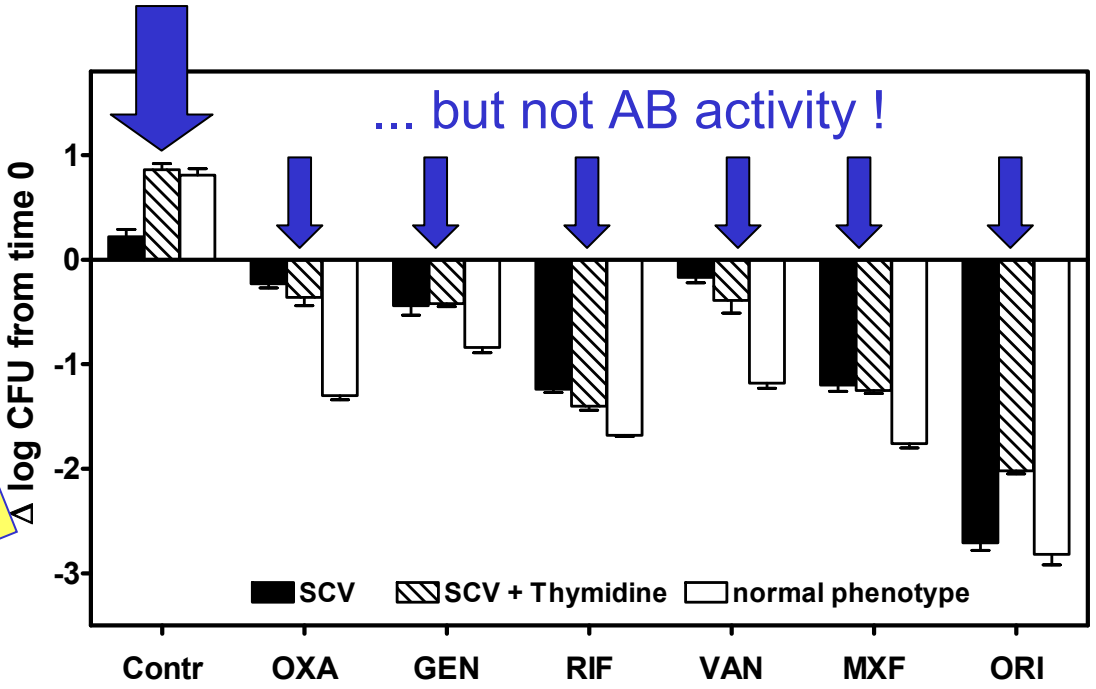
⇨ **Restore growth ?**

Complementing SCV of *S. aureus*



Thy restores
intracellular growth

... but not AB activity !



No systematic link between growth and activity…

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

How can we modulate potency ?

⇨ « PK-related » parameter

- accumulation ?
- bioavailability ?
- local environment ?

How can we modulate potency ?

⇨ « PK-related » parameter

- accumulation ?
- bioavailability ?
- local environment ?

⇨ **Inhibit efflux ?**

⇨ **Select low-binding drugs ?**

⇨ **Modulate pH / ROS ?**

How can we modulate potency ?

⇨ « PK-related » parameter

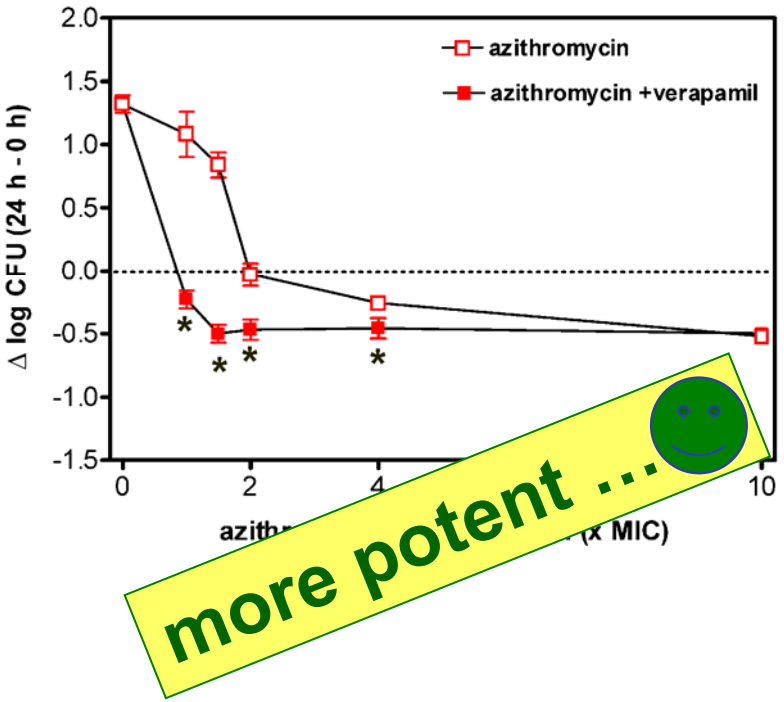
- accumulation ?
- bioavailability ?
- local environment ?

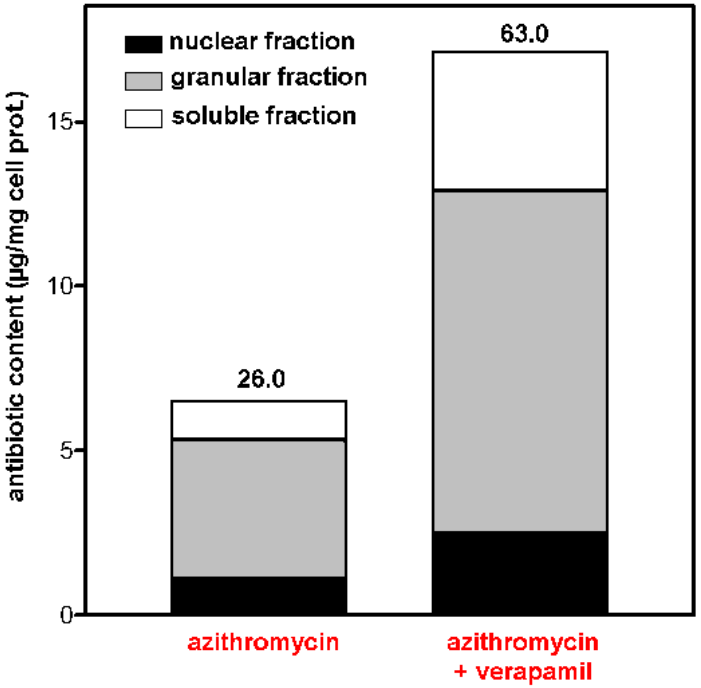
⇨ **Inhibit efflux ?**

Inhibition of active efflux

- intracellular activity
- accumulation in lysosomes

of azithromycin are increased by P-glycoprotein inhibitors





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

How can we modulate potency ?

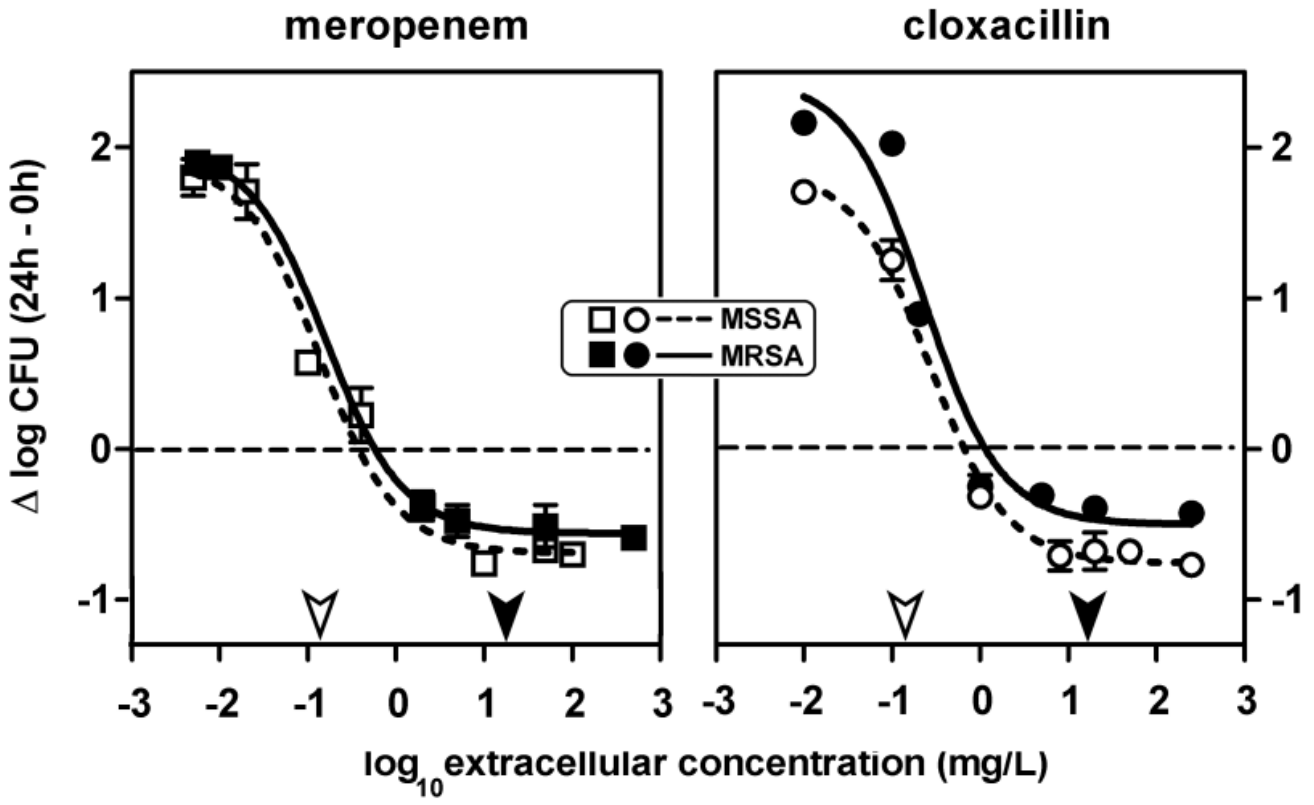
⇨ « PK-related » parameter

- accumulation ?
- bioavailability ?
- local environment ?

⇨ **Modulate pH / ROS ?**

Influence of intracellular pH

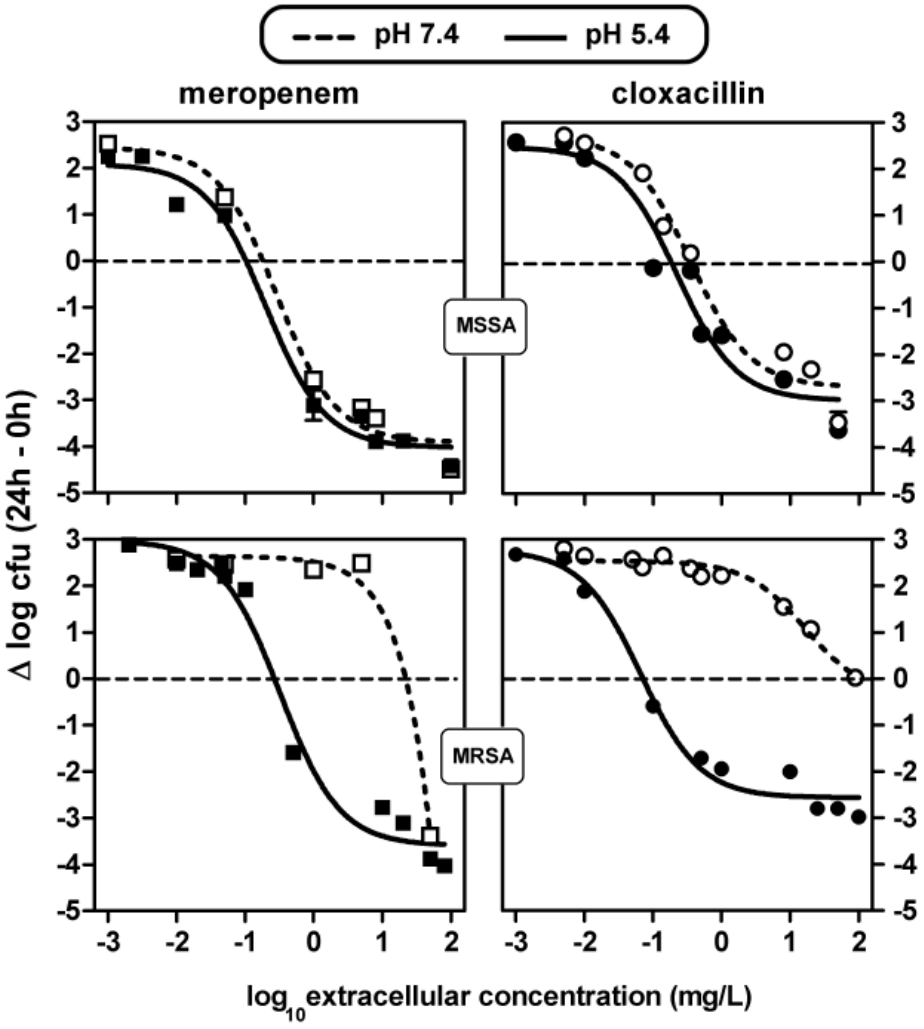
MRSA are as susceptible as MSSA to β-lactams when intracellular !



Lemaire et al., Antimicrob. Ag. Chemother. (2007) 51:1627-32

Influence of intracellular pH

MRSA are as susceptible as MSSA in broth at acidic pH



Lemaire et al., Antimicrob. Ag. Chemother. (2007) 51:1627-32

PBP2a conformation is modified by acidic pH

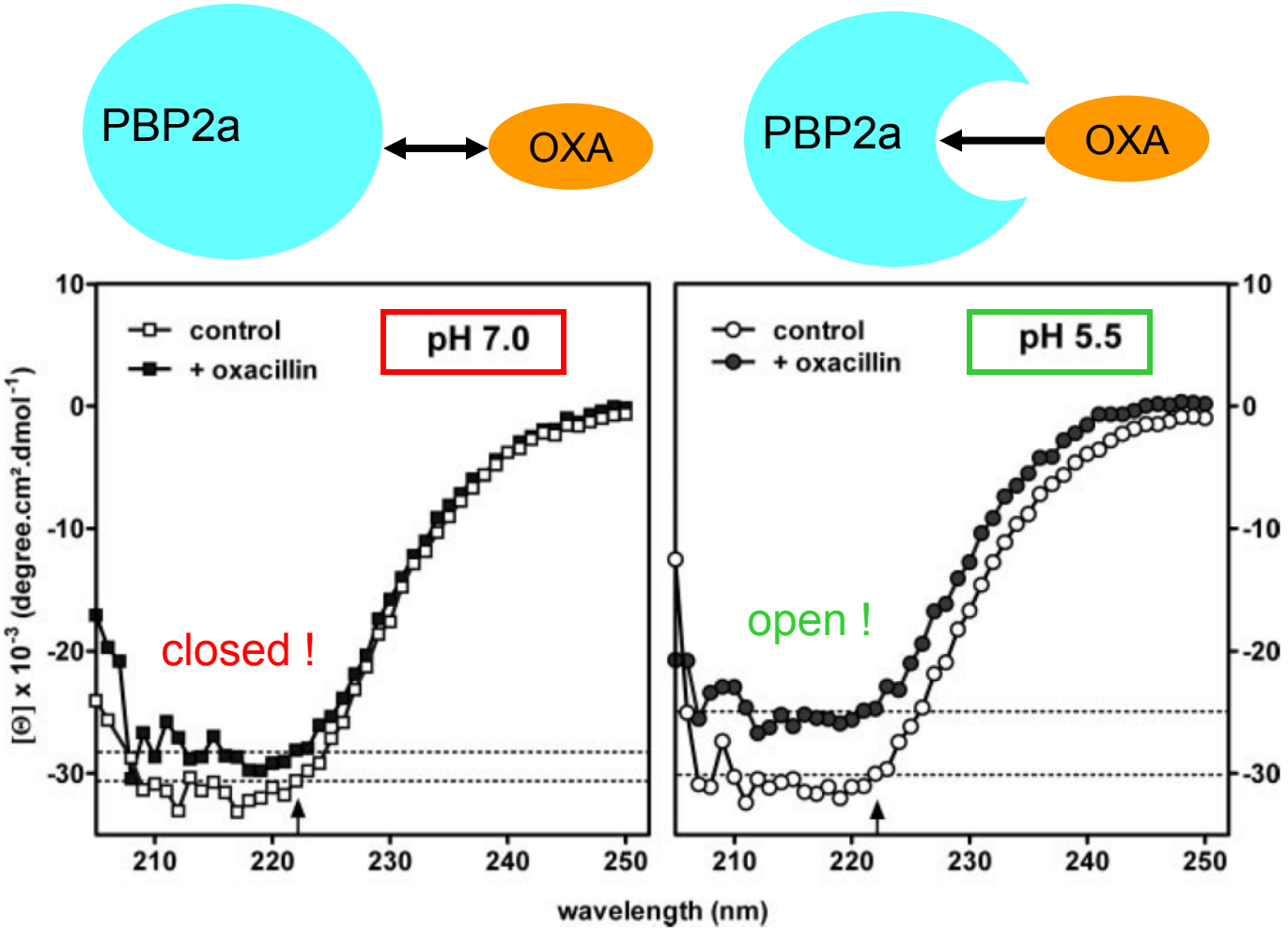
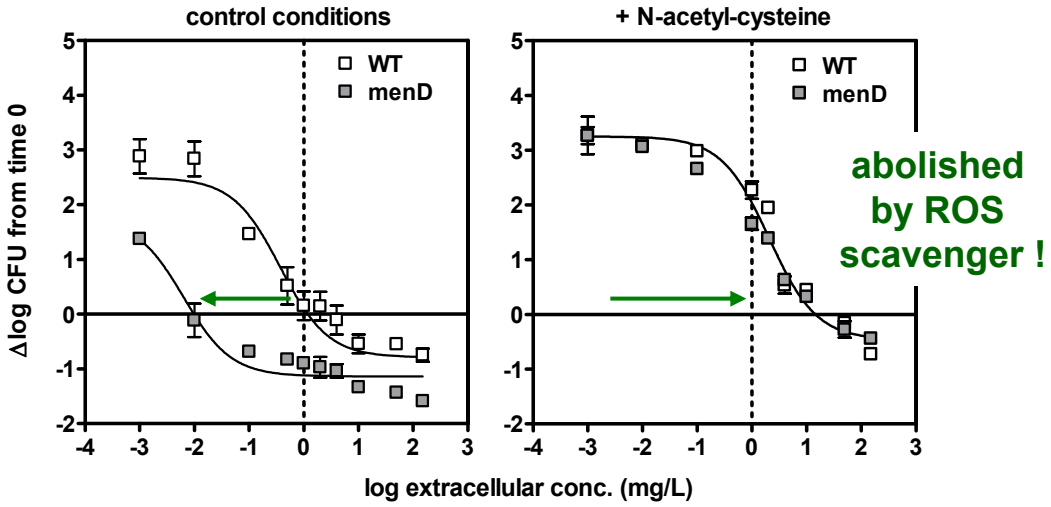


FIGURE 4. **Circular dichroic spectra of PBP 2a at pH 7. 0 (*left panel*) and pH 5.5 (*right panel*) in the absence (*open symbols*) and in the presence (*closed symbols*) of oxacillin (30 μM) for 30 min at 25 °C.** The *thin dotted lines* in each graph represent minima of PBP 2a molar ellipticity at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

Lemaire et al., J Biol Chem (2008) 283:12769-76

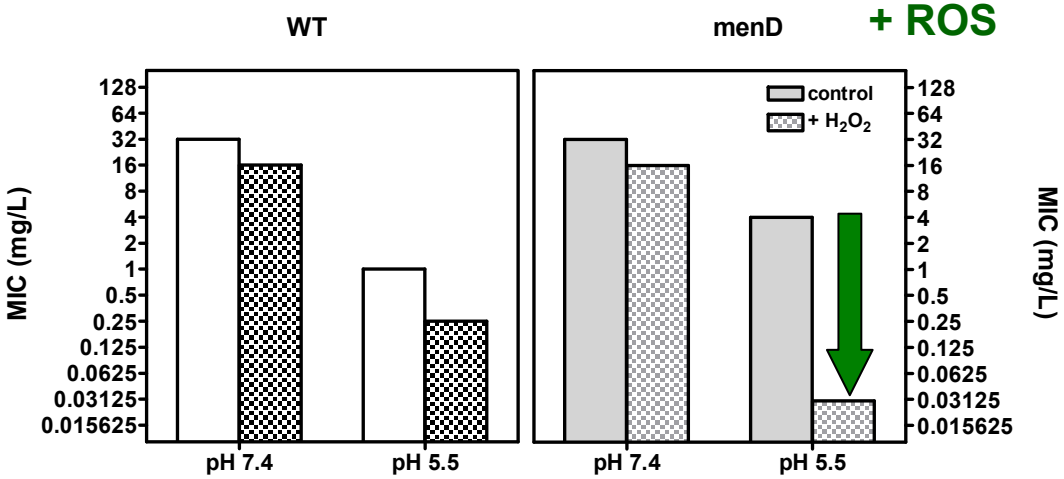
Cooperation between acid pH and ROS

Menadione-dependent SCV of MRSA are hypersusceptible to β-lactams intracellularly ….



abolished by ROS scavenger !

