Etude de l'activité d'antibiotiques vis-à-vis des formes intracellulaires de *Staphylococcus aureus* et *Legionella pneumophila*

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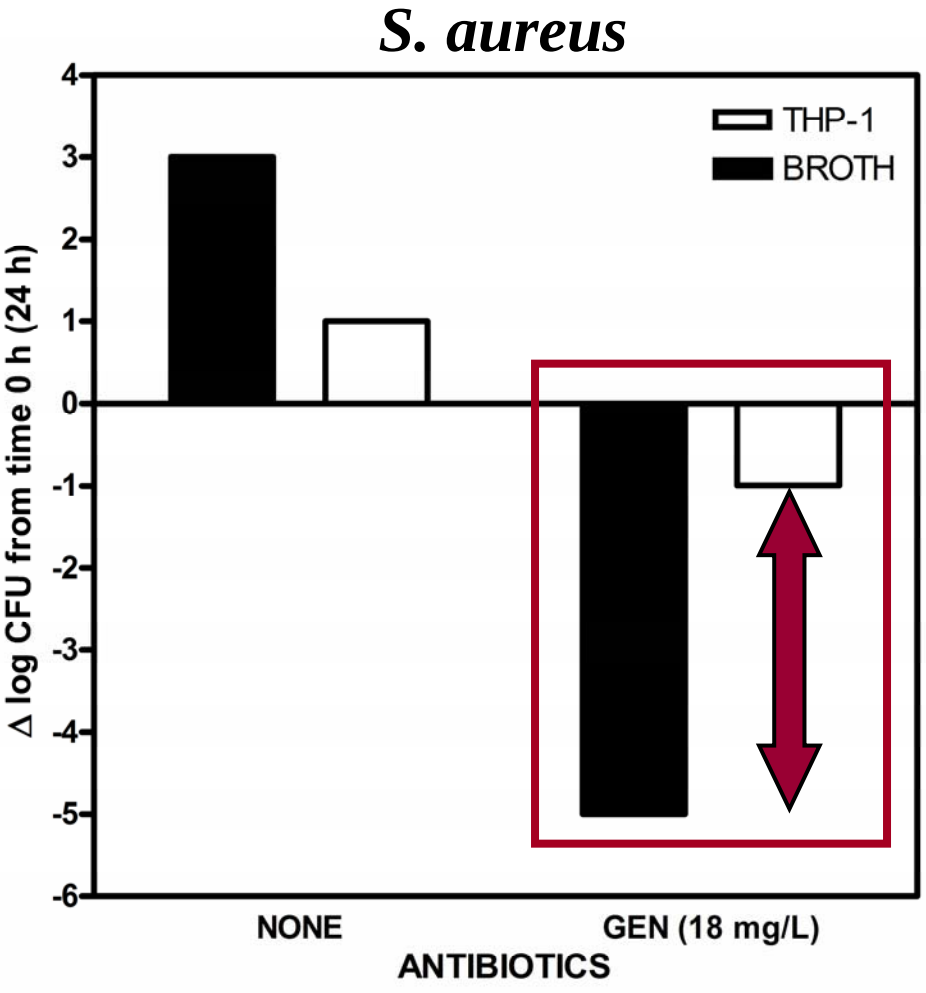
What makes bacteria so difficult-to-treat ?

- **Rising resistance to antimicrobials …**
  reaching the limit of clinical application …

- **Difficulty in eradicating intracellular forms, which probably results in recurrences, relapses and selection of drug-resistant organisms.**

Routine evaluation of antibiotic activity is usually performed on extracellular infections

