

AWARDS 2013



Foundation AstraZeneca

Infectiology award: Bacterial and cellular factors affecting antibiotic activity towards persistent infections

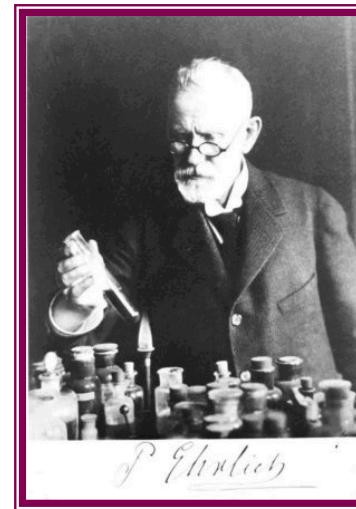
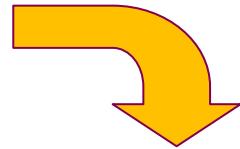
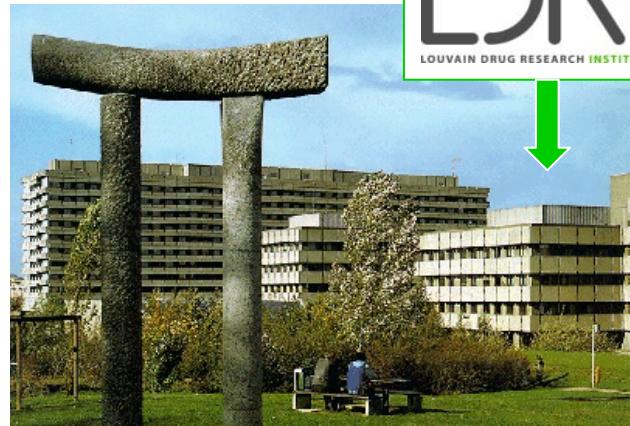
Françoise Van Bambeke
Louvain Drug Research Institute, UCL

AstraZeneca 
Foundation
Bringing science to society

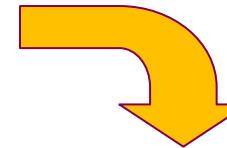
FWO
VLAANDEREN
Fonds Wetenschappelijk Onderzoek
Research Foundation – Flanders

fnrs
LA LIBERTÉ DE CHERCHER

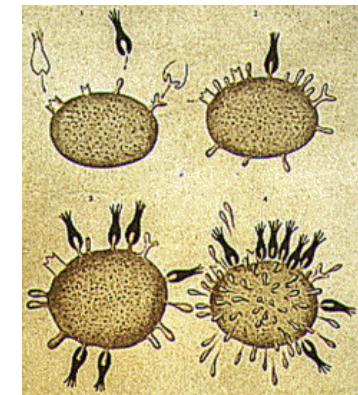
Paul Ehrlich, the father of anti-infective chemotherapy



"The aim is to find chemical substances that have special affinities for pathogenic organisms and that, like «magic bullets», go straight to their targets."