MIC ↘↘ if acid pH + ROS



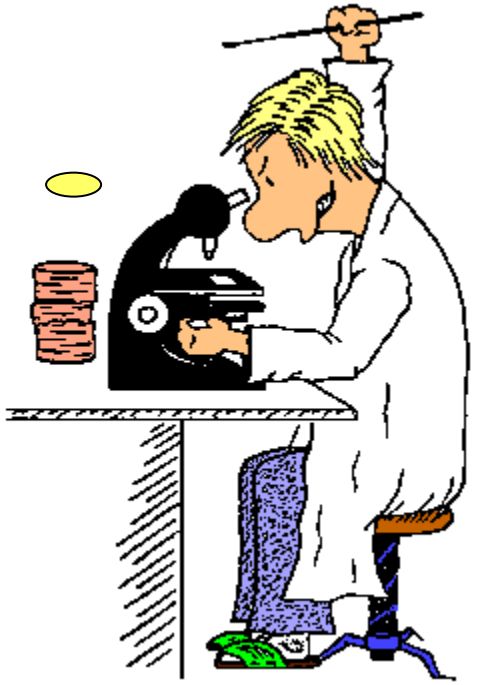
data shown for meropenem

Garcia et al., ICAAC 2012 ; A 596

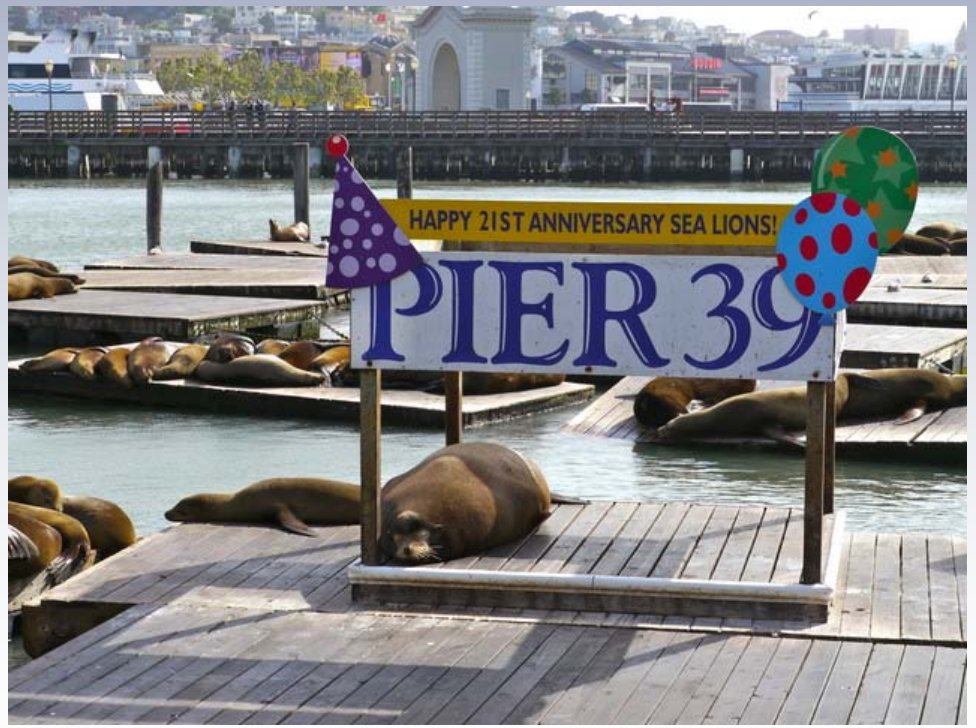
Conclusion: what have we learned so far ?

Now, I know how to catch them !

- high intracellular bioavailability
- 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



From in vitro to in vivo: is this a difficult exercise ?

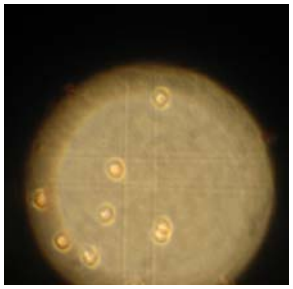
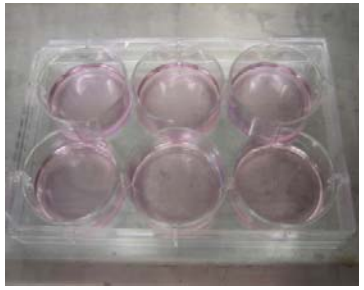


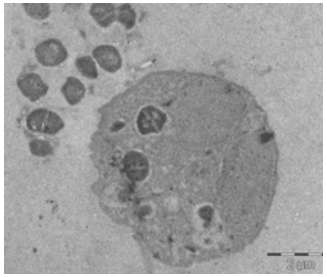
Why in vivo model?

- Appliance of a whole body system

↓

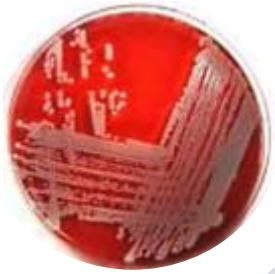
- Full functional immune defence system
- Whole body pharmacokinetics
- Closer to the clinical situation ????
- Performance of both intra- and extracellular PK/PD studies

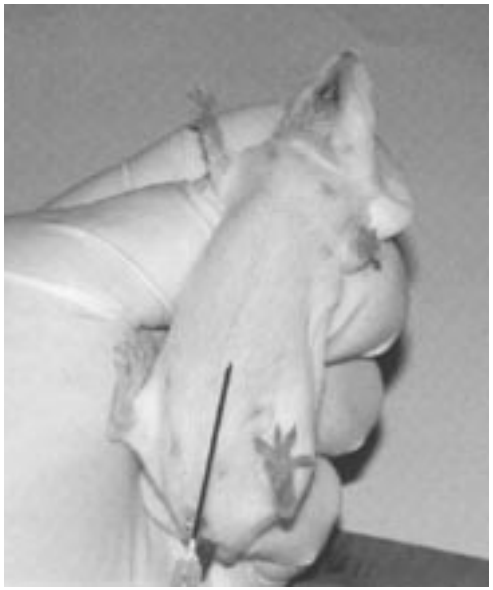






The mouse peritonitis model

Intra- and extracellular activity of antibiotics against *S. aureus*





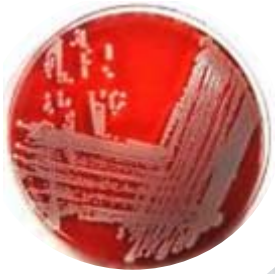
Inoculation:

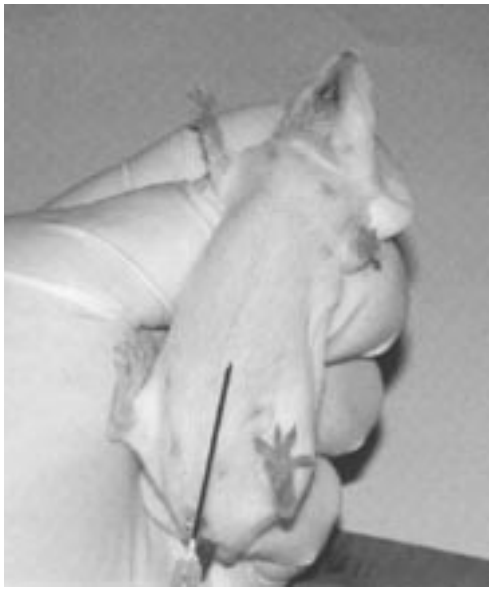
- Intraperitoneal injection of *S.aureus*

Sandberg et al., Antimicrob Agents Chemother (2009) 53:1874-1883

The mouse peritonitis model

Intra- and extracellular activity of antibiotics against *S. aureus*



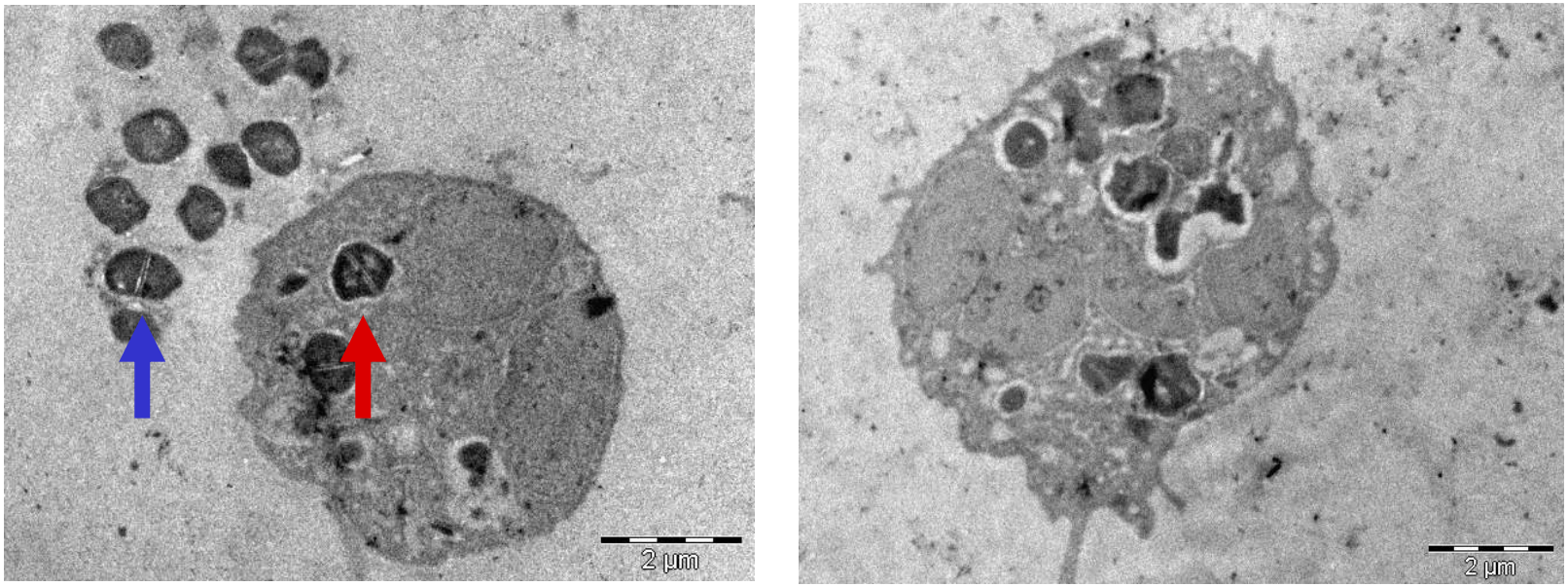


Inoculation:

- Intraperitoneal injection of *S.aureus* ⟶ peritonitis (2 hr)

Sandberg et al., Antimicrob Agents Chemother (2009) 53:1874-1883

The mouse peritonitis model



Electron microscopy of peritoneal fluid post infection with *S. aureus*

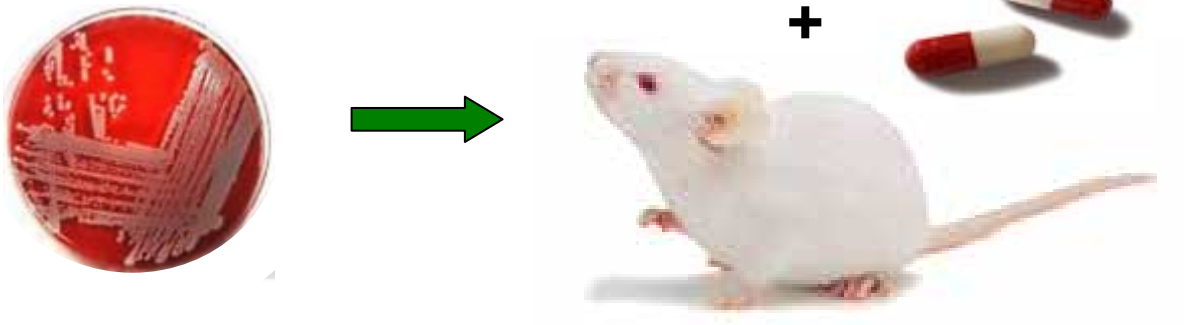
Extracellular *S. aureus*

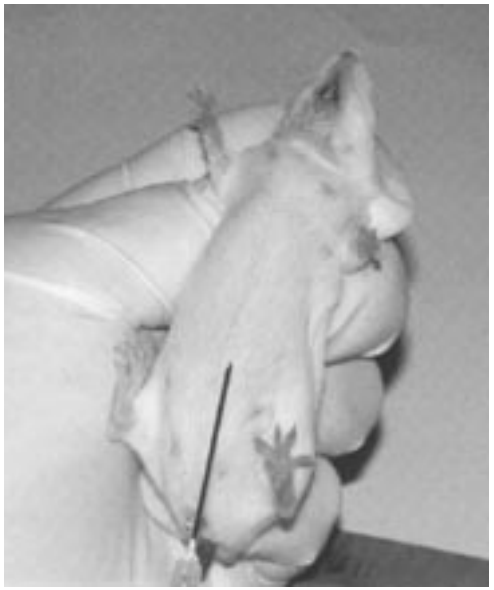
Intracellular *S. aureus*

Sandberg et al., Antimicrob Agents Chemother (2009) 53:1874-1883

The mouse peritonitis model

Intra- and extracellular activity of antibiotics against *S. aureus*





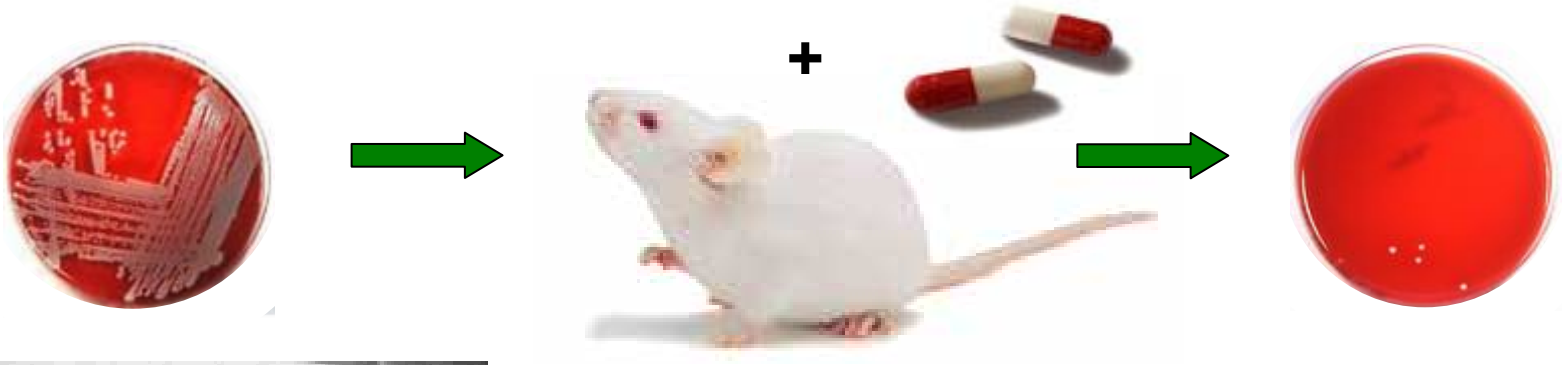
Antibiotic treatment

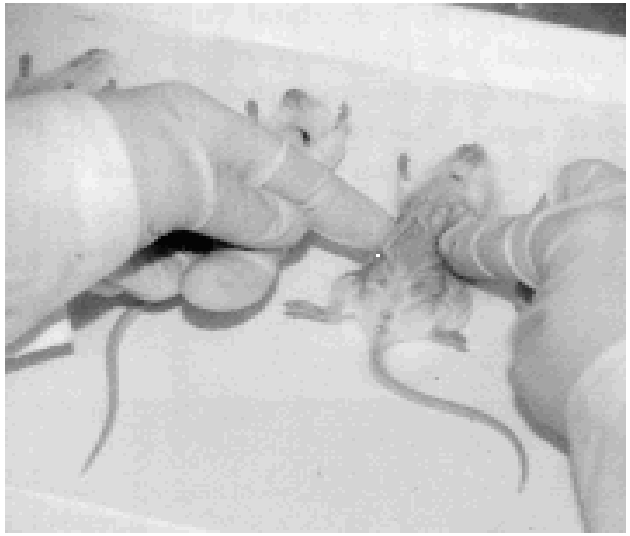
- Intraperitoneal injection of *S.aureus*
- Subcutaneous injection of antibiotic

Sandberg et al., Antimicrob Agents Chemother (2009) 53:1874-1883

The mouse peritonitis model

Intra- and extracellular activity of antibiotics against *S. aureus*





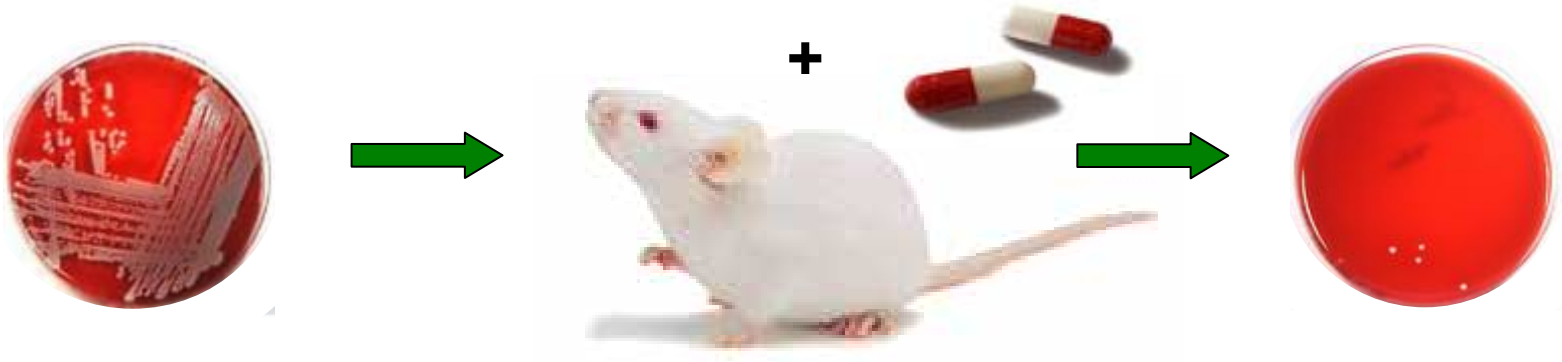
Sampling:

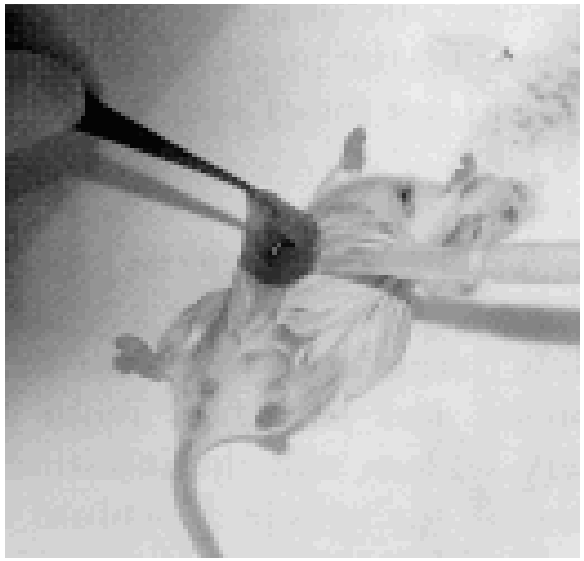
- Euthanasia
- Intraperitoneal injection of HBSS (2 ml) and mix