Intracellular bacteria



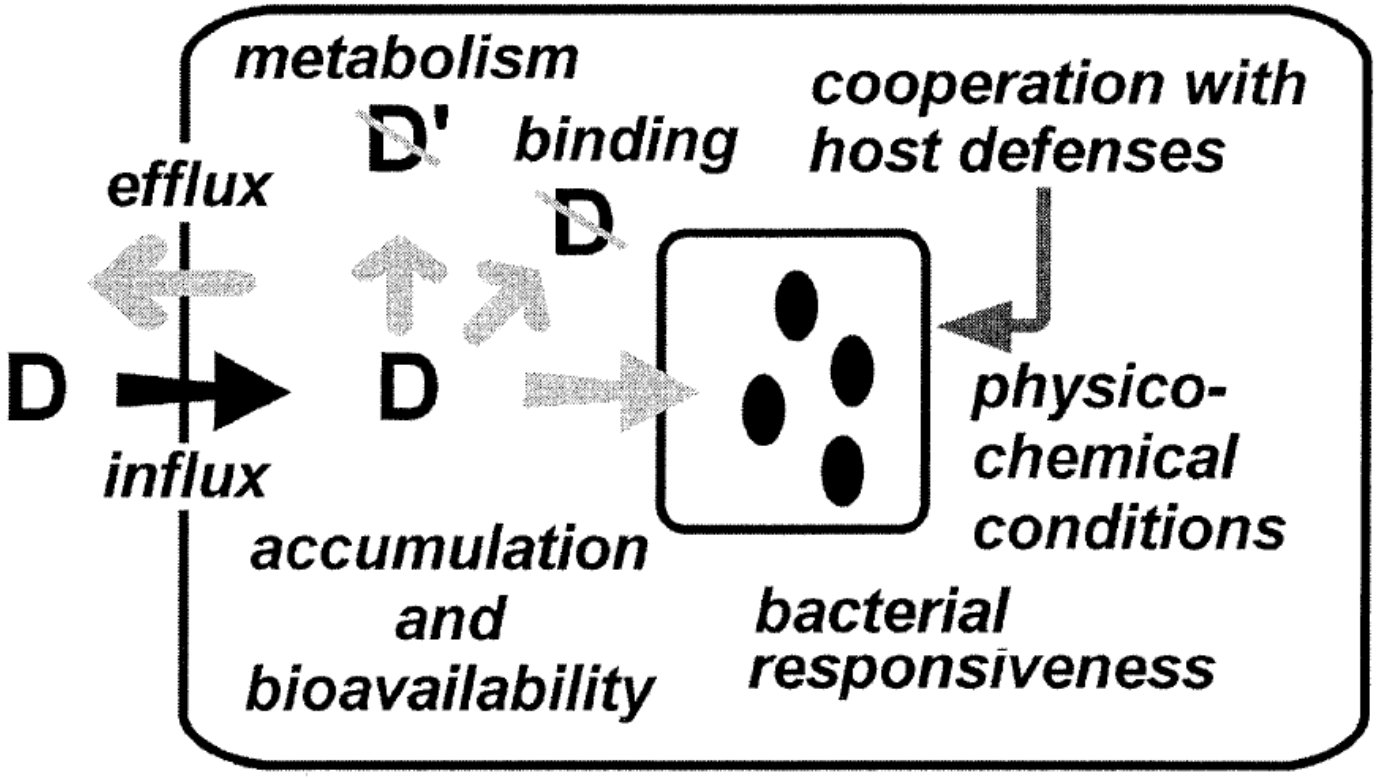
The intracellular environment:

- **delays the growth rate of this organism**
- **protects bacteria from the lethal action of most antimicrobials**

Barcia-Macay et al., Antimicrob. Agents and Chemother, 2006

Treatment of intracellular infections

An effective treatment requires antibiotics that efficiently accumulate within the infected intracellular compartment, and express activity therein



Aim of the study

Evaluation of the activity of antibiotics against bacteria sojourning intracellularly in distinct subcellular compartment, such as

- *L. pneumophila* (phagosomal model of infection)
- or *S. aureus* (phagolysosomial model of infection)

Antibiotics	Accumulation (Cc/Ce)
Azithromycin	**+++ (> 40)**
Clindamycin, tetracycline, fluoroquinolones	**++ (5 to 20)**
Linezolid	**+ (1 to 2)**

Intracellular *L. pneumophila*

Intracellular replication of *Legionella pneumophila*
Horwitz and Silverstein SC, J Exp Med (1981), 153:398-406

Intracellular multiplication of *Legionella pneumophila* in cultured human embryonic lung fibroblasts
Wong and Peacock, Infect Immun (1980), 28:1014-8.