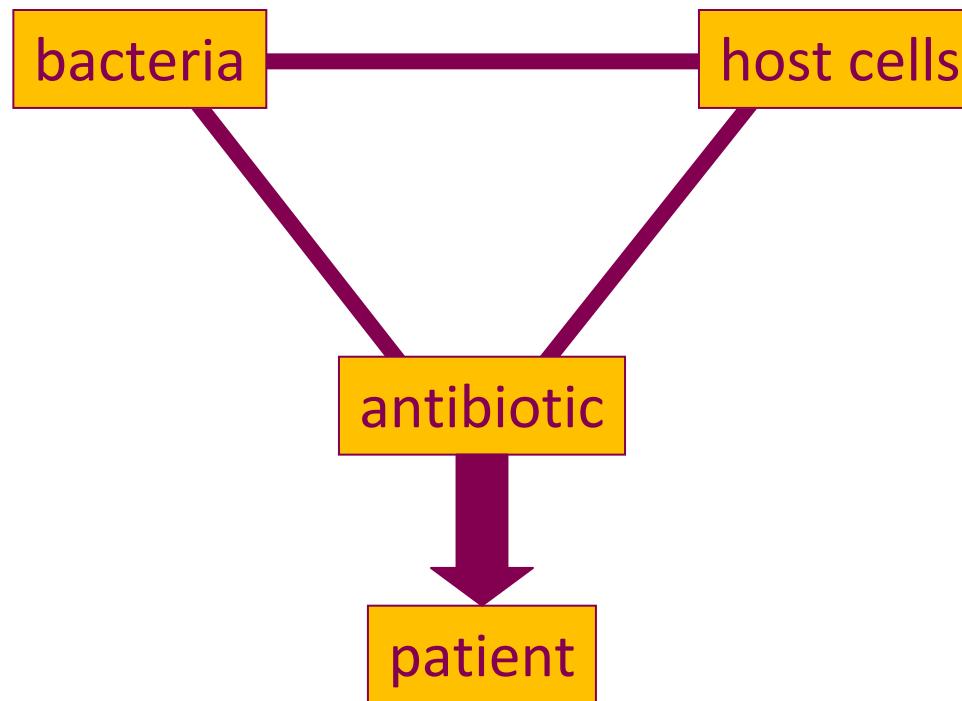


"corpora non agunt nisi fixata"

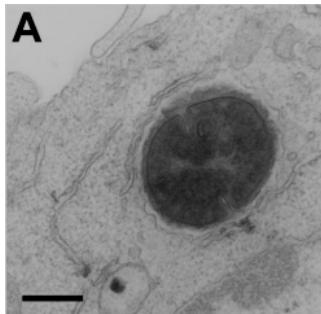
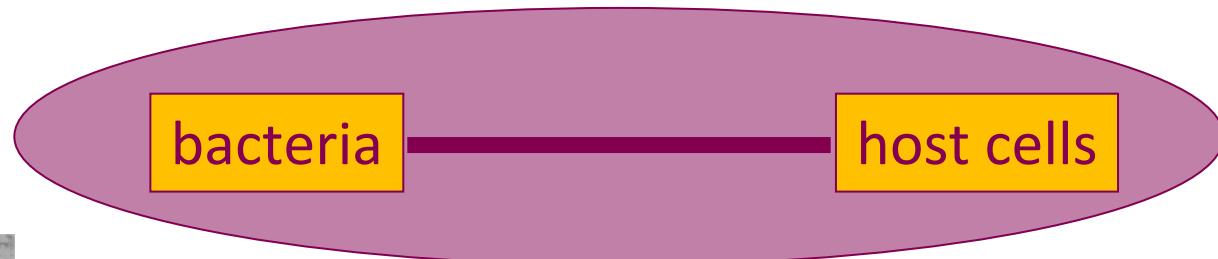


Ehrlich's "magic bullet" theory

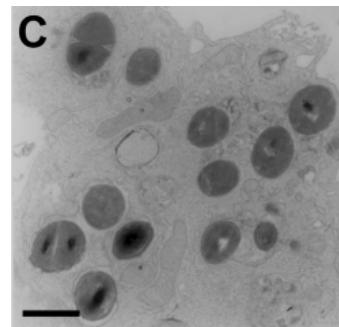
Studying anti-infective pharmacology to improve antibiotic treatment



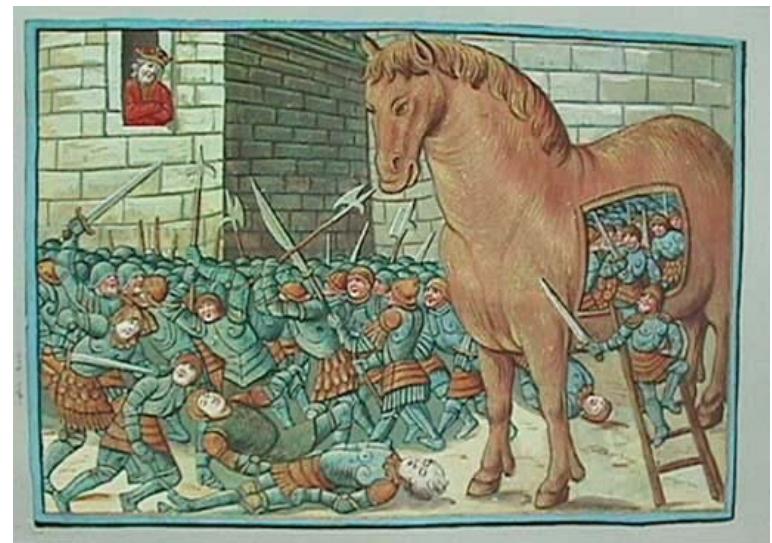
Specific lifestyles associated with persistent infections