Sandberg et al., Antimicrob Agents Chemother (2009) 53:1874-1883

The mouse peritonitis model

Intra- and extracellular activity of antibiotics against *S. aureus*



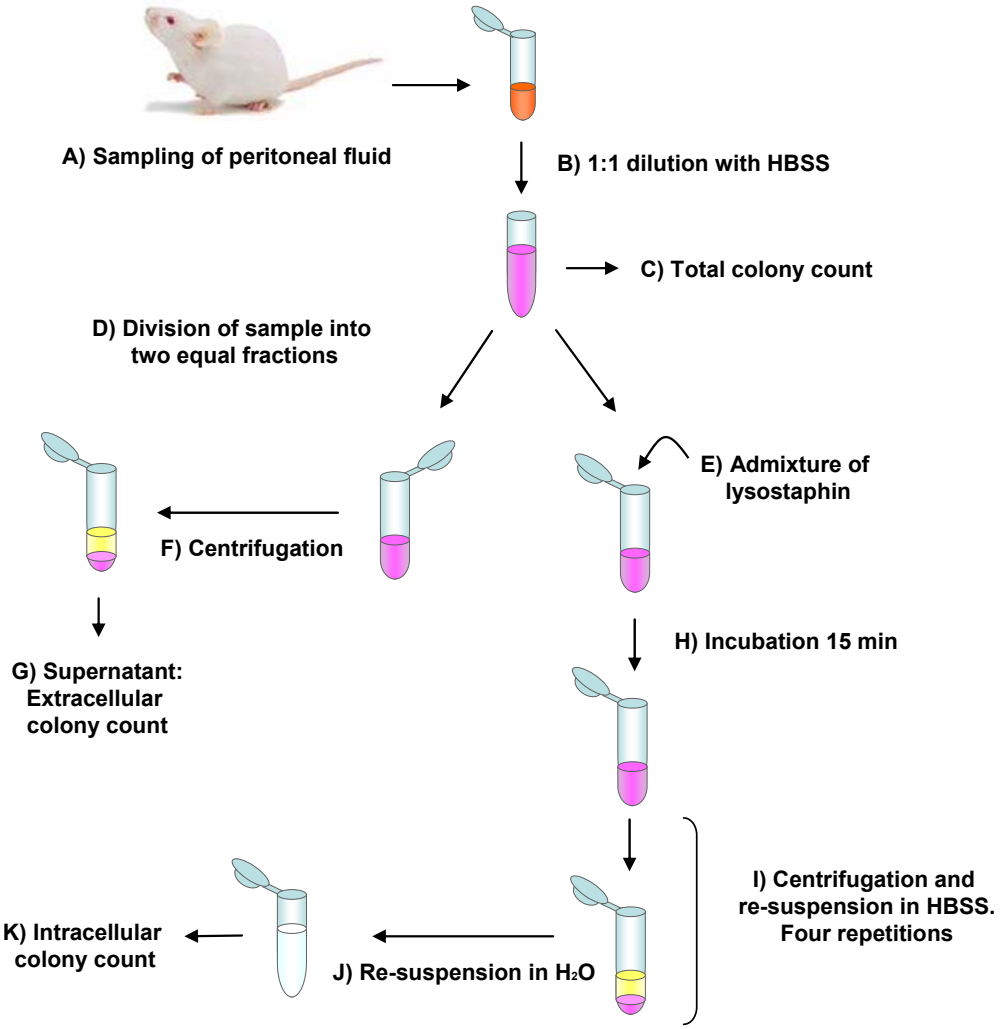


Sampling:

- Euthanasia
- Intraperitoneal injection of HBSS (2 ml) and mix
- Collection of peritoneal fluid through incision

Sandberg et al., Antimicrob Agents Chemother (2009) 53:1874-1883

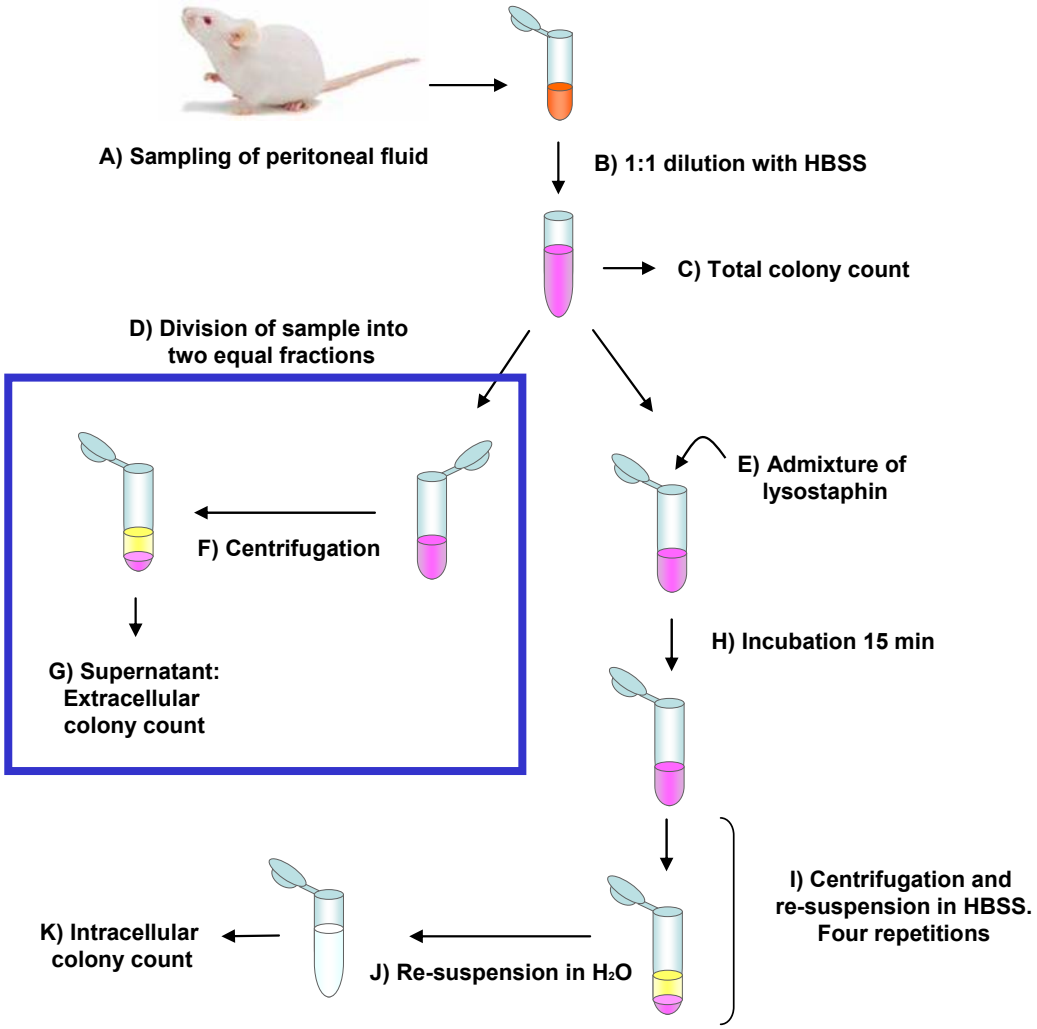
Separation of intra- and extracellular bacteria



Division of sample into two equal fractions

Sandberg et al., Antimicrob Agents Chemother (2009) 53:1874-1883

Separation of intra- and extracellular bacteria

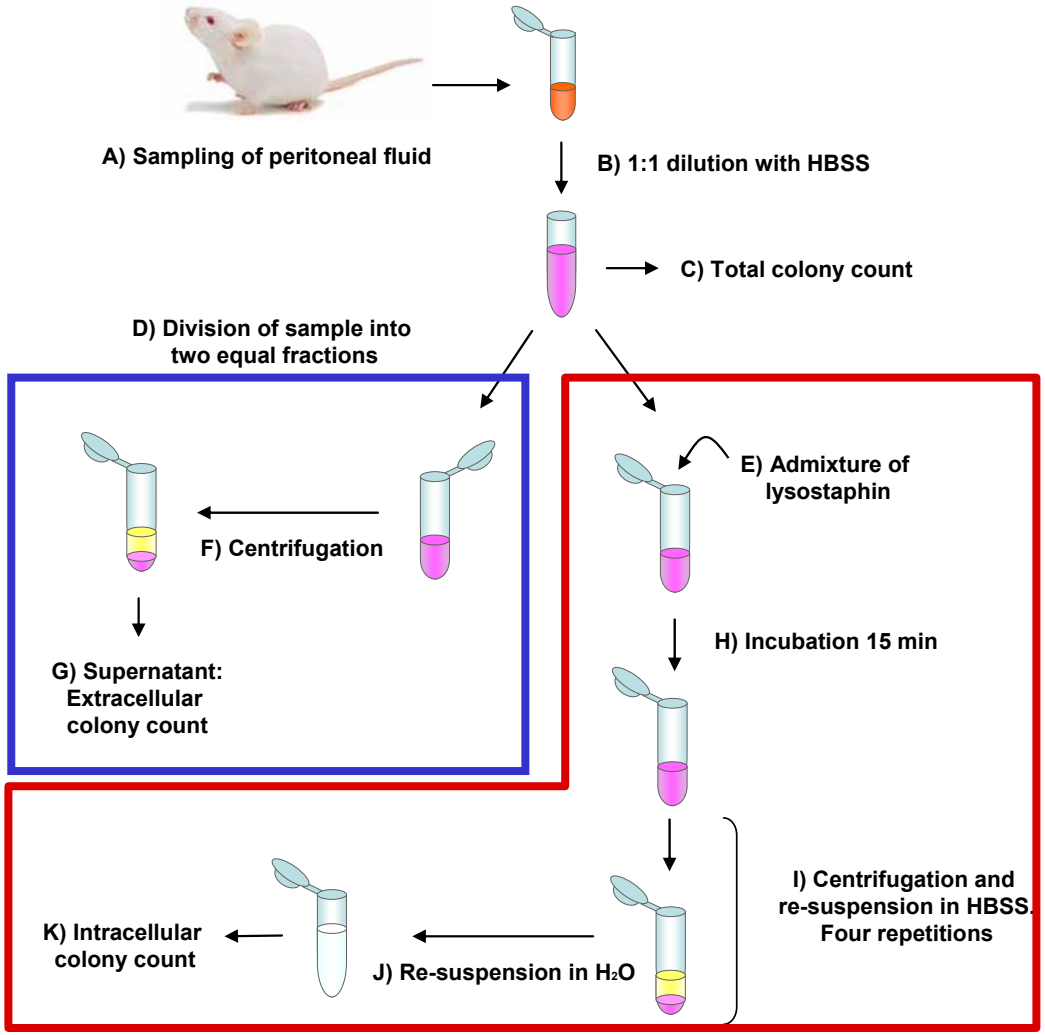


Division of sample into two equal fractions

Fraction A:
Extracellular *S. aureus* estimated from supernatant after centrifugation

Sandberg et al., Antimicrob Agents Chemother (2009) 53:1874-1883

Separation of intra- and extracellular bacteria



Division of sample into two equal fractions

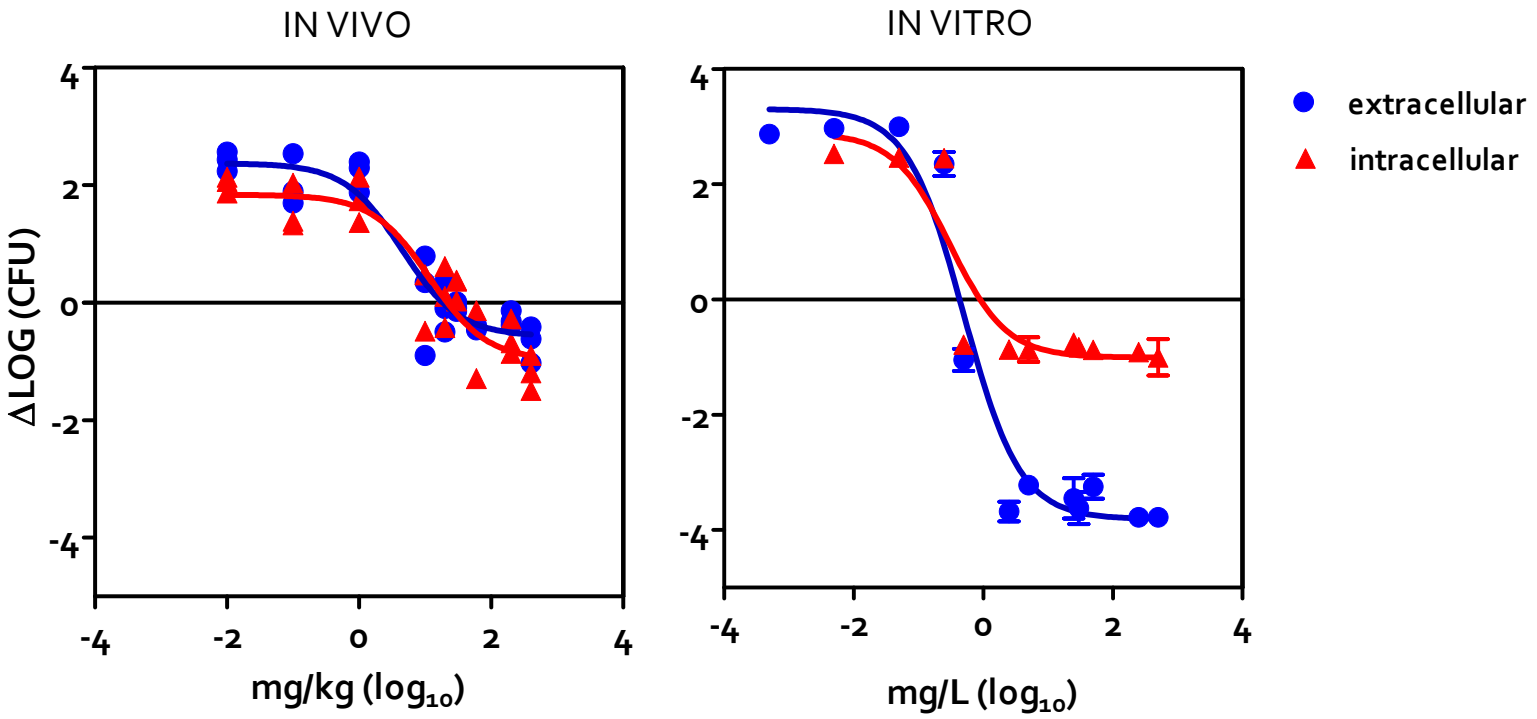
Fraction A:
Extracellular *S. aureus* estimated from supernatant after centrifugation

Fraction B:
Intracellular *S. aureus* estimated after incubation with lysostaphin, lysostaphin wash-out, and lysis with H_2O

Sandberg et al., Antimicrob Agents Chemother (2009) 53:1874-1883

Dose-response studies

DICLOXACILLIN vs. *S. aureus*

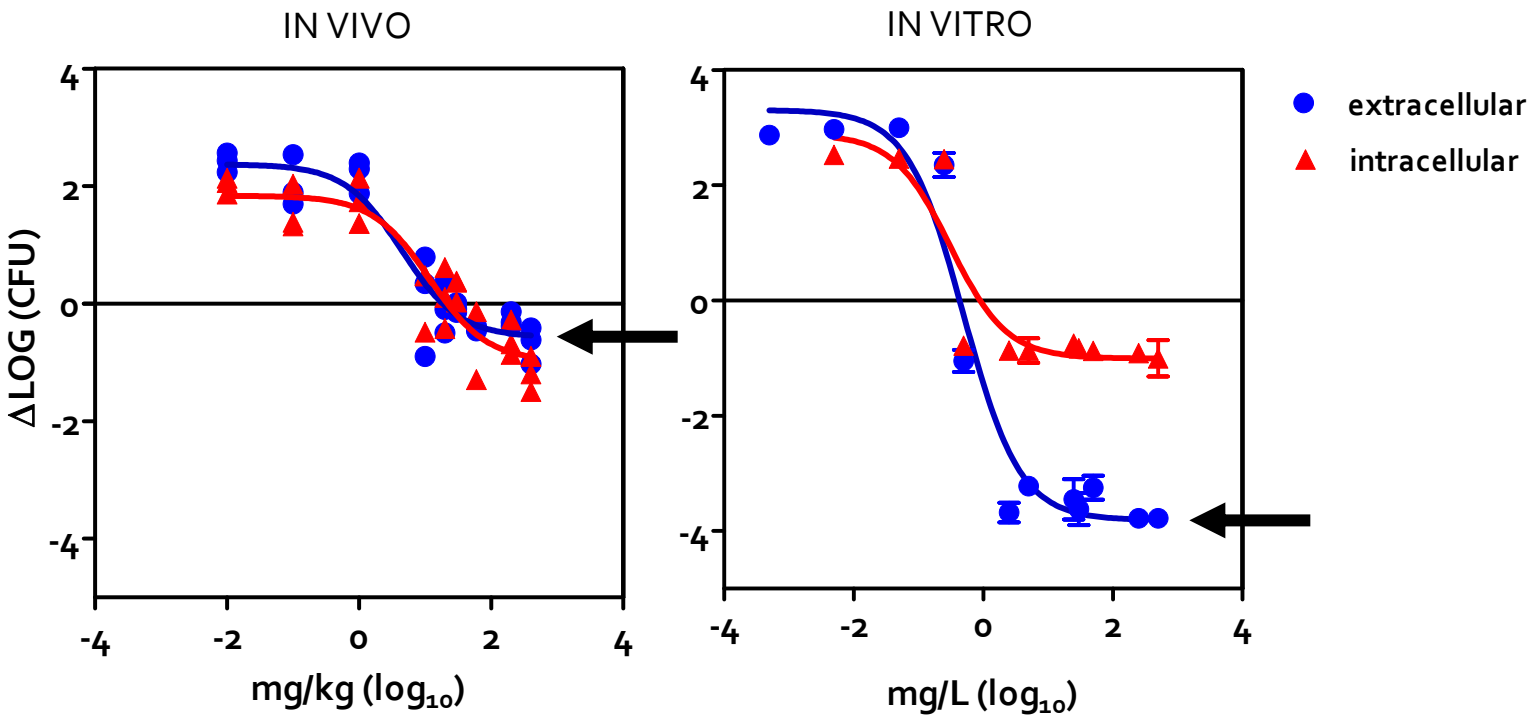


Δlog(CFU) = changes in colony counts compared to the original inoculum (treatment outcome)