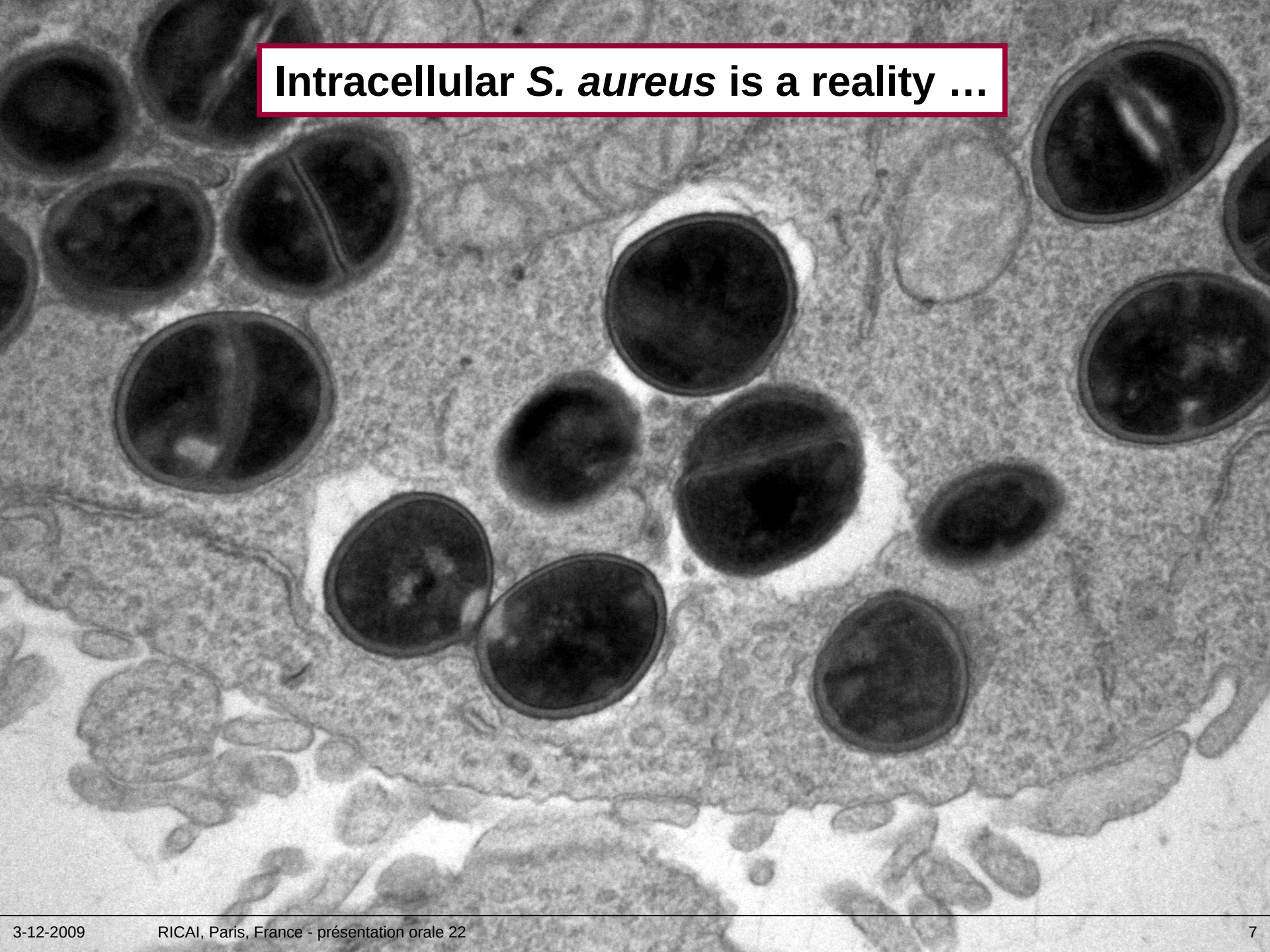
Cell-mediated immunity in Legionnaire's disease
Horwitz, J Clin Invest (1983), 6:1686-97

Adhesion, penetration and intracellular replication of *Legionella pneumophila*: an in vitro model of pathogenesis
Oldham and Rodgers FG, J Gen Microbiol (1985), 131:697-706.

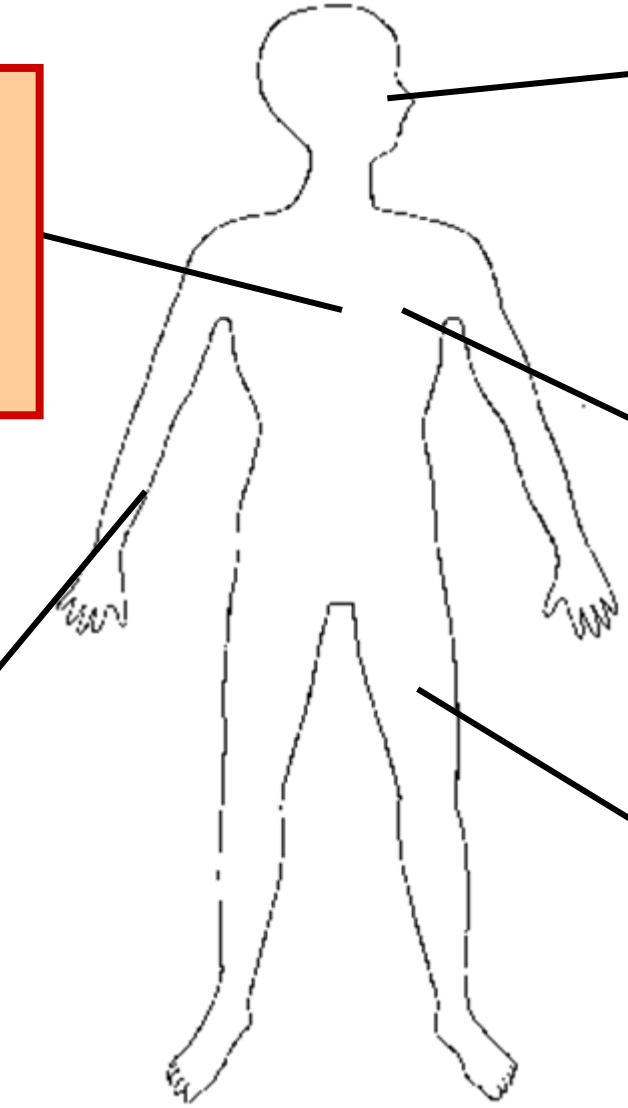
Intracellular survival and expression of virulence determinants of Legionella pneumophila
Hacker et al, Infection (1991), 4:S198-201