A
Intracellular
Staphylococcus aureus

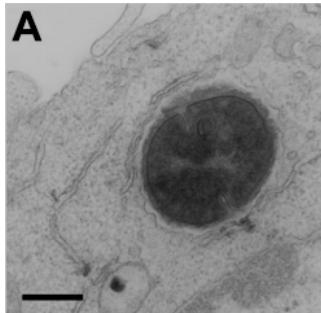


C
Intracellular infection: an application of the Trojan horse strategy

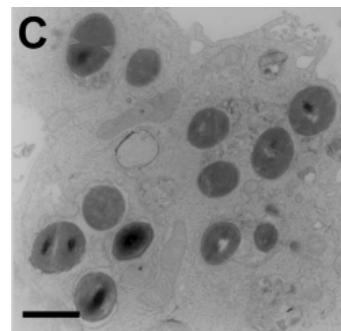


Seral et al, AAC 1993

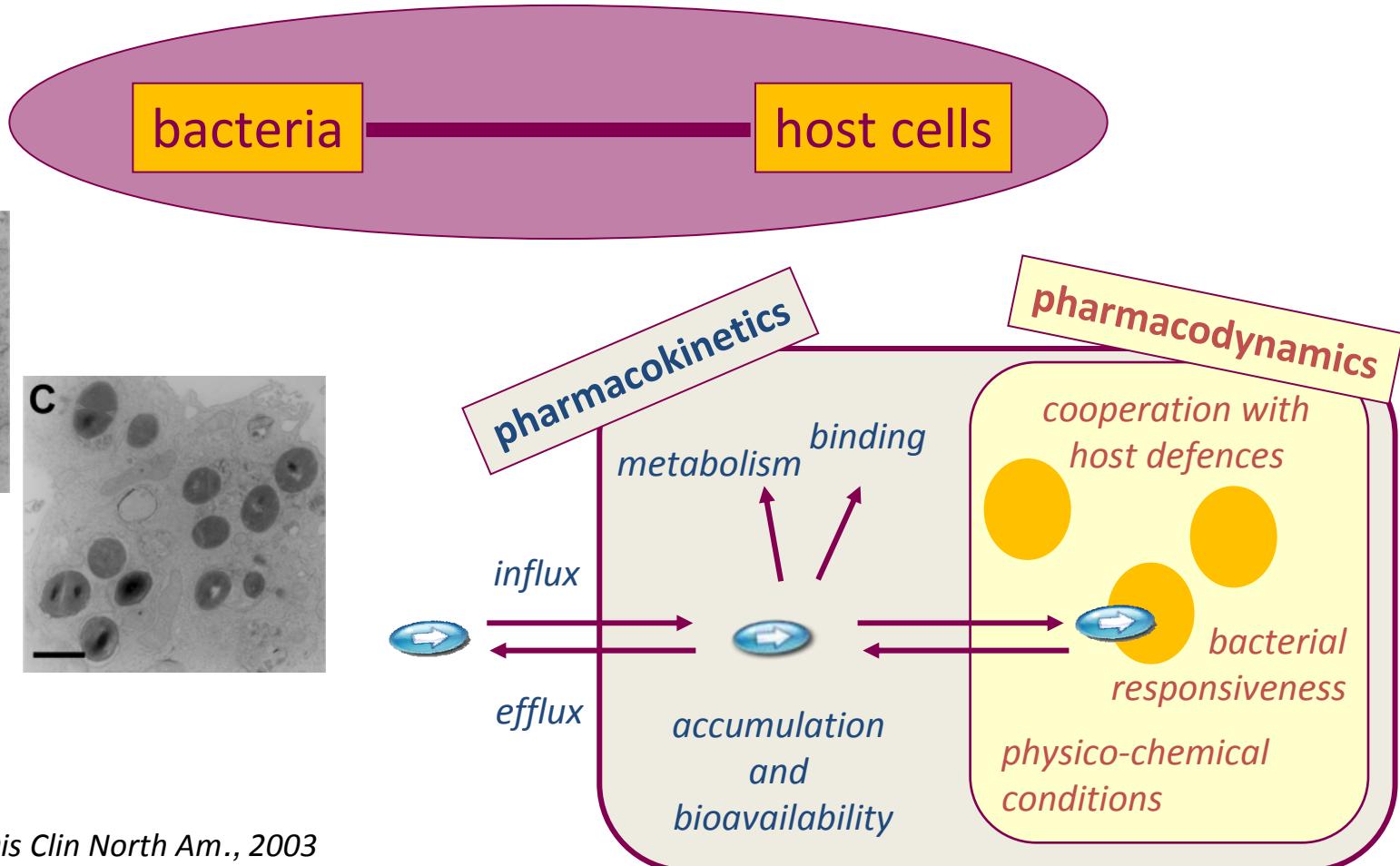
Specific lifestyles associated with persistent infections