Sandberg et al., Antimicrob Agents Chemother (2010) 54:2391-2400

Dose-response studies

DICLOXACILLIN vs. *S. aureus*

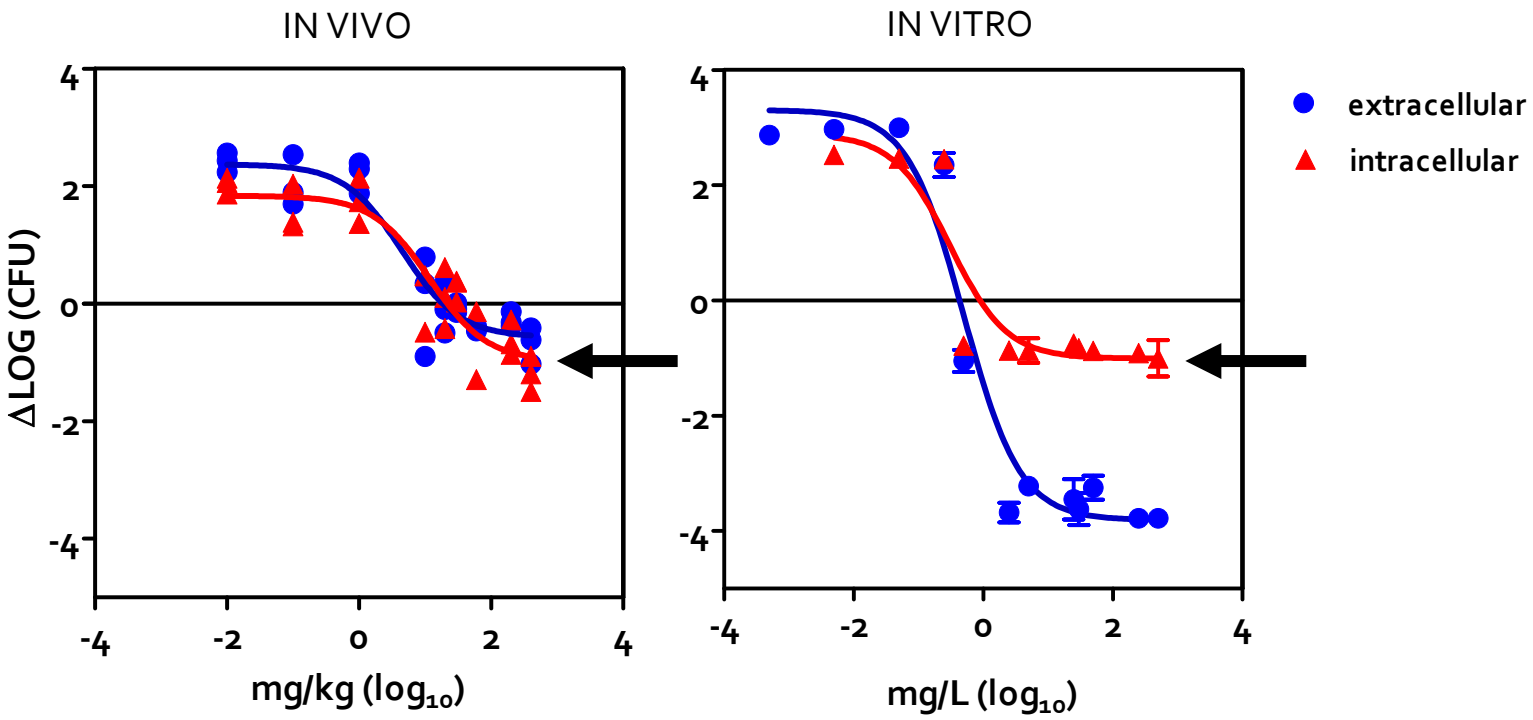


Extracellular activity: dissimilar results were obtained in vitro and in vivo

Sandberg et al., Antimicrob Agents Chemother (2010) 54:2391-2400

Dose-response studies

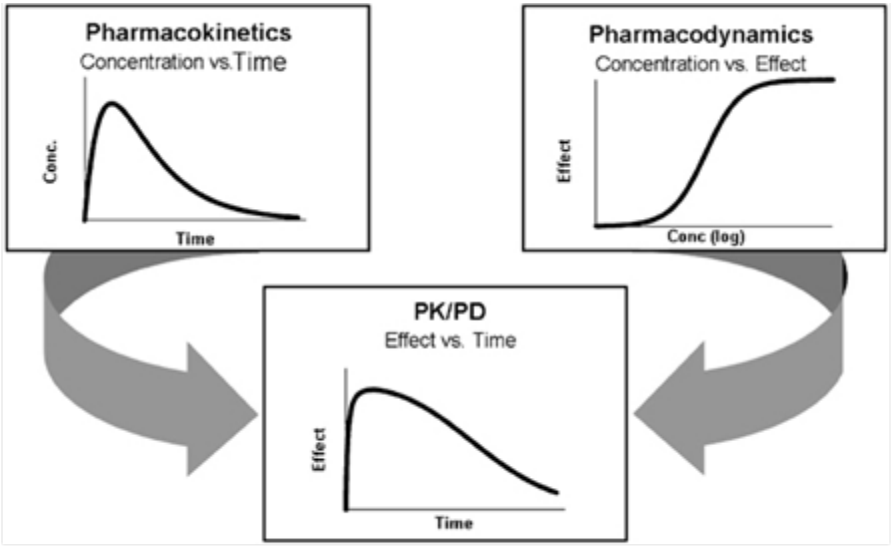
DICLOXACILLIN vs. *S. aureus*



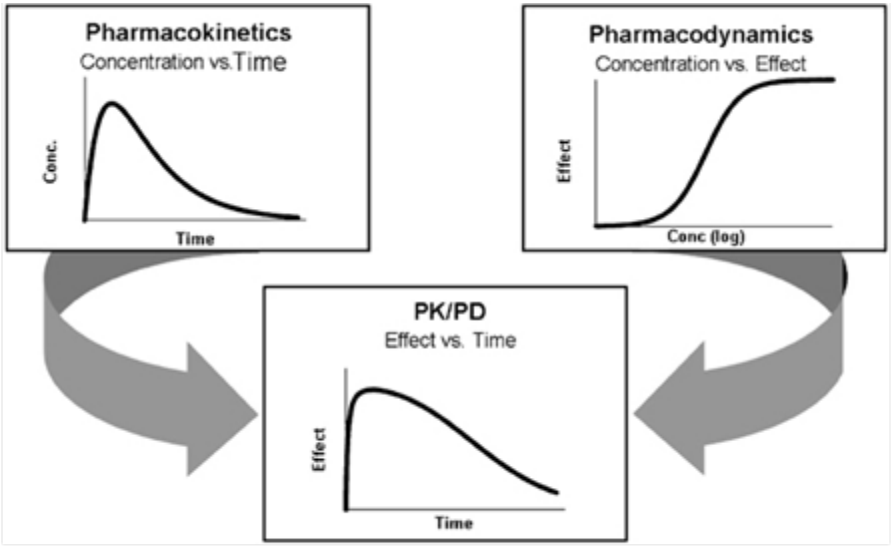
Intracellular activity: similar results were obtained in vitro and in vivo

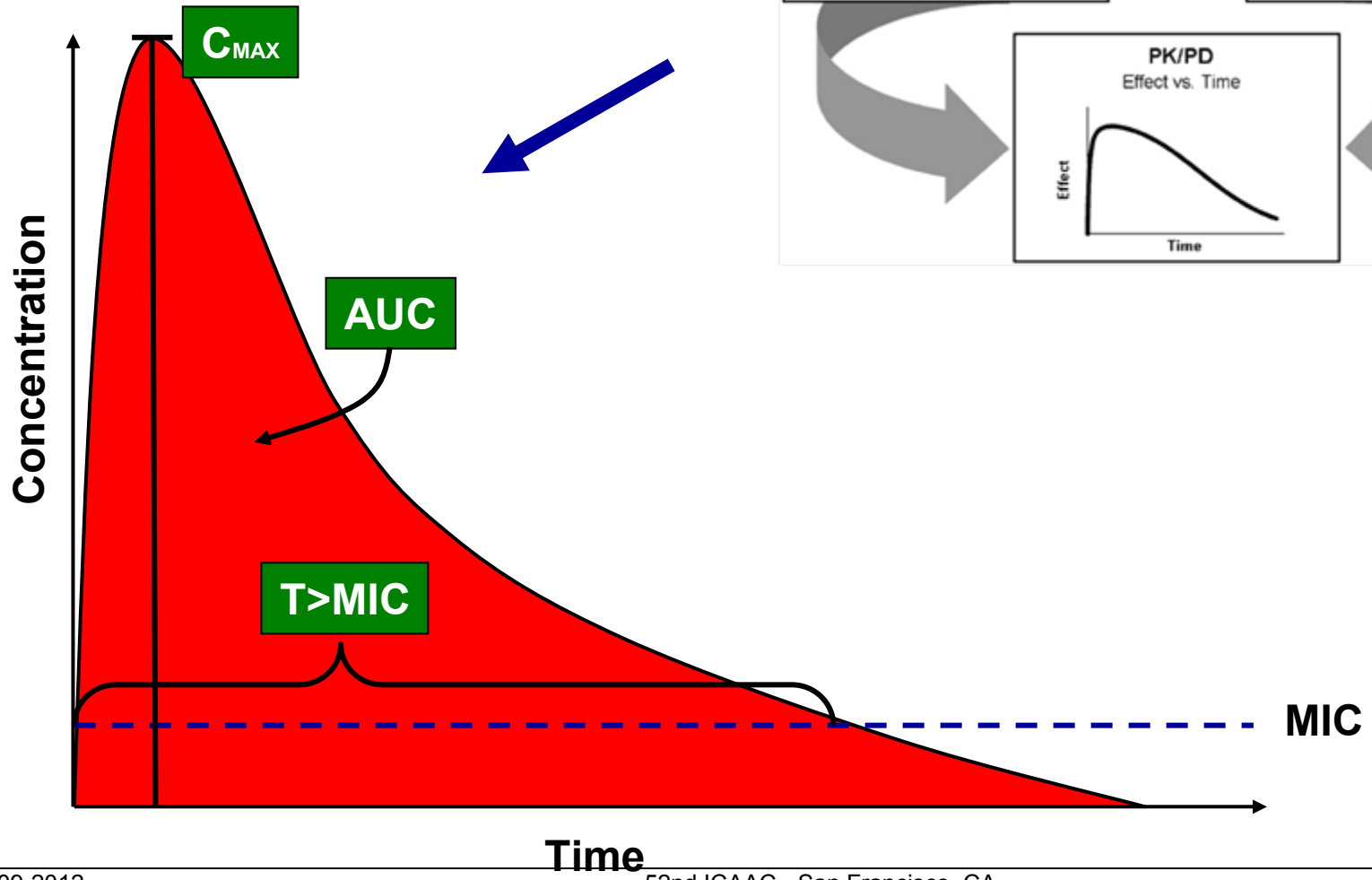
Sandberg et al., Antimicrob Agents Chemother (2010) 54:2391-2400

PK/PD studies in practice…….

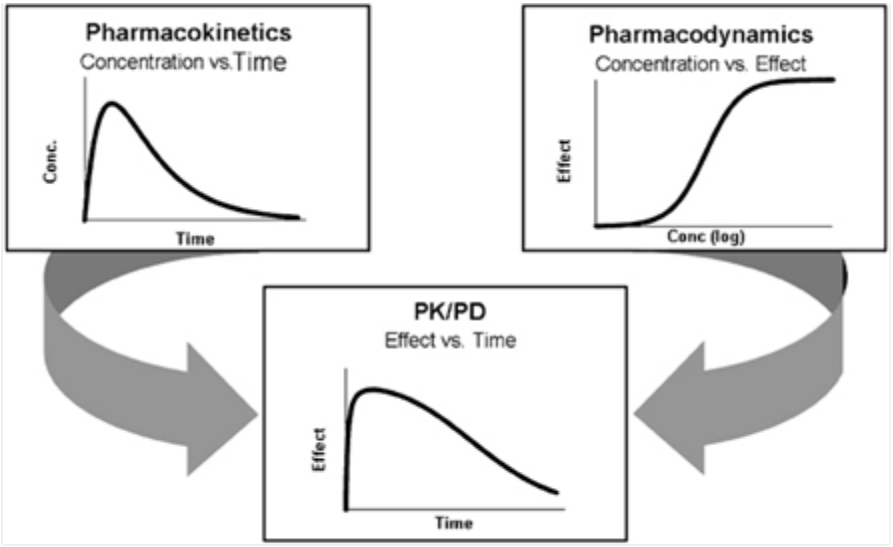


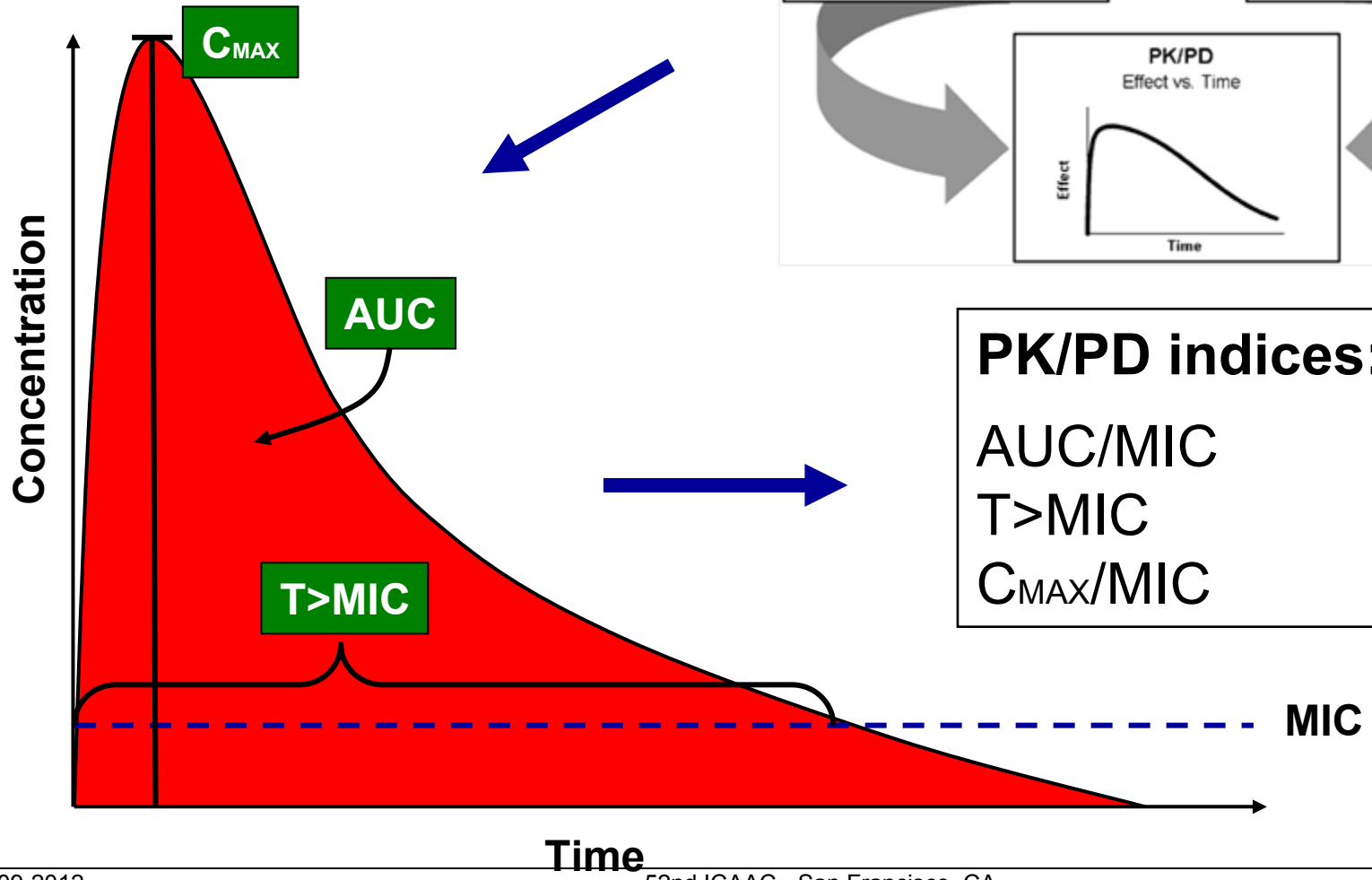
PK/PD studies in practice…….





PK/PD studies in practice…….

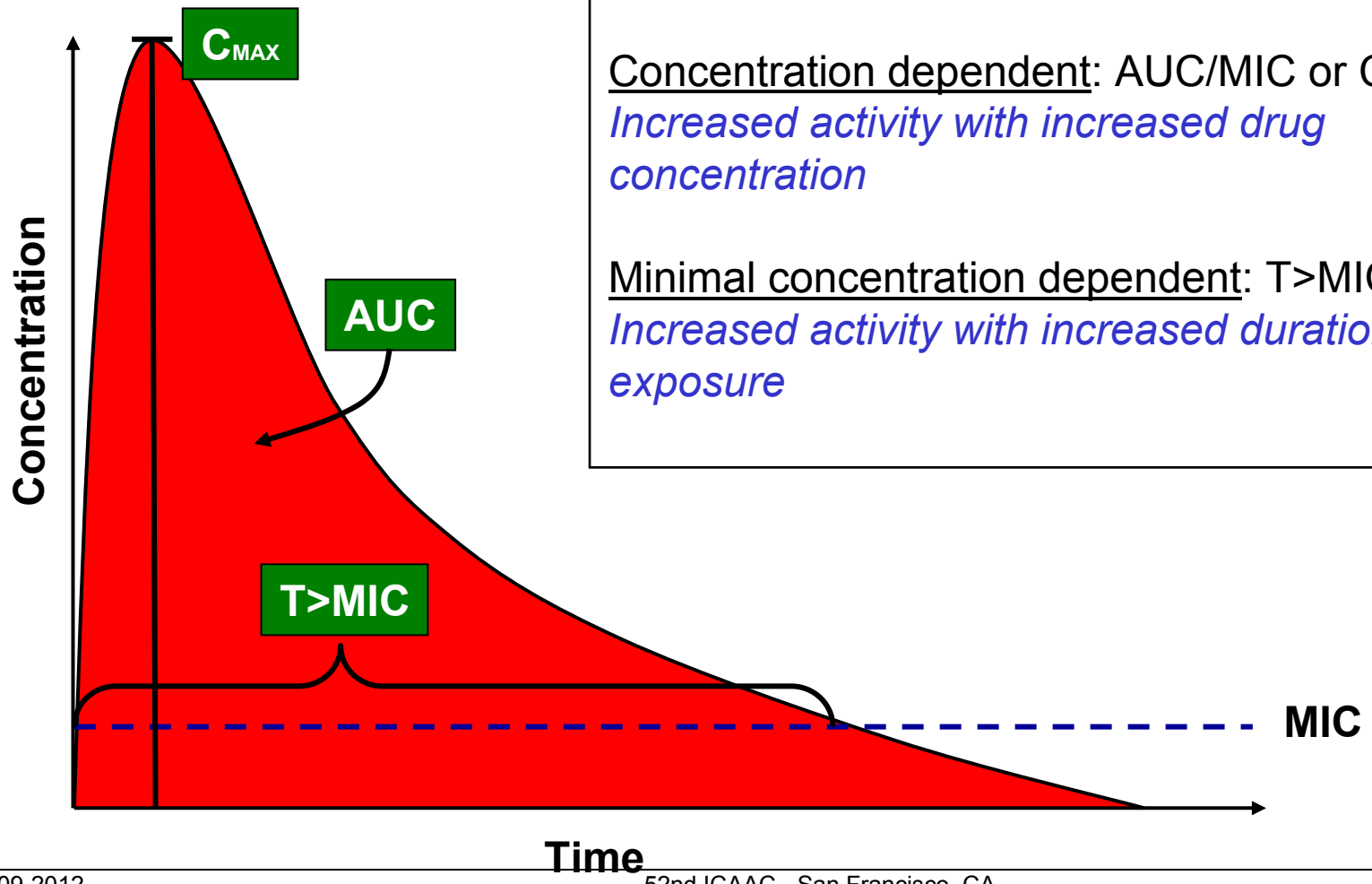




PK/PD indices:

AUC/MIC
T>MIC
C_{MAX}/MIC

PK/PD studies in practice…….

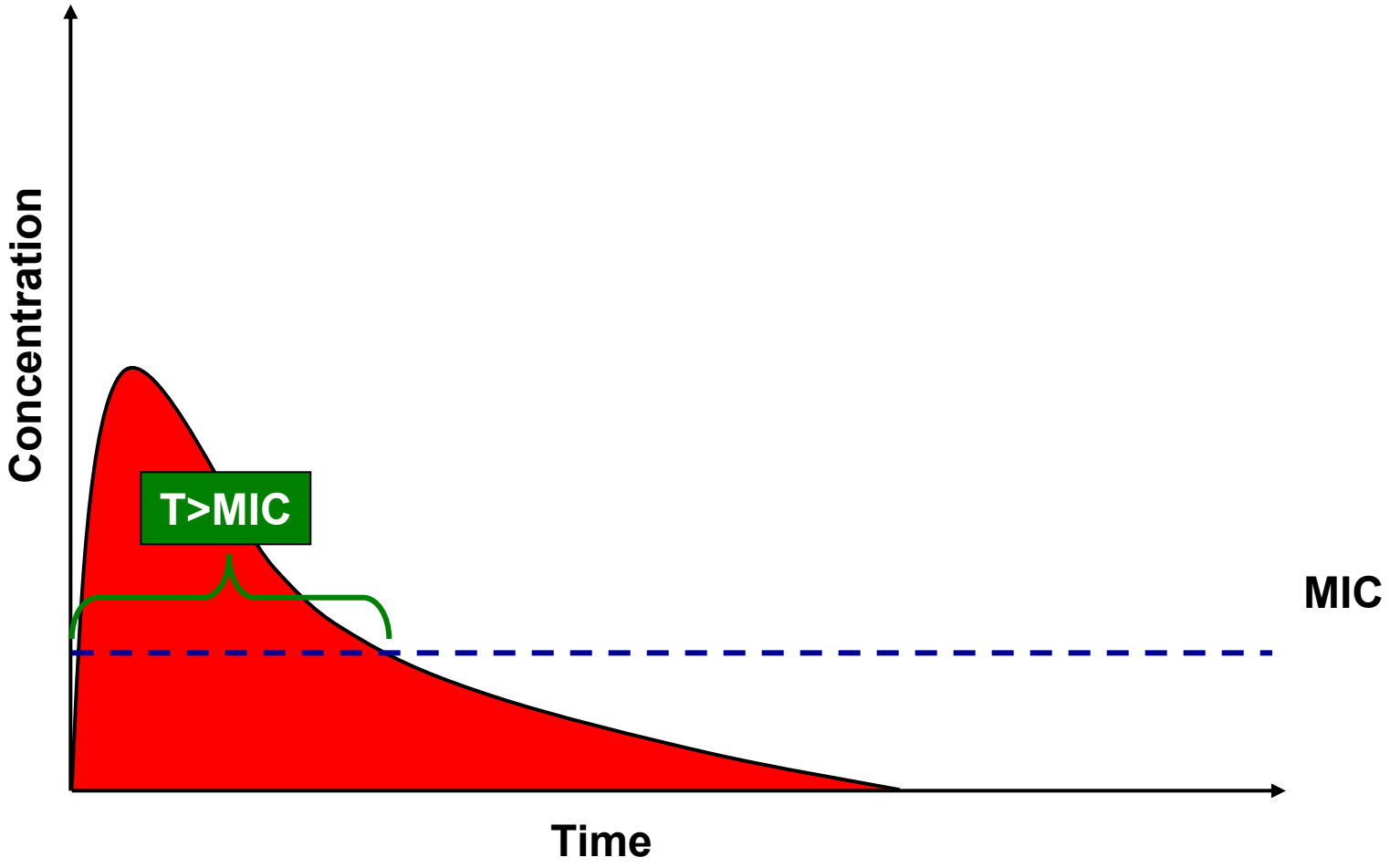


PK/PD indices related to antibiotic activity:

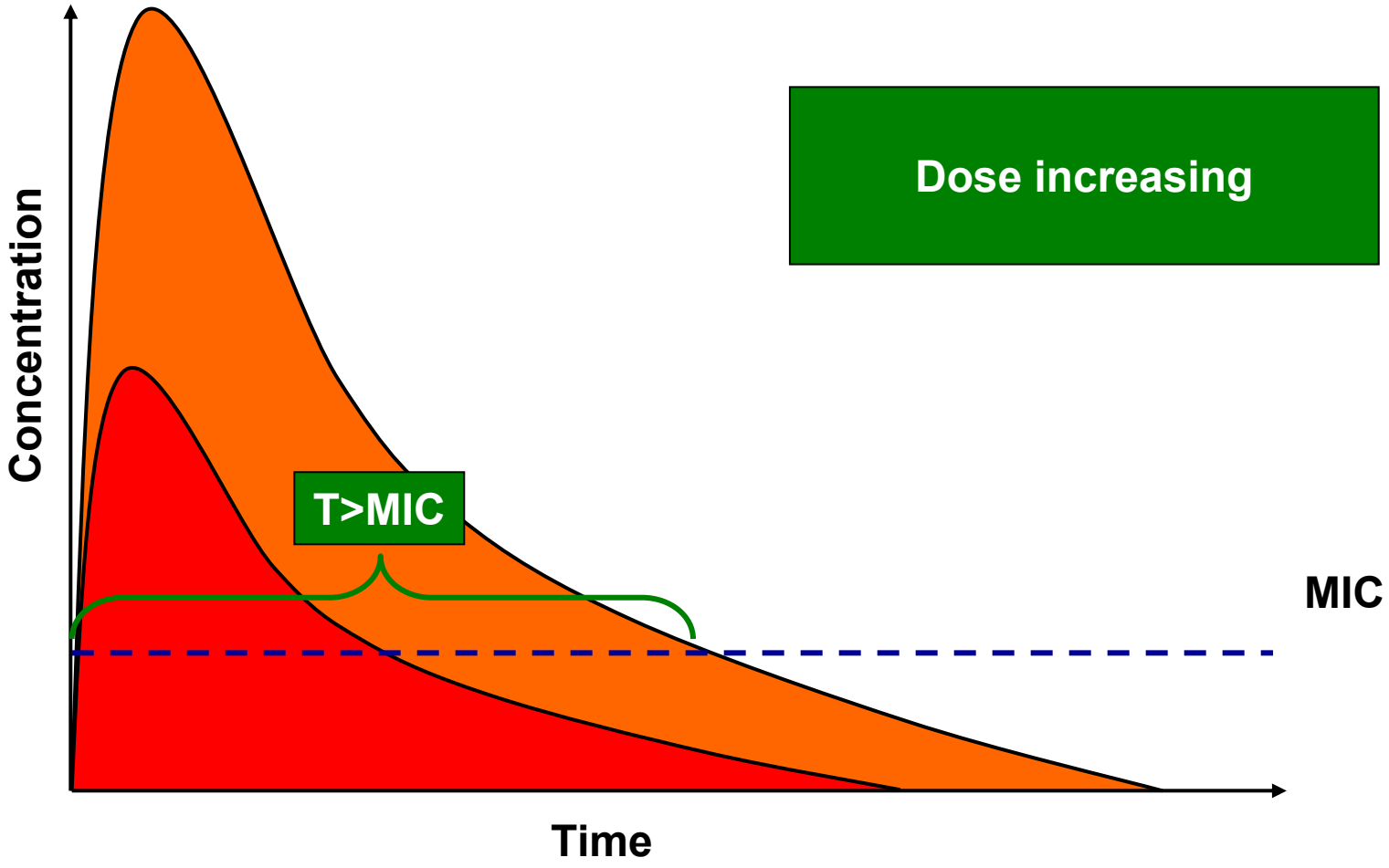
Concentration dependent: AUC/MIC or C_{MAX}/MIC
Increased activity with increased drug concentration

Minimal concentration dependent: T>MIC
Increased activity with increased duration of drug exposure

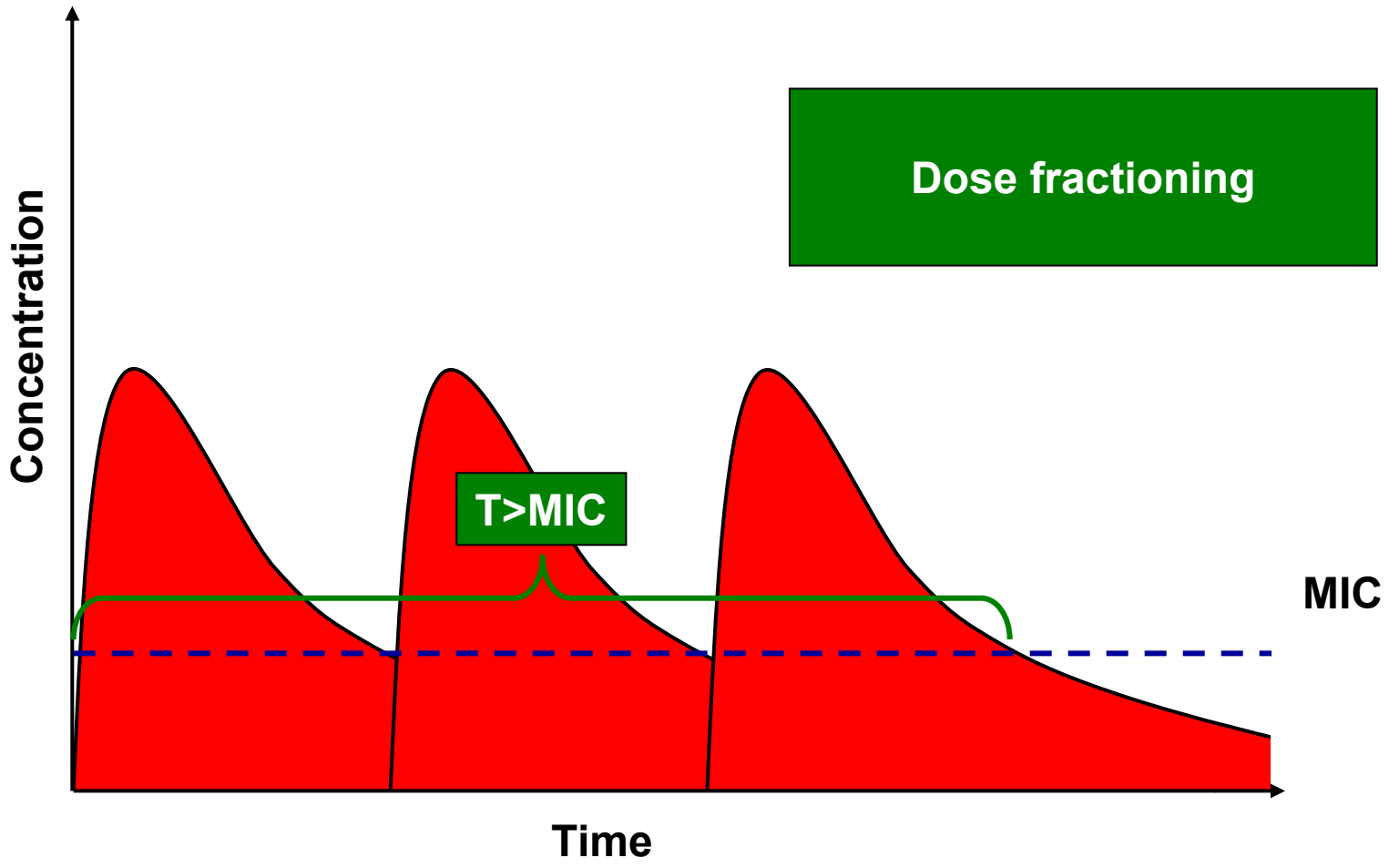
Increase of drug exposure (T>MIC)



Increase of drug exposure (T>MIC)



Increase of drug exposure (T>MIC)



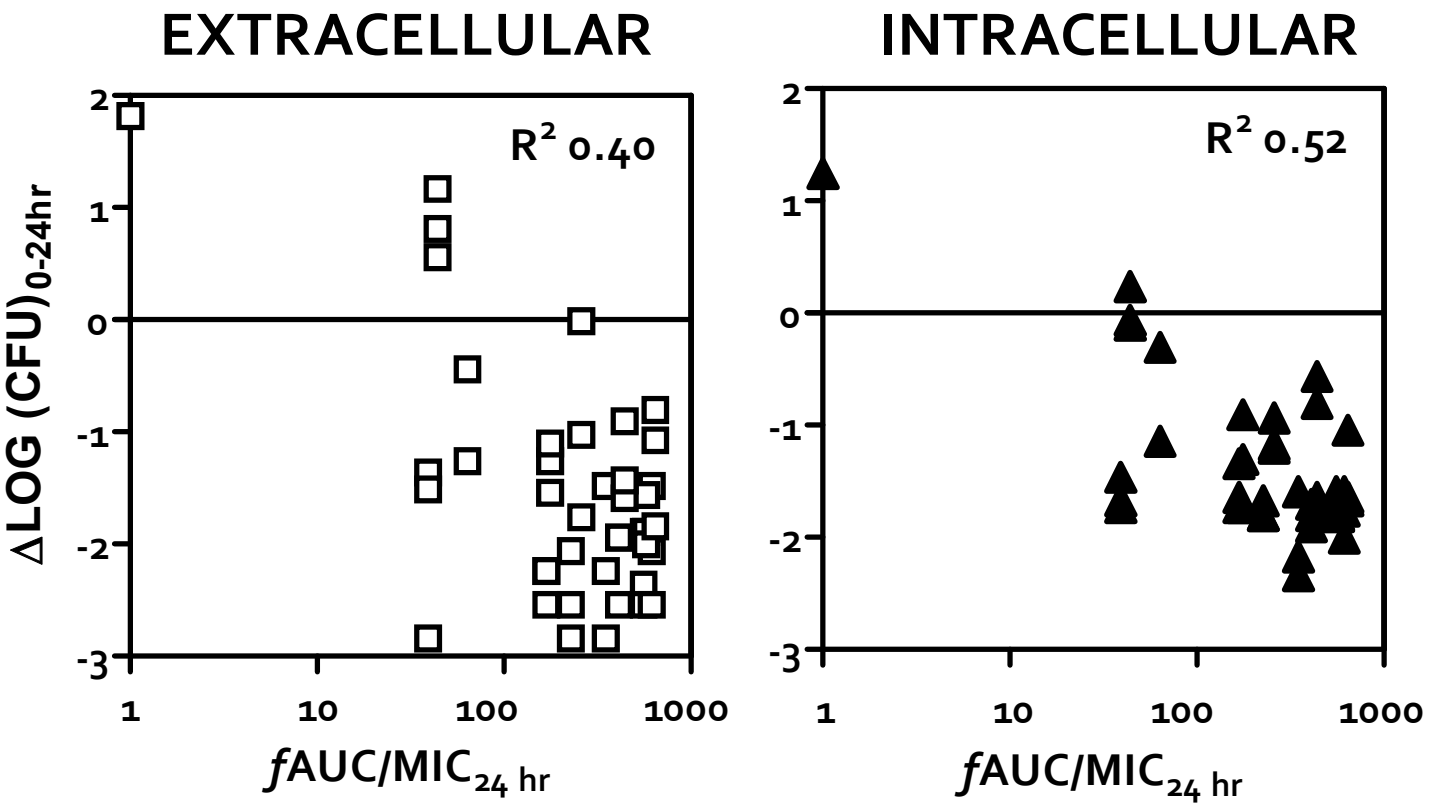
PK/PD studies: Dicloxacillin vs *S. aureus*

Pharmacokinetic studies → **PKPDsim** ← **Protein binding studies**

MATHEMATICAL MODELLING OF PHARMACOKINETICS AND PHARMACODYNAMICS (PK/PD)

Dosing regimen no.	Dose (mg/kg)	Dosing interval[a]	Cumulative total dose (mg/24 kg · h)	Result for the following PK/PD index (free drug):		
				% fT_{MIC}	$fAUC_{24}$/MIC (h)	fC_{max}/MIC
1	76	q2h	912	100.00	227.43	28.18
2	30	q2h	360	45.83	39.32	6.27
3	80	q3h	640	66.67	168.69	30.64
4	120	q3h	960	75.56	350.73	61.60
5	160	q3h	1,280	96.11	556.40	81.58
6	200	q4h	1,200	86.25	616.07	101.92
7	80	q8h	240	25.00	63.49	30.64
8	240	q6h	960	65.28	572.83	136.18
9	200	q6h	800	57.50	411.61	101.92
10	120	q6h	480	37.78	175.82	61.60
11	400	q12h	800	35.97	644.84	291.53
12	300	q12h	600	35.28	439.15	196.76
13	340	q24h	340	17.78	259.37	233.35
14	120	q24h	120	9.44	43.95	61.60

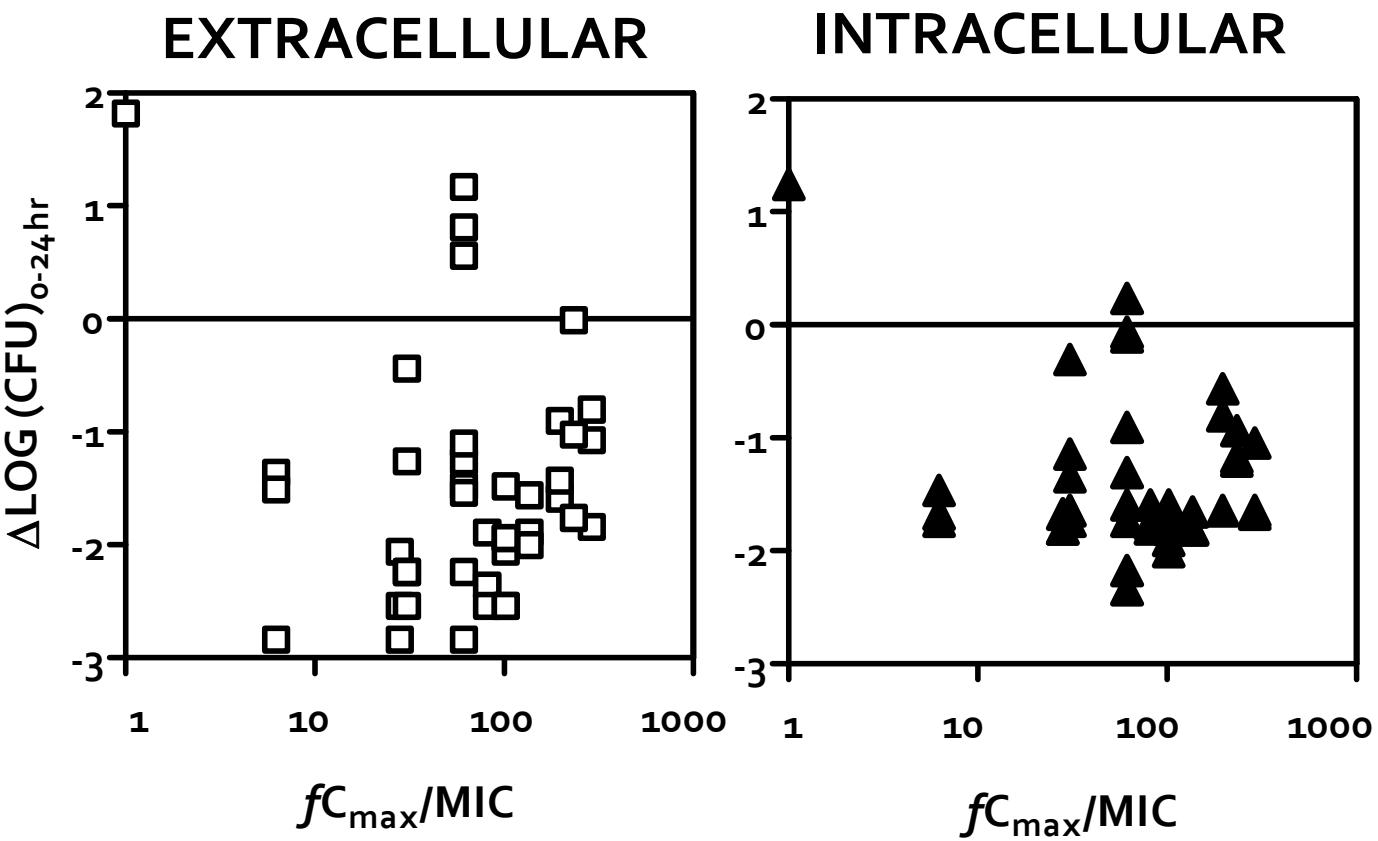
PK/PD studies: Dicloxacillin vs *S. aureus*



No correlation between treatment outcome and the AUC/MIC index

Sandberg et al., Antimicrob Agents Chemother (2010) 54:2391-2400

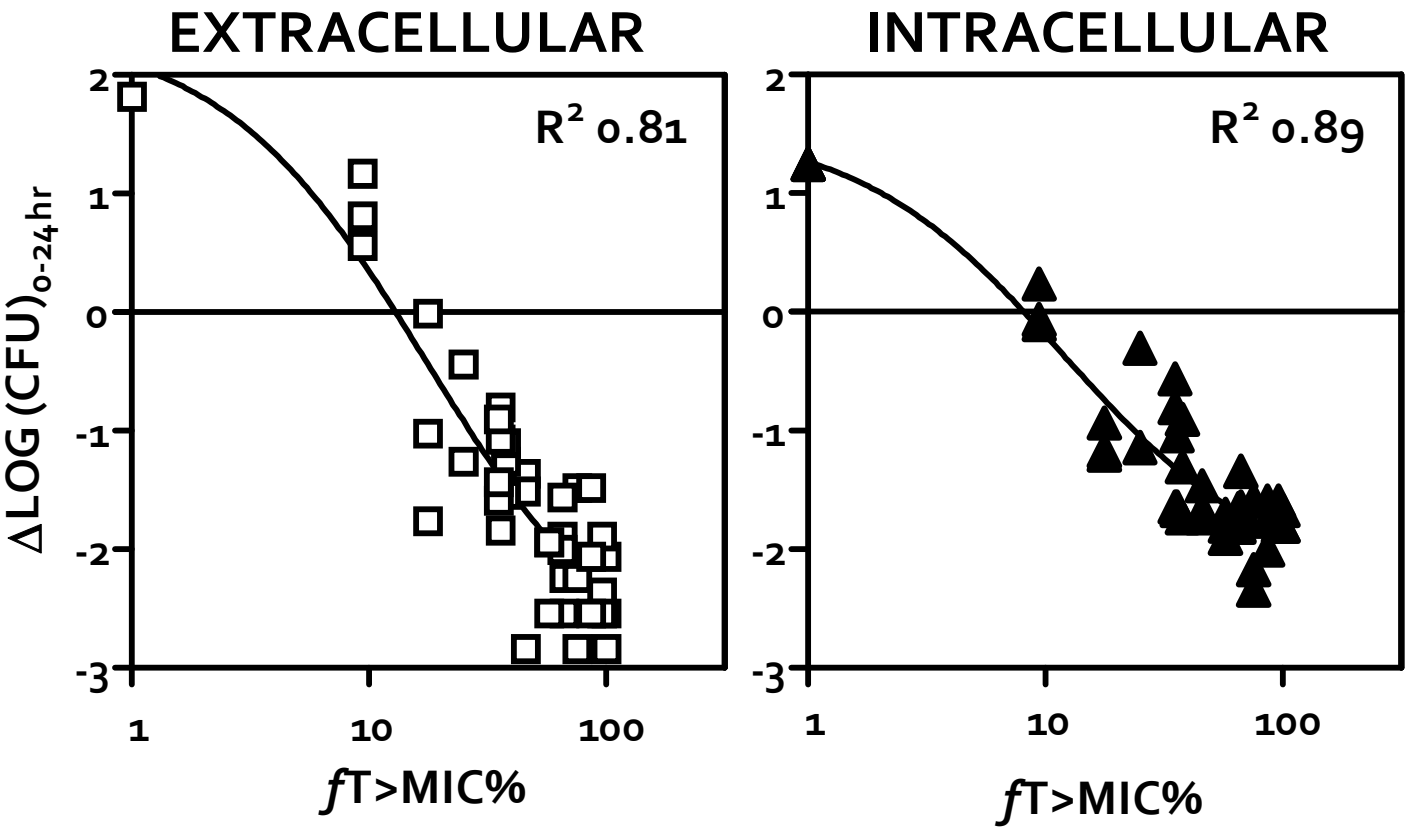
PK/PD studies: Dicloxacillin vs *S. aureus*