Modulation of caspases and their not-apoptotic functions by Legionella pneumophila
Amer AO, Cell Microbiol (2009), in press

Intracellular *S. aureus* is a reality …



Intracellular *S. aureus*



PULMONARY INFECTIONS ASSOCIATED WITH CYSTIC FIBROSIS
Jarry and Cheung, Infect Immun, 2006

RECURRENT RHINOSINUSITIS
Clement et al, J Infect Dis, 2005

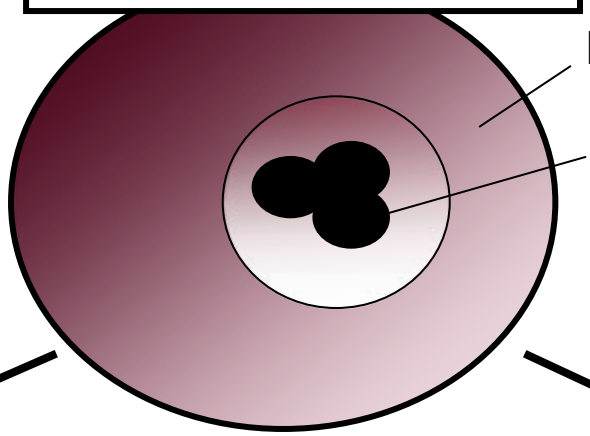
ENDOCARDITIS
Sinha and Herrmann, Thromb Haemost, 2005

COMPLICATED SKIN INFECTIONS
Mempel et al, Br J Dermatol., 2002

OSTEOMYELITIS
Ellington et al, J Bone Joint Surg Br., 2003
Wright and Nair, Int J Med Microbiol; 2009

S. aureus reprograms its transcriptome once it reaches the cellular environment …

2 h post-phagocytosis



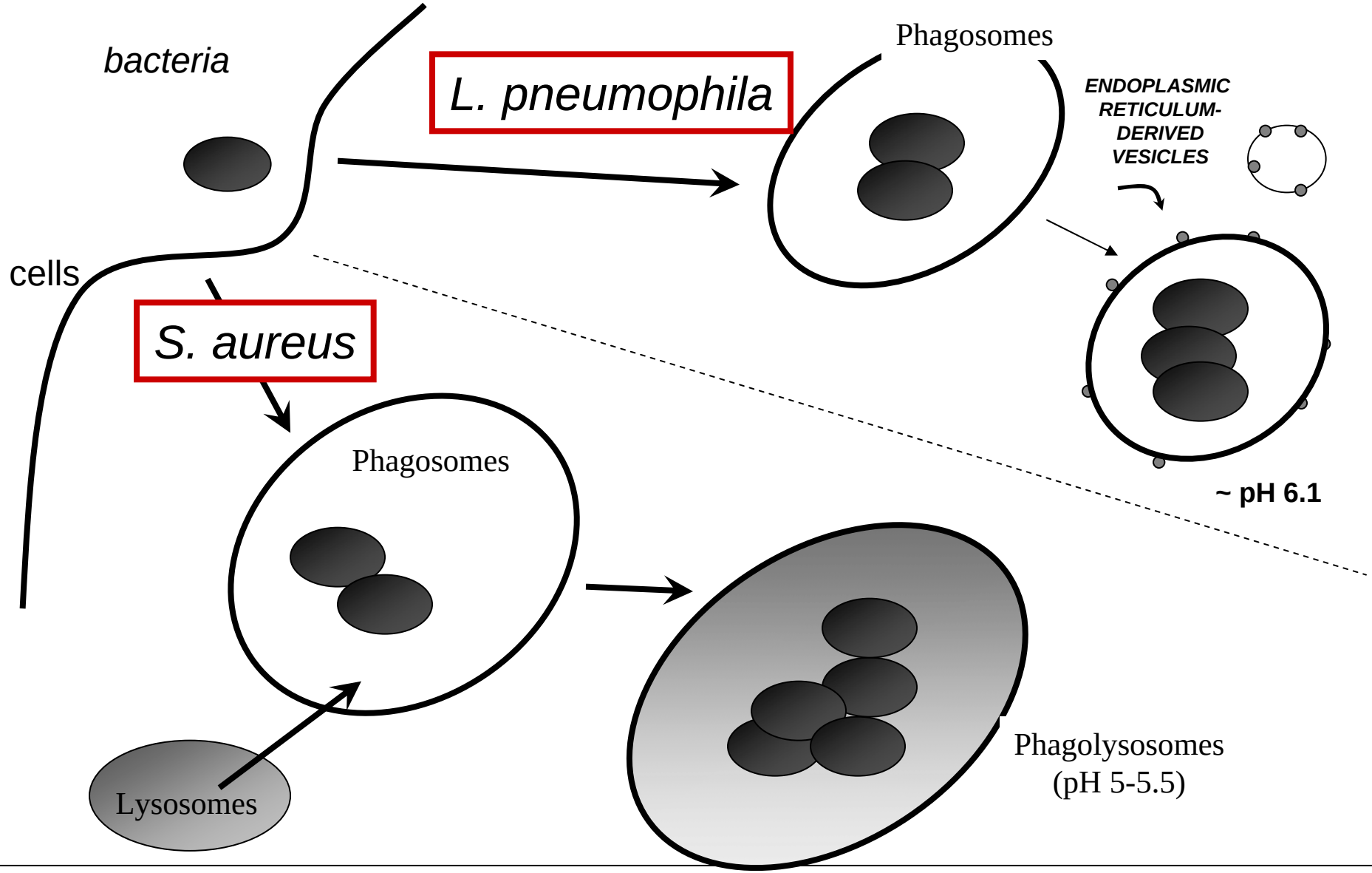
Down-regulation of major metabolic pathways (cell division, nutrient transport, …)