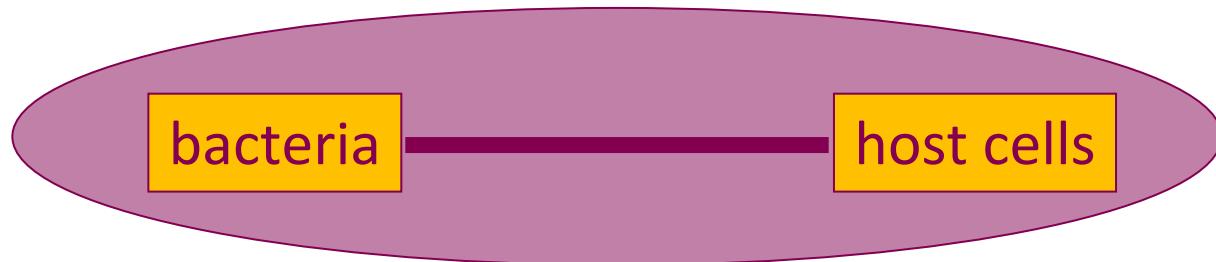
Intracellular
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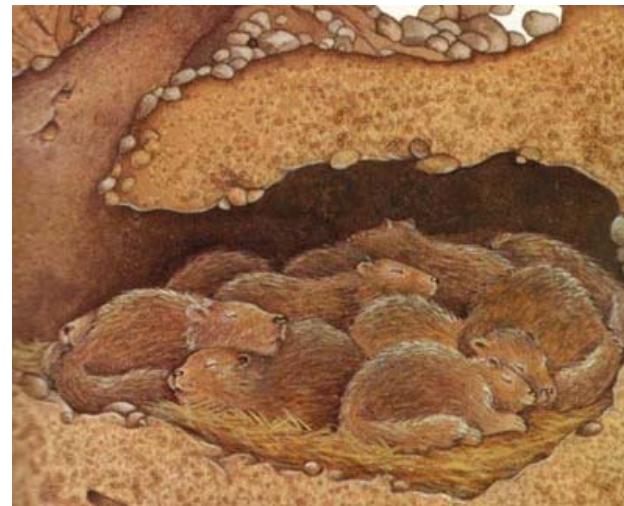
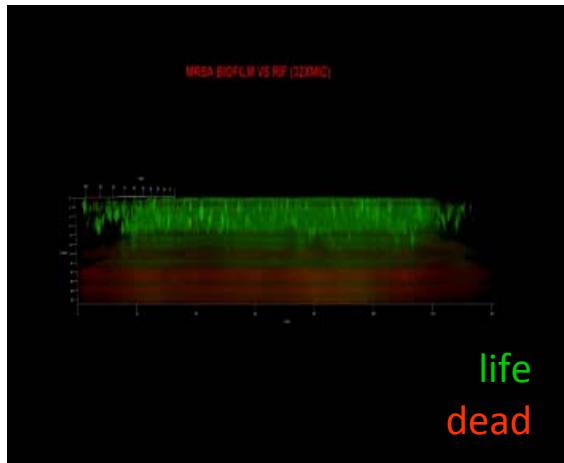
Carryn et al, Infect Dis Clin North Am., 2003