No correlation between treatment outcome and the C_{max} /MIC index

Sandberg et al., Antimicrob Agents Chemother (2010) 54:2391-2400

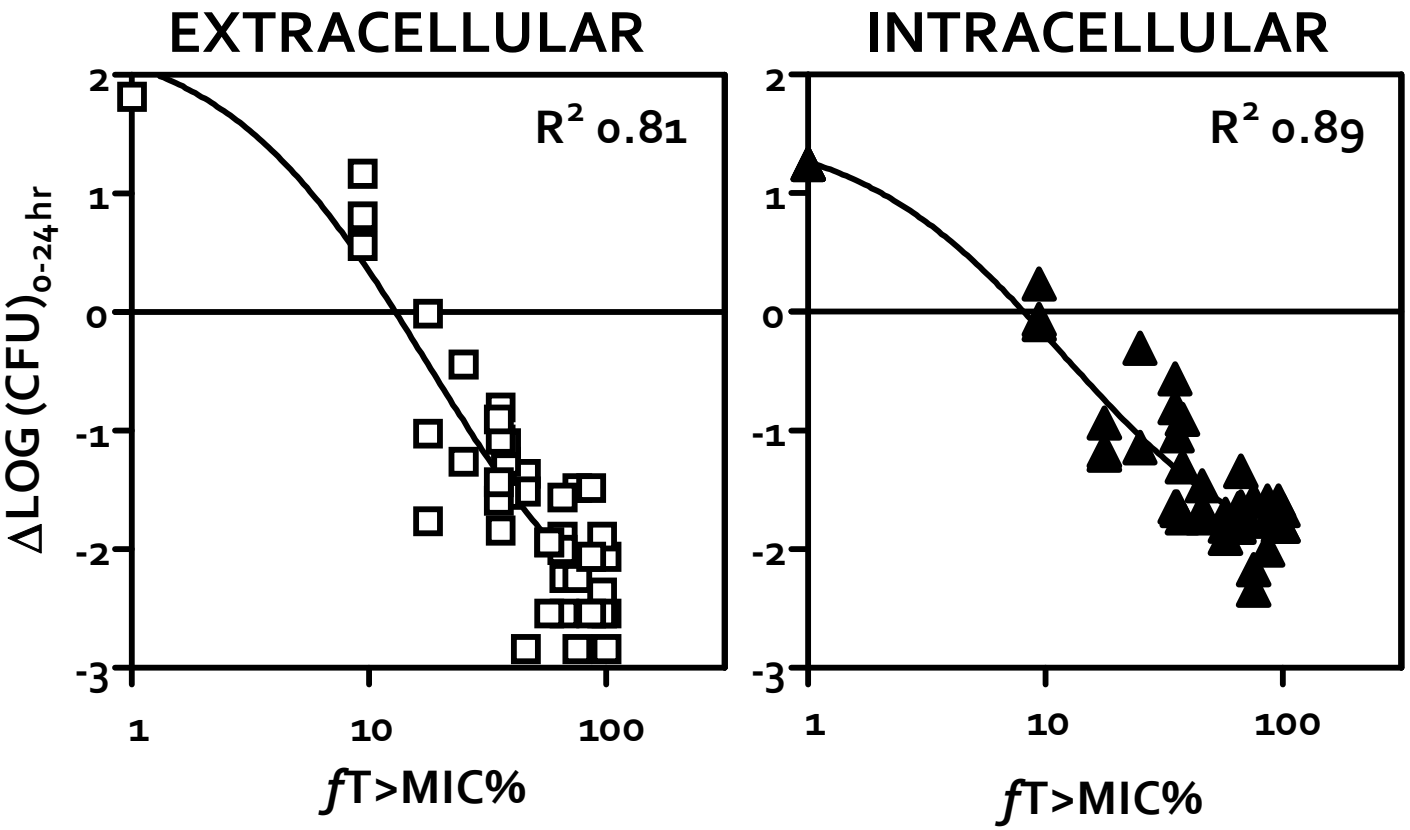
PK/PD studies: Dicloxacillin vs *S. aureus*



Correlation between treatment outcome and the T>MIC index

Sandberg et al., Antimicrob Agents Chemother (2010) 54:2391-2400

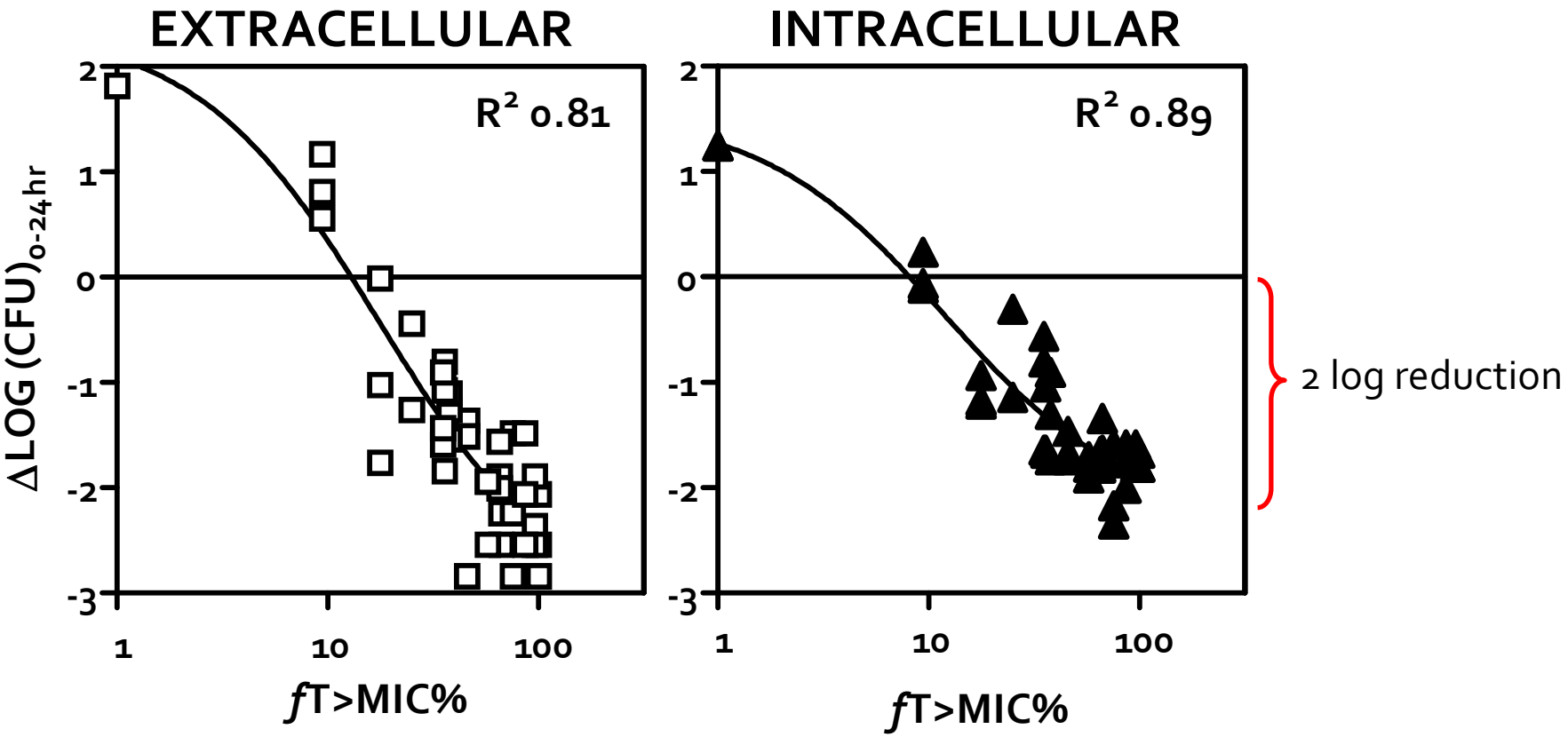
PK/PD studies: Dicloxacillin vs *S. aureus*