Up-regulation of bacterial genes contributing to oxidative stress protection

Down-regulation of toxin genes known to affect host cell integrity (i.e. *hla*)

Garzoni et al, BMC Genomics, 2007

Intracellular lifestyle of *S. aureus and L. pneumophila*



Methods

THP-1 macrophages

L. pneumophila
Opsonized *S. aureus*

phagocytosis

Removal of extracellular bacteria (washings)

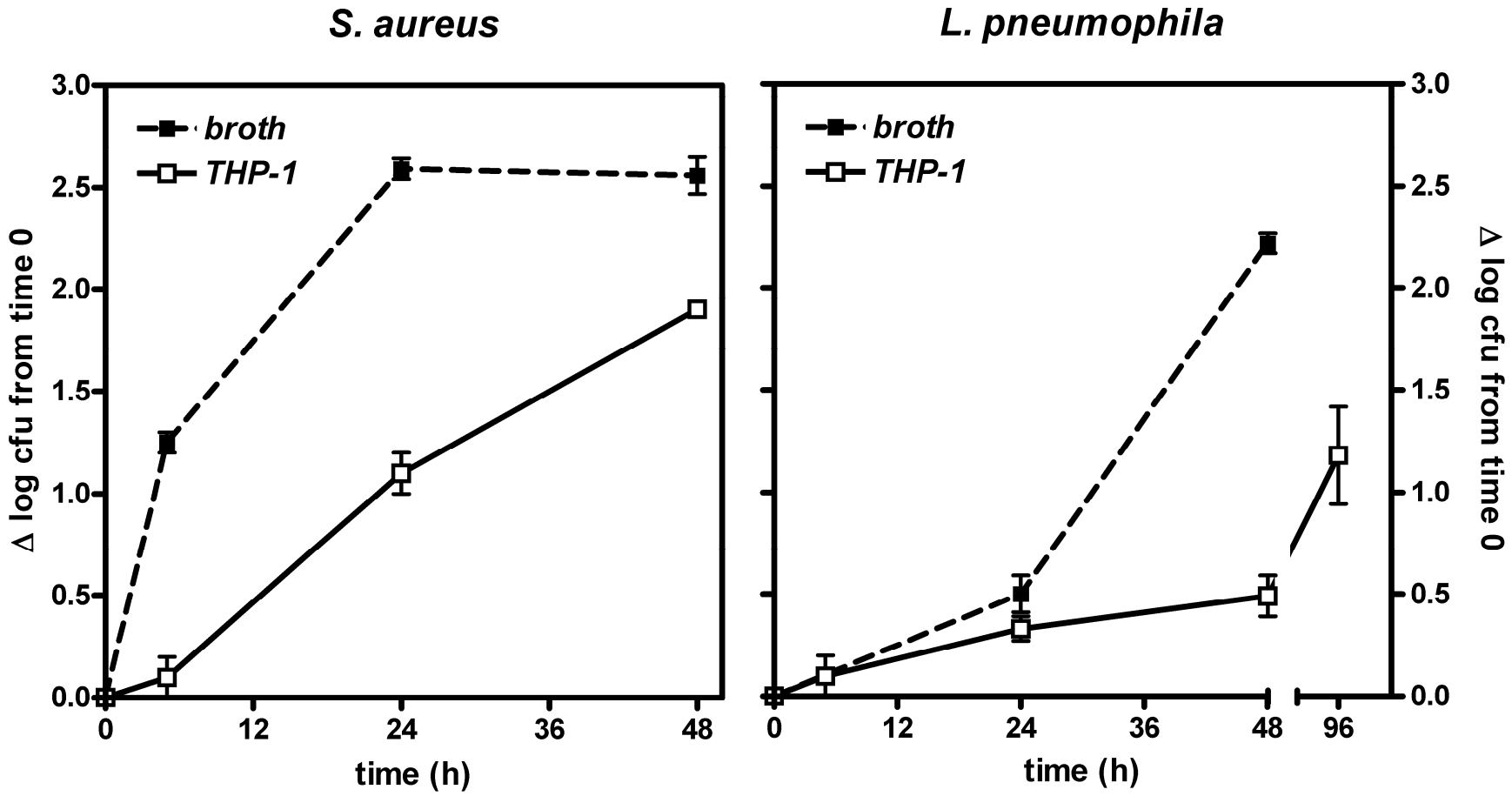
Incubation with antibiotics
(equivalent to human Cmax)

At 24h (SA) and 48 h (LP): removal of extracellular bacteria/antibiotics and cell lysis

CFU counting and assay of protein content

Lemaire et al, JAC, 2005; Barcia-Macay et al, AAC, 2006

Bacterial growth



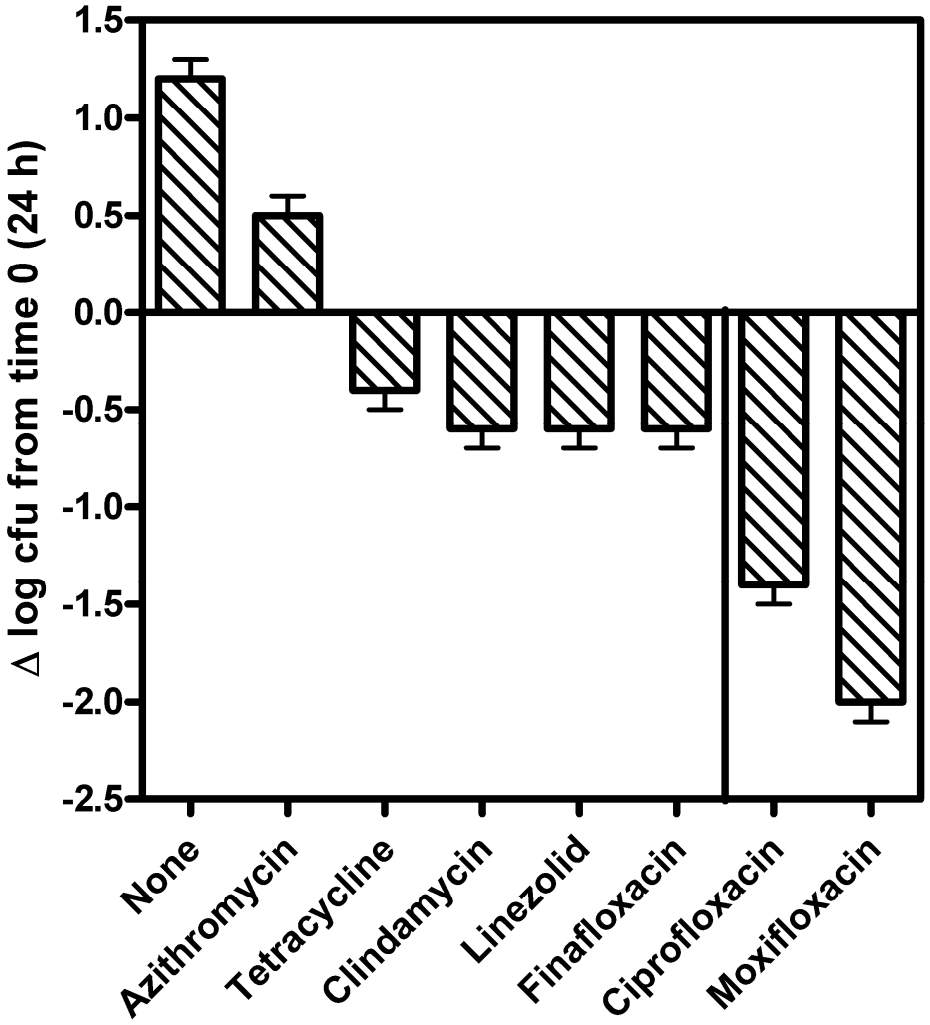
The intracellular environment delays the growth of both bacteria

Intrinsic activities (MICs)

Antibiotic	Human Cmax (mg/L)	*S. aureus* ATCC 25923*		*L. pneumophila* ATCC 33153**
		pH 7.4	pH 5.5	pH 6.9
azithromycin	0.5	0.5	**256**	0.01
tetracycline	5	0.5	N.D.	16
clindamycin	20	0.125	**4**	4
linezolid	20	1-2	1-2	4
ciprofloxacin	4	0.125	**0.5**	< 0.01
finafloxacin	10	0.06	**0.01**	< 0.01
moxifloxacin	4	0.03	**0.125**	< 0.01