T>MIC is the predicting PK/PD index both intra- and extracellularly

Sandberg et al., Antimicrob Agents Chemother (2010) 54:2391-2400

PK/PD studies: Dicloxacillin vs *S. aureus*

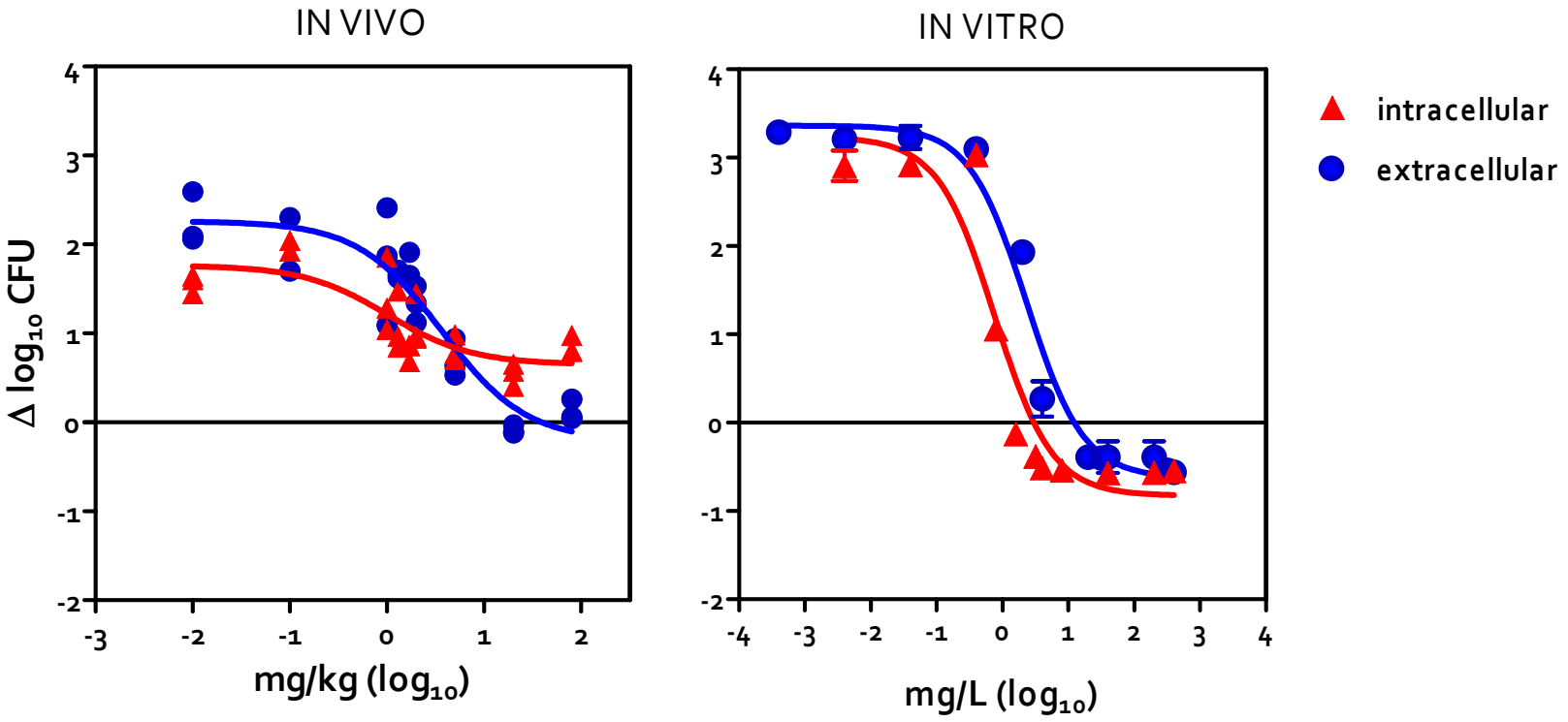


A reduction of 2 logs was obtained intracellularly with optimal dosing

Sandberg et al., Antimicrob Agents Chemother (2010) 54:2391-2400

Dose-response studies

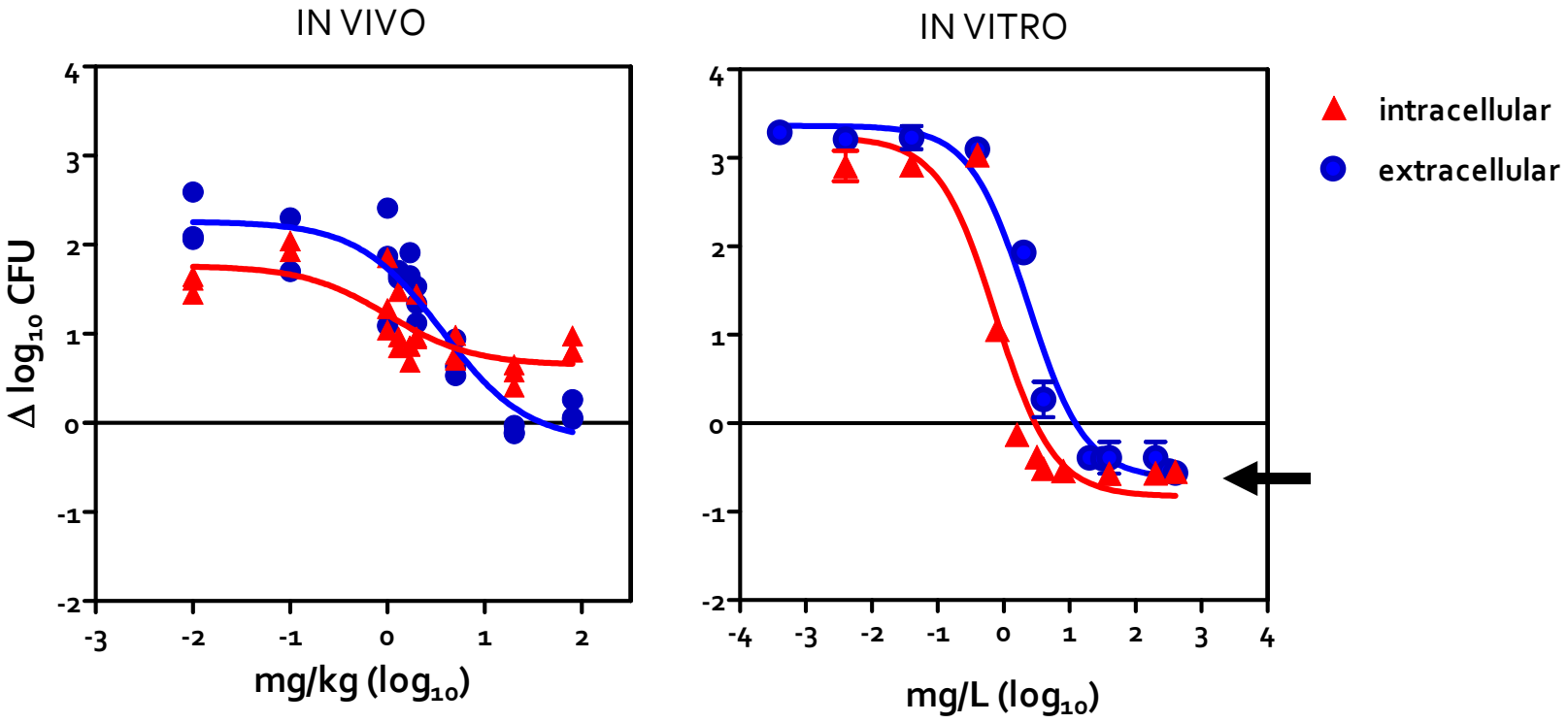
LINEZOLID vs. *S. aureus*



Sandberg et al., J. Antimicrob. Chemother (2010) 65:962-973

Dose-response studies

LINEZOLID vs. *S. aureus*

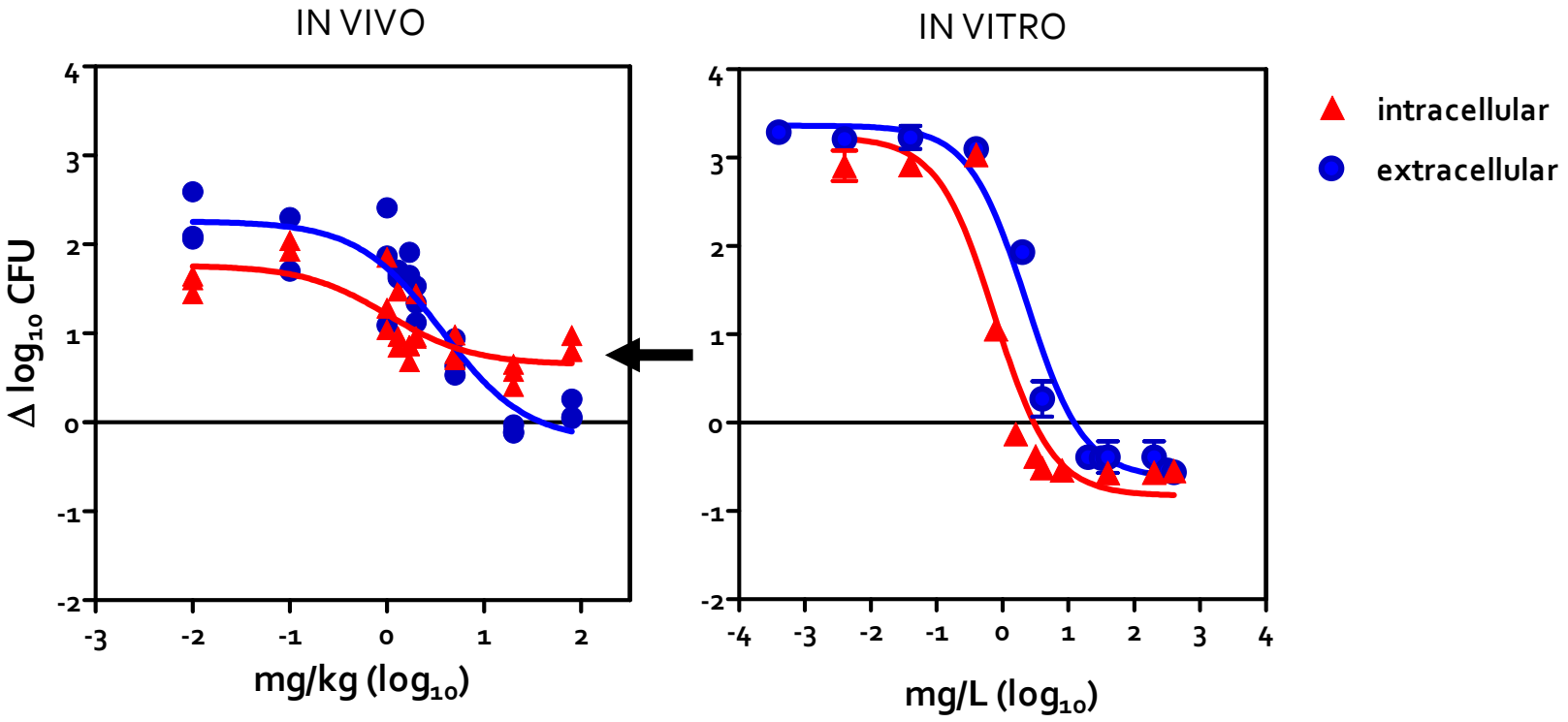


No decreased intracellular activity in vitro

Sandberg et al., J. Antimicrob. Chemother (2010) 65:962-973

Dose-response studies

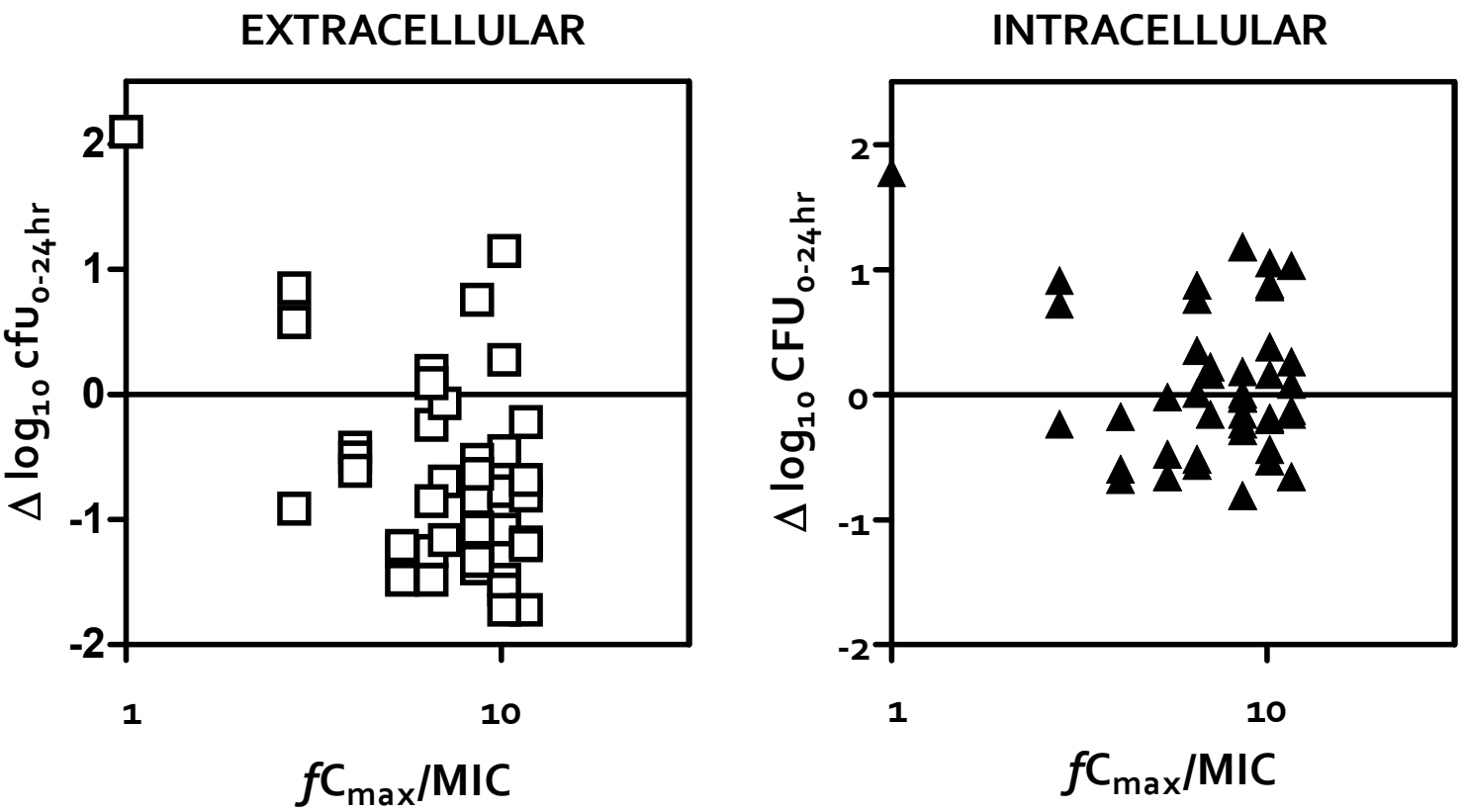
LINEZOLID vs. *S. aureus*



No reduction of the original intracellular inoculum in vivo

Sandberg et al., J. Antimicrob. Chemother (2010) 65:962-973

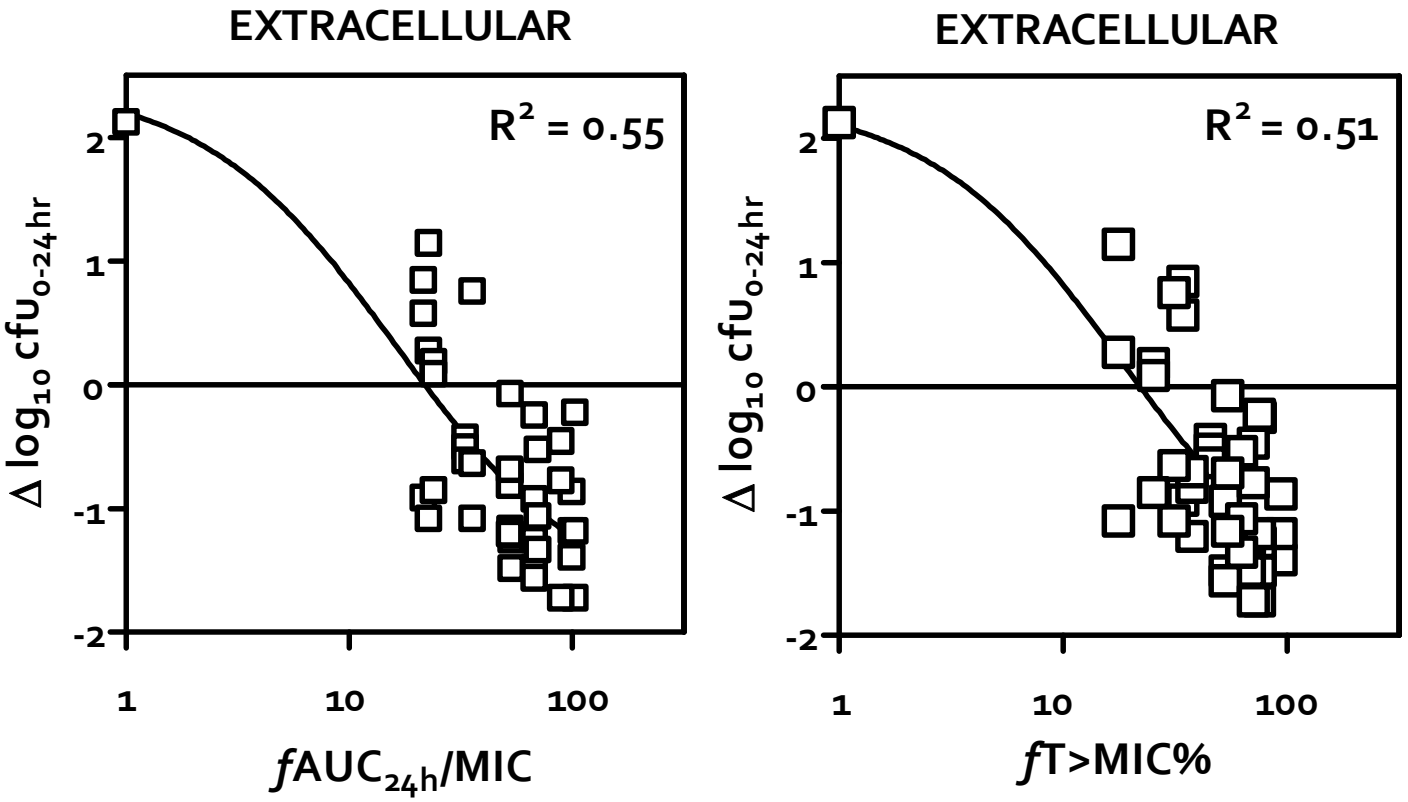
PK/PD studies: Linezolid vs *S. aureus*



No correlation between treatment outcome and the C_{max} /MIC index

Sandberg et al., J. Antimicrob. Chemother (2010) 65:962-973

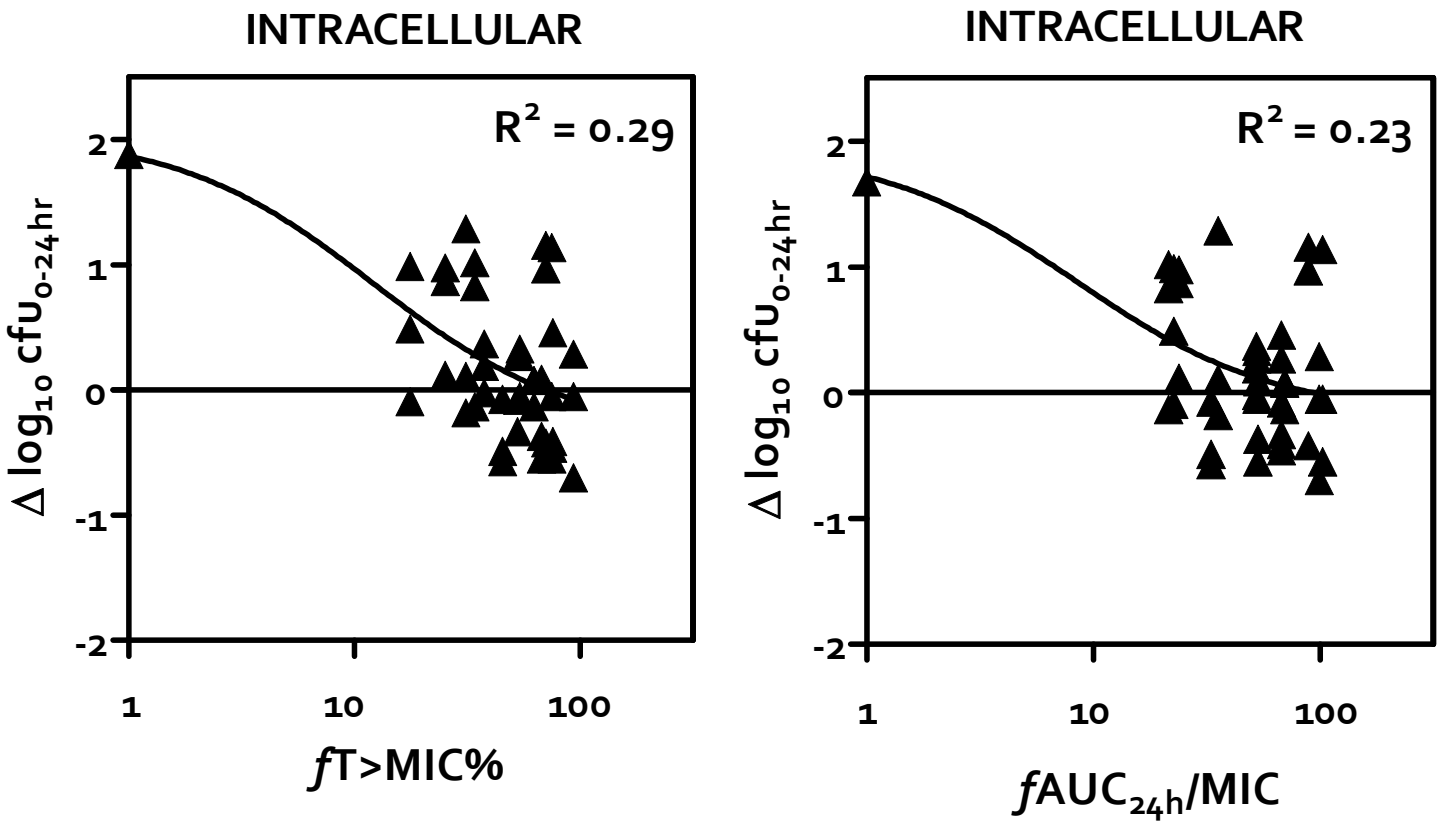
PK/PD studies: Linezolid vs *S. aureus*



Both AUC and T>MIC correlated equally to the extracellular outcome

Sandberg et al., J. Antimicrob. Chemother (2010) 65:962-973

PK/PD studies: Linezolid vs *S. aureus*

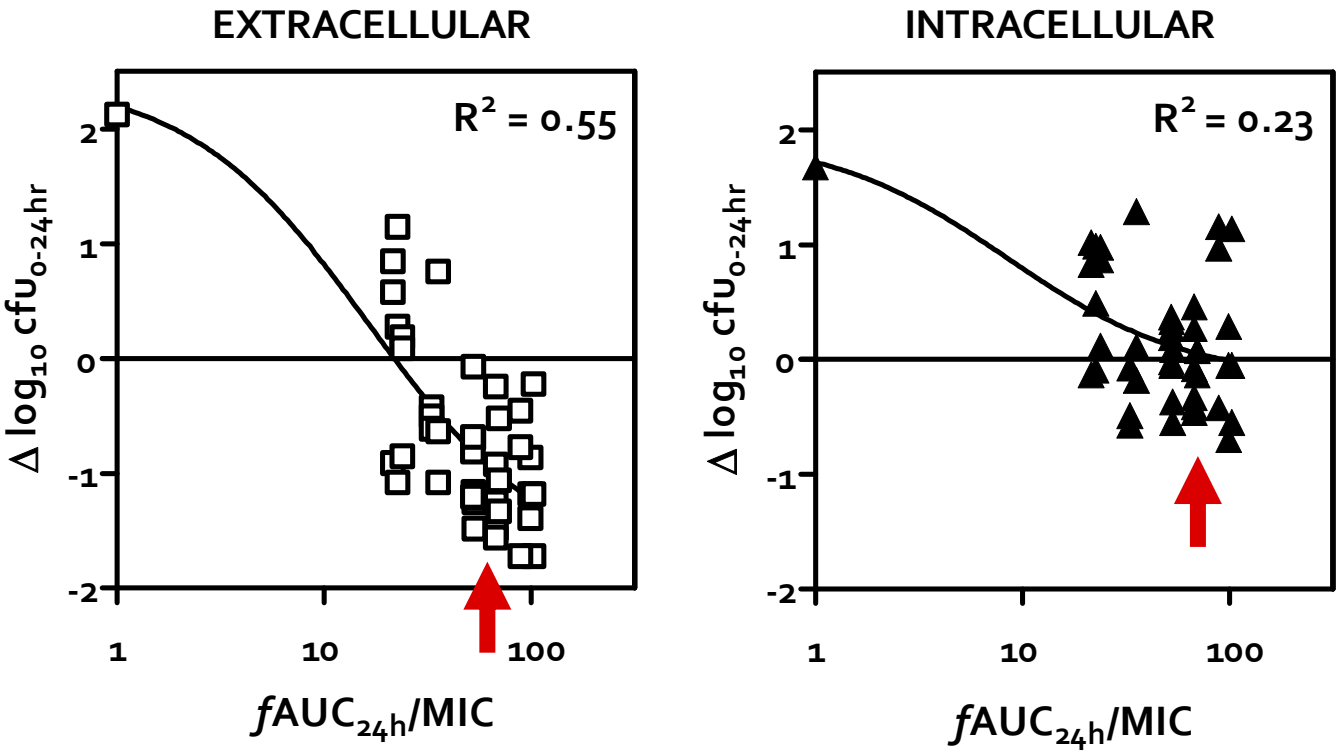


Poor correlation between PK/PD indices and the intracellular outcome

Sandberg et al., J. Antimicrob. Chemother (2010) 65:962-973

PK/PD studies: Linezolid vs *S. aureus*

Conventional dose: 600 mg twice daily ⟶ AUC/MIC = 80



Acceptable extracellular effect but questionable intracellular effect with conventional dose

Sandberg et al., J. Antimicrob. Chemother (2010) 65:962-973

Conclusions and perspectives:

In vivo models for intracellular activity studies?

- Useful for the study of intracellular activity of antibiotics

  ⟶ adds on PK and immune system

Conclusions and perspectives:

In vivo models for intracellular activity studies?

- Useful for the study of intracellular activity of antibiotics
  → adds on PK and immune system
- Equal conclusions according to intracellular activity were obtained compared to the in vitro model → screening in vitro?

Conclusions and perspectives:

In vivo models for intracellular activity studies?

- Useful for the study of intracellular activity of antibiotics
  → adds on PK and immune system
- Equal conclusions according to intracellular activity were obtained compared to the in vitro model → screening in vitro?
- Enables PK/PD studies → useful for optimizing the antibiotic treatment of intracellular infections

Conclusions and perspectives:

In vivo models for intracellular activity studies?

- Useful for the study of intracellular activity of antibiotics
  → adds on PK and immune system
- Equal conclusions according to intracellular activity were obtained compared to the in vitro model → screening in vitro?
- Enables PK/PD studies → useful for optimizing the antibiotic treatment of intracellular infections
- Dicloxacillin was active against intracellular *S. aureus* when dosed optimally (increased time exposure)

Conclusions and perspectives:

In vivo models for intracellular activity studies?

- Useful for the study of intracellular activity of antibiotics
  → adds on PK and immune system
- Equal conclusions according to intracellular activity were obtained compared to the in vitro model → screening in vitro?
- Enables PK/PD studies → useful for optimizing the antibiotic treatment of intracellular infections
- Dicloxacillin was active against intracellular *S. aureus* when dosed optimally (increased time exposure)
- The use of linezolid for intracellular *S. aureus* was more questionable !

Conclusions and perspectives:

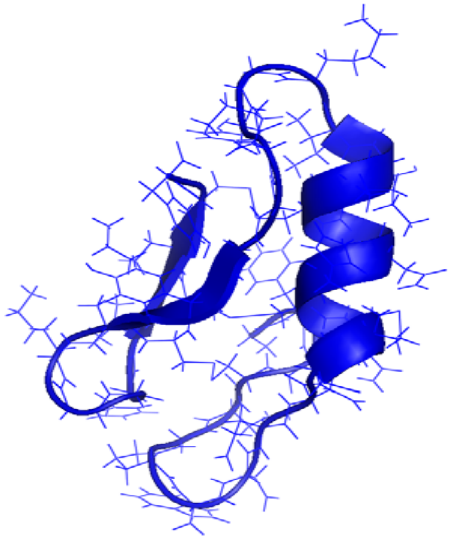
In vivo models for intracellular activity studies?

- Useful for the study of intracellular activity of antibiotics
  → adds on PK and immune system
- Equal conclusions according to intracellular activity were obtained compared to the in vitro model → screening in vitro?
- Enables PK/PD studies → useful for optimizing the antibiotic treatment of intracellular infections
- Dicloxacillin was active against intracellular *S. aureus* when dosed optimally (increased time exposure)
- The use of linezolid for intracellular *S. aureus* was more questionable !
- In vivo models for other types of intracellular infections (bacteria and infection site)

Directions for future research : where do we go ?

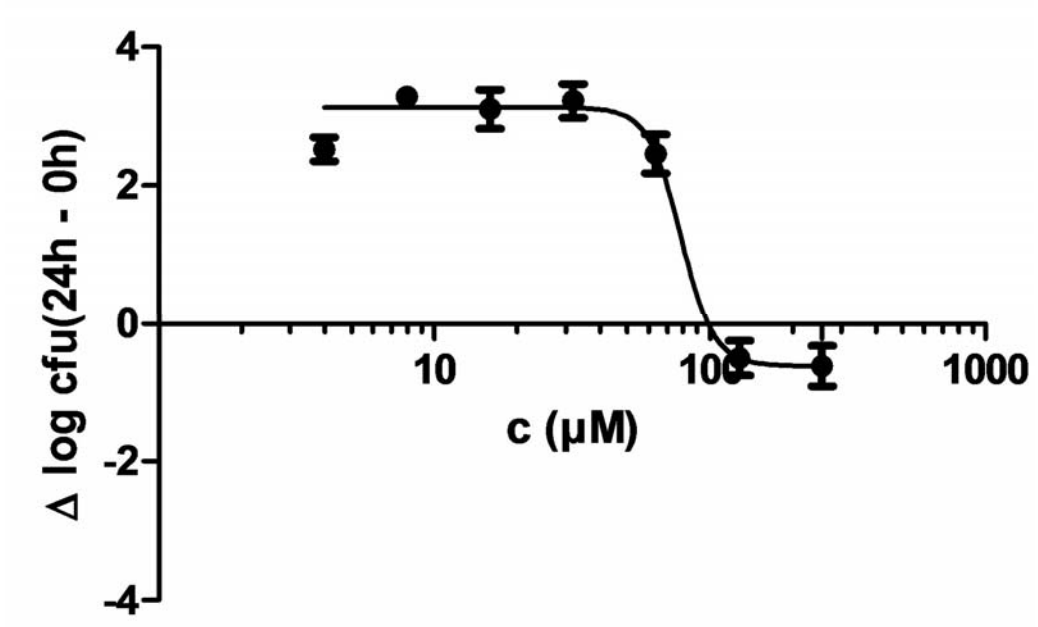
Use of peptides for treatment of intracellular infections ?

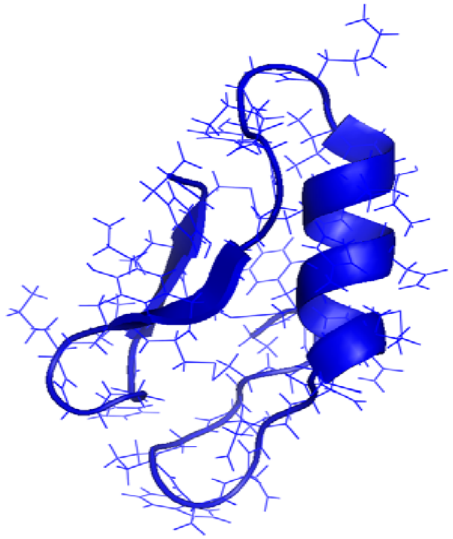
- Screening of cell-penetrating peptides (CPPs) with antimicrobial activity



Use of peptides for treatment of intracellular infections ?

- Screening of cell-penetrating peptides (CPPs) with antimicrobial activity
- Test of a well-known CPP against intracellular *S. aureus* in the in vitro model

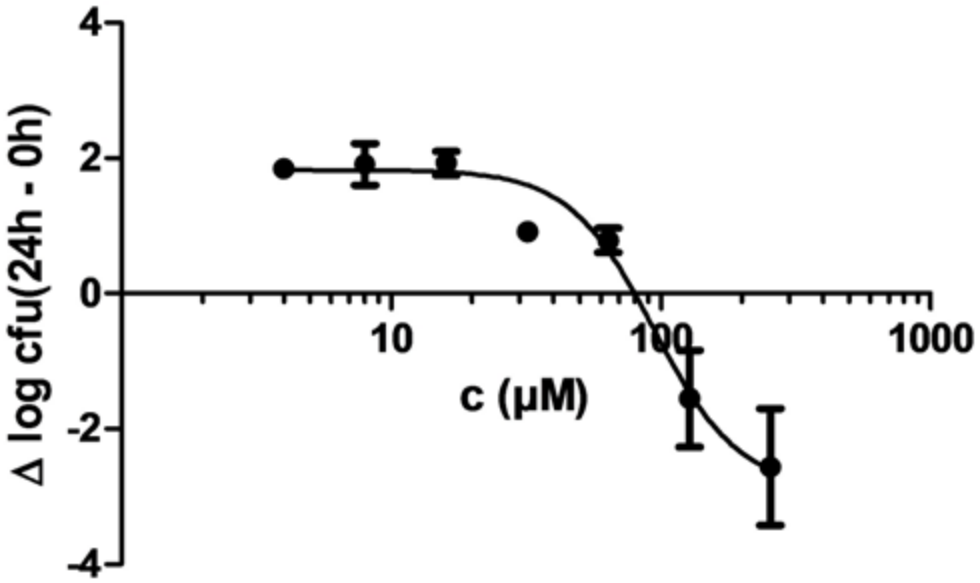


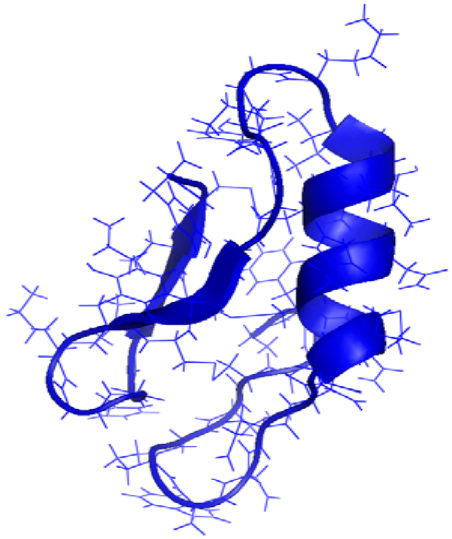


Bahnsen et al.(2012), Data not published

Use of peptides for treatment of intracellular infections ?