* Mueller Hinton broth (pH 7.4);
** Buffered yeast extract supplemented with a-ketoglutarate (pH 6.9)

Intracellular activity of antibiotics against *S. aureus*

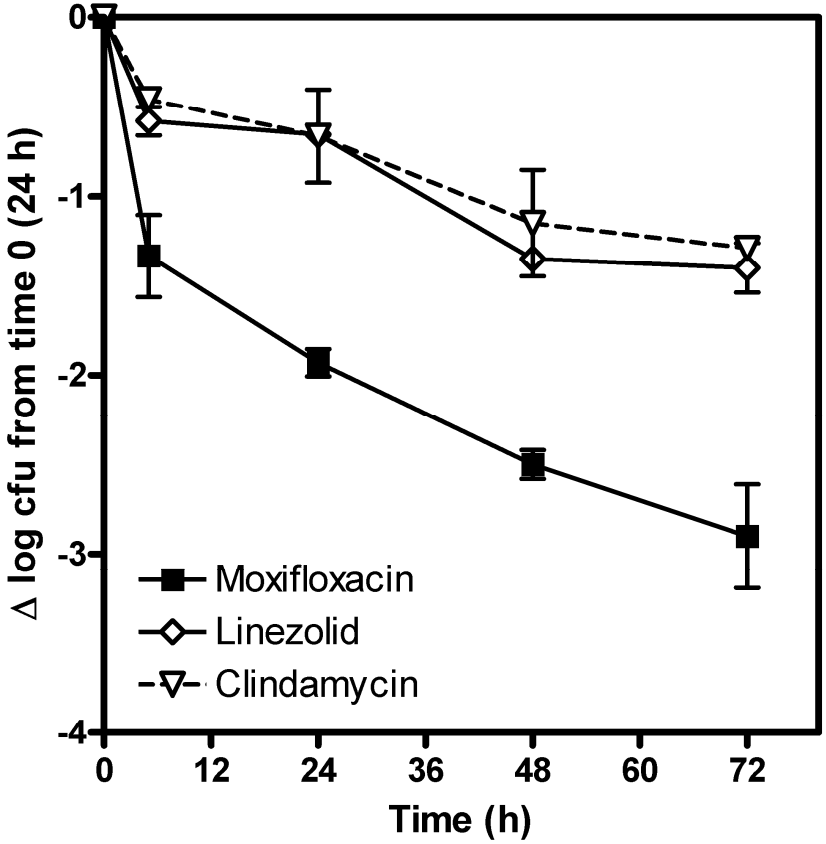


- **Azithromycin fails to reduce the intracellular foci (probably in relation with the deleterious effect of acidic pH on its antibacterial activity)**

- **Poor intracellular activity of tetracycline, clindamycin, linezolid and finafloxacin**

- **Higher reduction of the intracellular load obtained for ciprofloxacin- and moxifloxacin-treated cells**

Intracellular activity of antibiotics against *S. aureus*

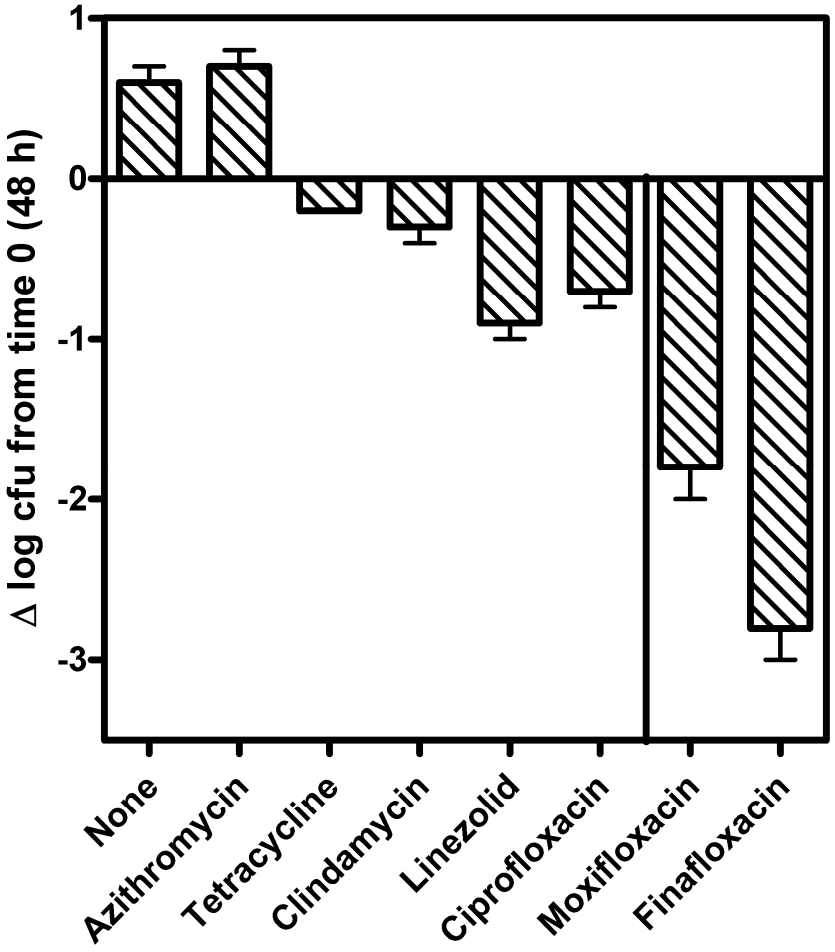


Increasing the incubation time (to 72 h) is associated with:

- a slight increased of clindamycin and linezolid activities

- a true bactericidal effect (≥ 3 log cfu decrease) of moxifloxacin against the intraphagocytic forms of *S. aureus*

Intracellular activity against *L. pneumophila*



- **Azithromycin fails to reduce the intracellular foci**
- **Poor intracellular activity of tetracycline, clindamycin, linezolid and ciprofloxacin**
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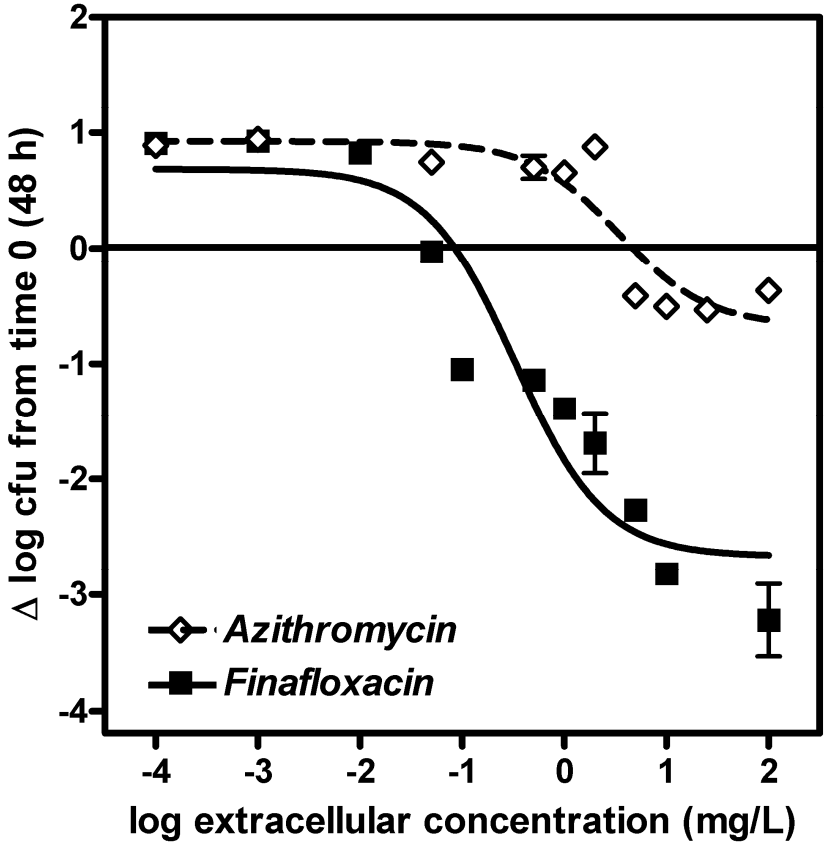
Dose-response activities against *L. pneumophila*



- **both antibiotics exerted concentration-dependent activities against intraphagocytic *L. pneumophila***

- **Pertinent pharmacological descriptors of antibiotic activity for finafloxacin:**

  - **Static concent. : 0.06 mg/L**
  - **EC_{50}: 0.33 mg/L**
  - **Emin : 0.9 log CFU**
  - **Emax: - 2.7 log CFU**

Discussion and perspectives

Conclusions

- Despite of their marked cellular accumulation,
  - azithromycin fails to prove efficacy against intracellular organisms
  - tetracycline and clindamycin shows only poor intracellular activity

- Potential advantages of moxifloxacin (for intracellular *S. aureus*) and finafloxacin (for intracellular *L. pneumophila*)

Conclusions

- Absence of direct correlation between the cellular accumulation of antibiotics and the expression of antibiotic efficacy

- Routine evaluation of antibiotic efficacy (which is useful to predict the therapeutic outcome when dealing with extracellular bacteria), does not allow foreseeing efficacy against intracellular organisms

- It is crucial to critically examine the cellular pharmacodynamics of antibiotics against intracellular pathogens

Thank you for your attention !

Thanks to ...

• Unité de pharmacologie cellulaire et moléculaire & Université catholique de Louvain (Bruxelles, Belgique)

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