- Screening of cell-penetrating peptides (CPPs) with antimicrobial activity
- Test of a well-known CPP against intracellular *S. aureus* in the in vitro model
- CPP modified (Lys → Arg)

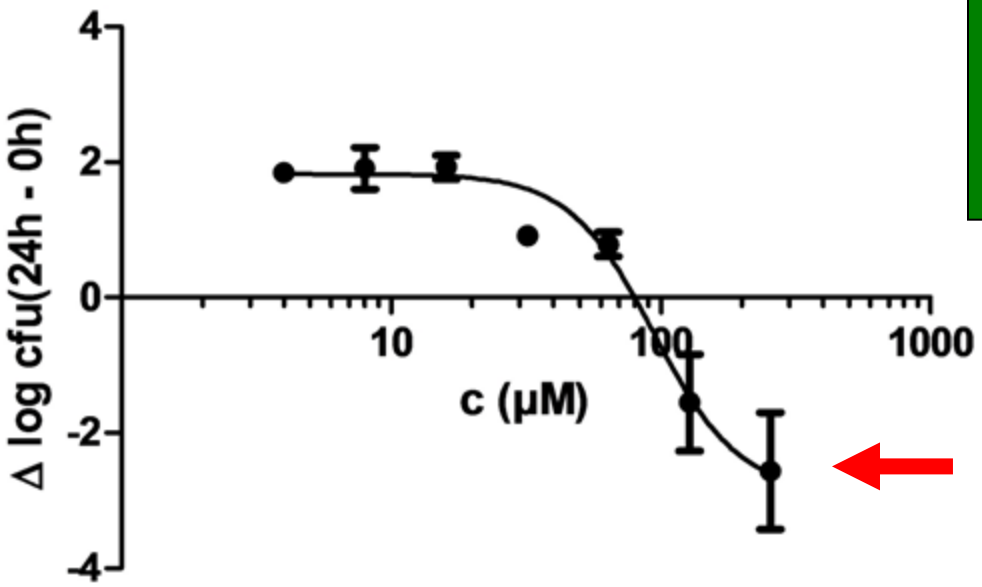




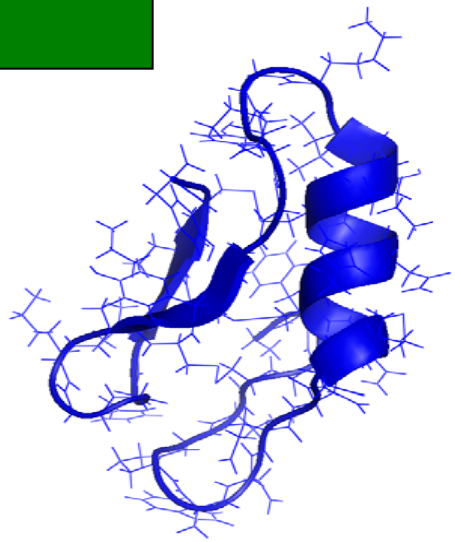
Bahnsen et al.(2012), Data not published

Use of peptides for treatment of intracellular infections ?

- Screening of cell-penetrating peptides (CPPs) with antimicrobial activity
- Test of a well-known CPP against intracellular *S. aureus* in the in vitro model
- CPP modified (Lys → Arg)



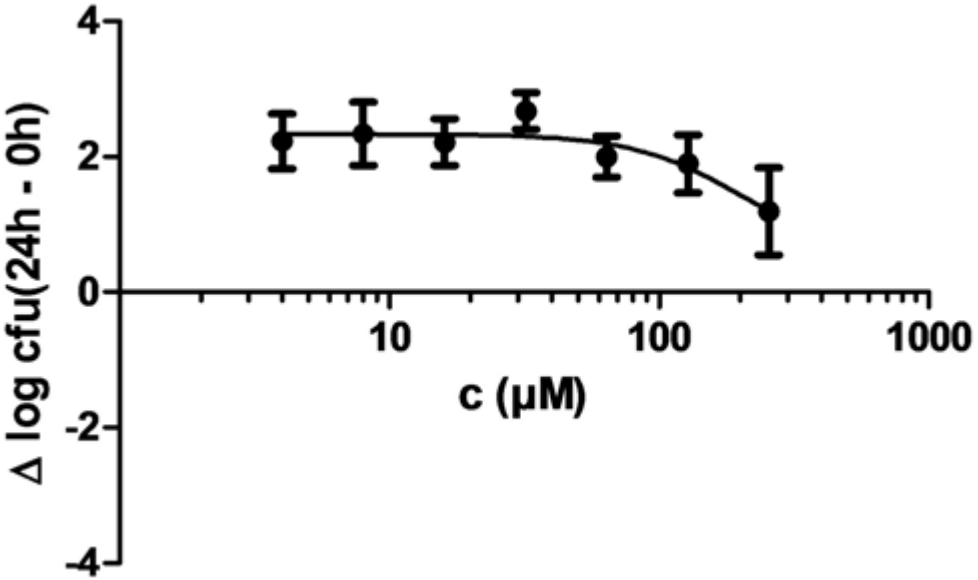
Increased efficacy



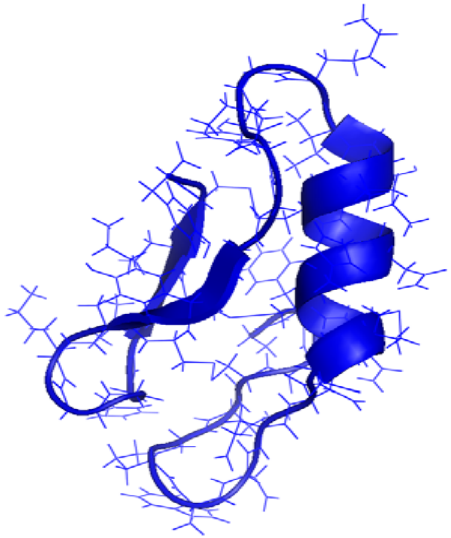
Bahnsen et al.(2012), Data not published

Use of peptides for treatment of intracellular infections ?

- Screening of cell-penetrating peptides (CPPs) with antimicrobial activity
- Test of a well-known CPP against intracellular *S. aureus* in the in vitro model
- CPP modified (Arg → Lys)

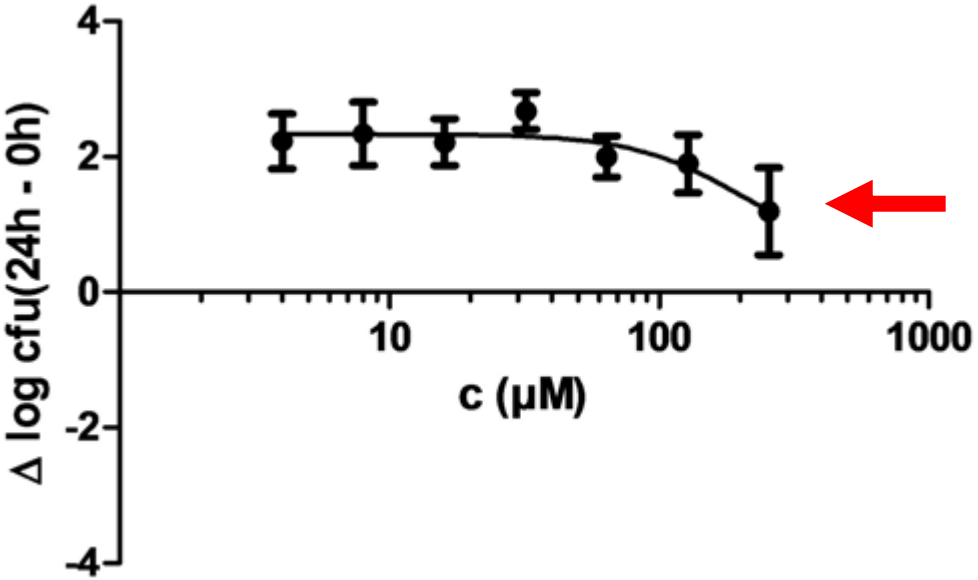


Bahnsen et al.(2012), Data not published

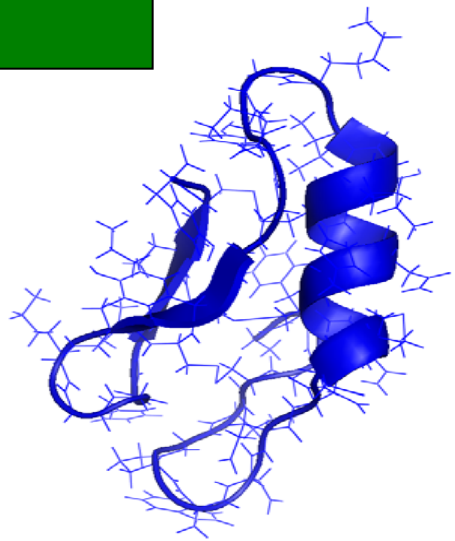


Use of peptides for treatment of intracellular infections ?

- Screening of cell-penetrating peptides (CPPs) with antimicrobial activity
- Test of a well-known CPP against intracellular *S. aureus* in the in vitro model
- CPP modified (Arg → Lys)



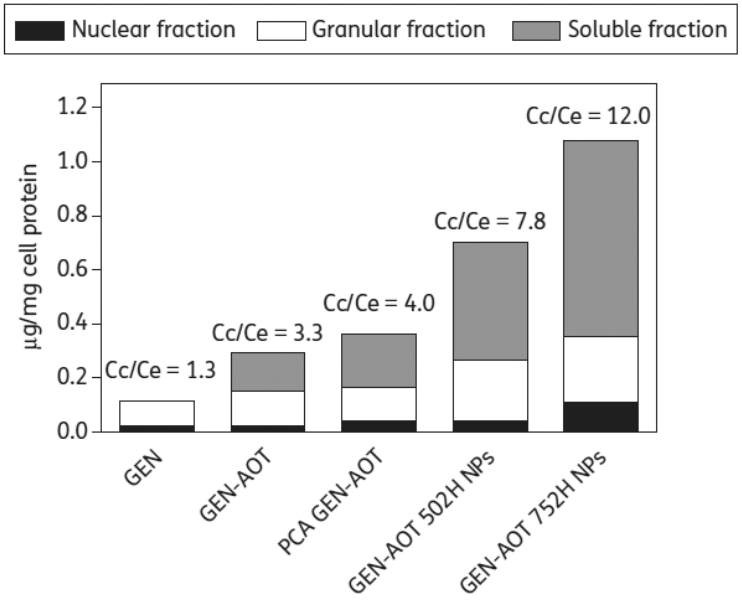
Decreased efficacy

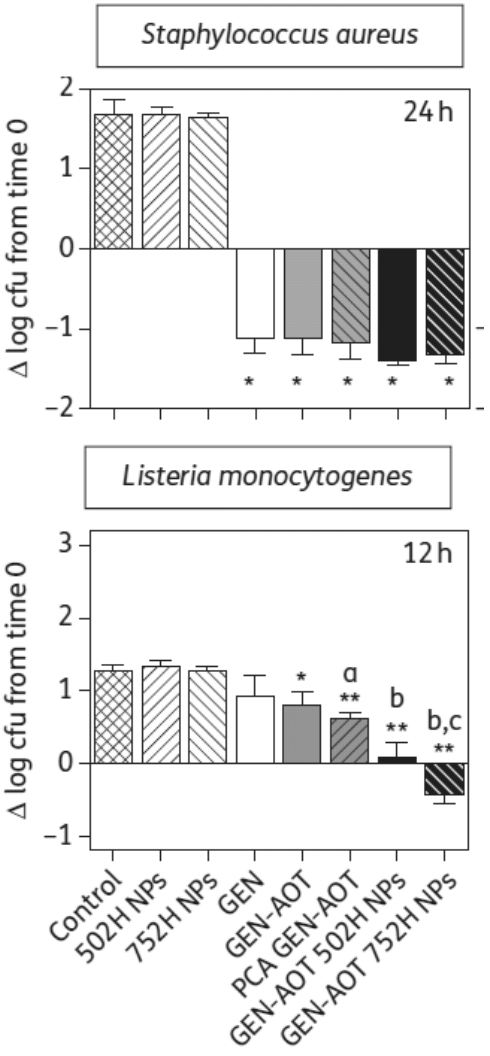


Bahnsen et al.(2012), Data not published

Passive targeting of drugs: nanoparticles

Gentamicin-loaded NP allow for cytosolic accumulation and increase activity on intracellular *Listeria*



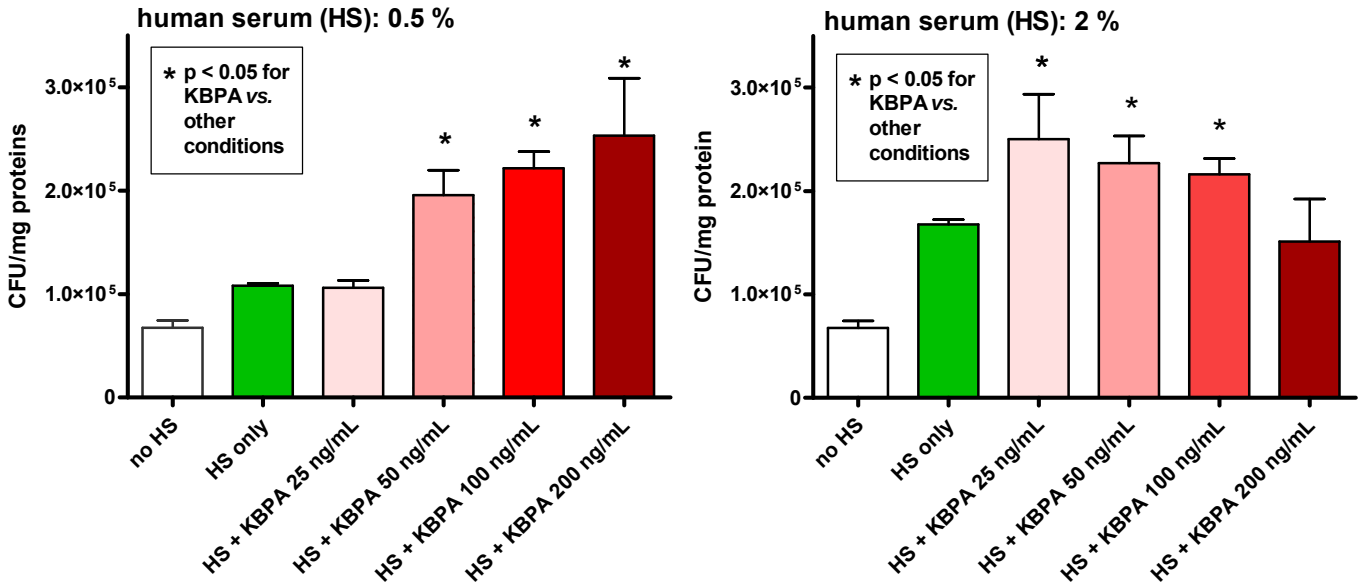


Imbuluzqueta et al, J. Antimicrob. Chemother. (2012): 67:2158-64

Active targeting of bacteria: antibodies

Panobacumab* increases *P. aeruginosa* phagocytosis

* fully human IgM monoclonal antibody antibody derived from an immortalized human lymphocyte raised against the O11 serotype PA



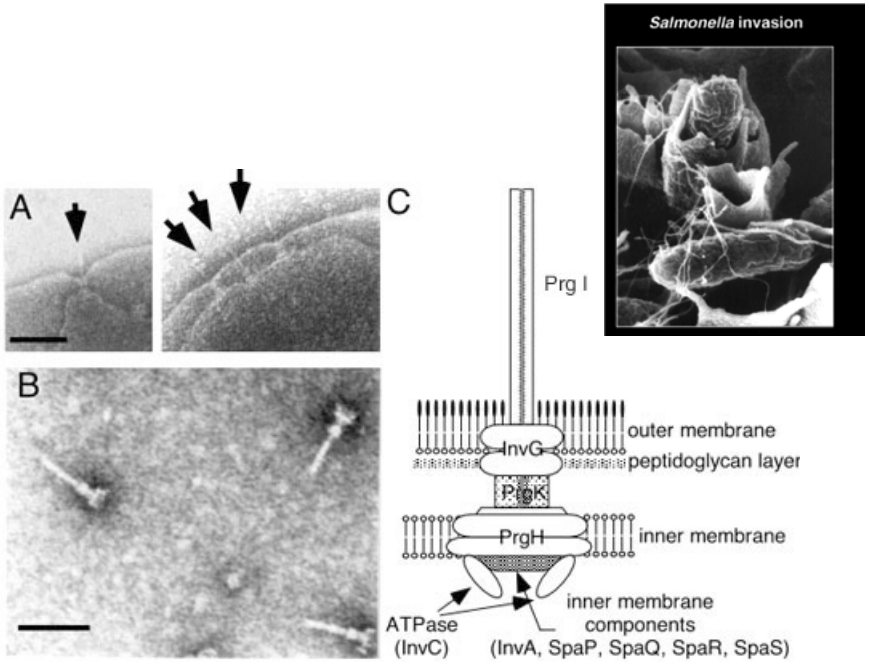
⇨ may help to expose extracellular/opportunistic pathogens to host defenses

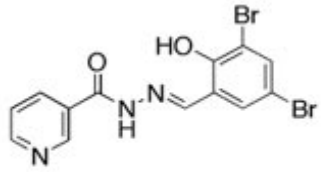
Jacqmin et al., ICAAC 2012; A1278

Preventing phagocytosis: inhibitors of virulence

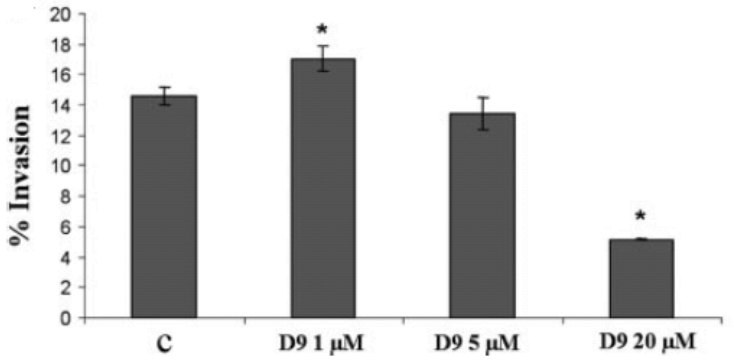
Inhibitors of TTSS prevent invasion of cells by *S. enterica*

Type III secretion system,
a molecular syringe





D9: INP0010



⇨ may help to impair invasion by obligate/facultative pathogens

Negrea et al. Antimicrob Ag. Chemother. (2007) 51: 2867–76

Conclusions: "take home" messages

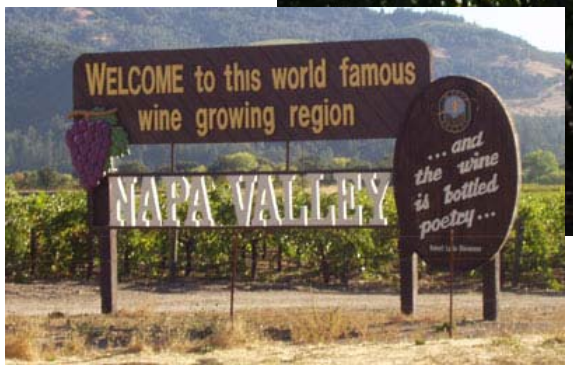
Appropriate models for studying intracellular infections

- Elimination of extracellular bacteria is a critical step
- Each model needs to be optimized
  - Bacteria:cells ratio or infecting inoculum in vivo
  - Time of phagocytosis, time of infection, …
- Antibiotic concentrations and time of incubation should be selected carefully
  - Pharmacodynamic parameters
  - Clinically-meaningful conclusions

The ideal antibiotic for intracellular infections

- Pharmacokinetics
  - Appropriate distribution (infected tissue / subcellular compartment)
  - If time-dependent, prolonged residence at the infection site
- Pharmacodynamics
  - Bactericidal character
  - Active over broad pH range
  - Cooperation with host defence mechanisms

Thank you for your attention



… hoping the session has provided you with food for thought …