Specific lifestyles associated with persistent infections

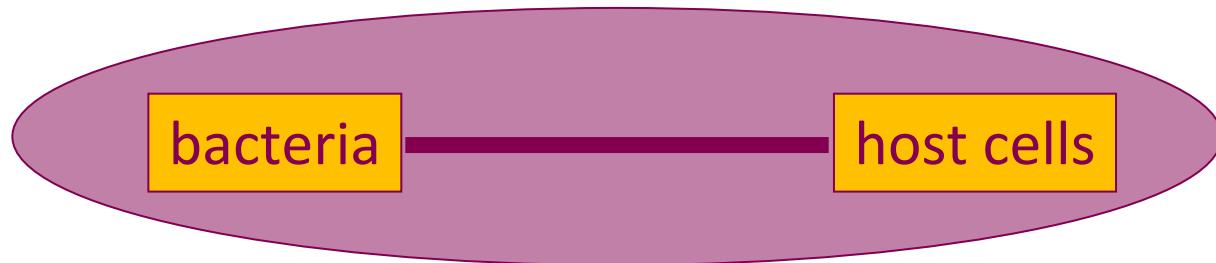


Biofilms: bacterial hibernation

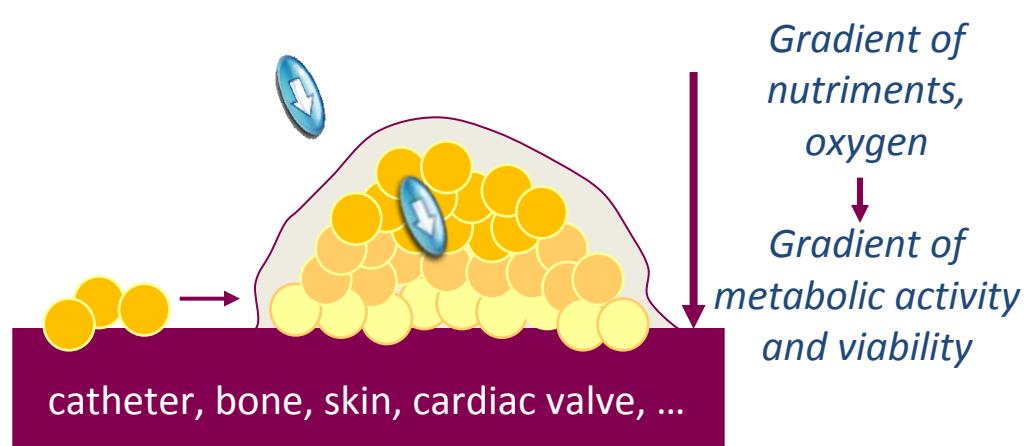
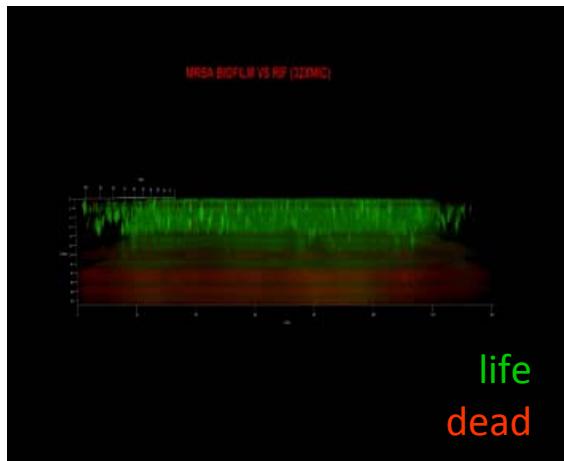


Bauer, Siala et al, AAC 2013

Specific lifestyles associated with persistent infections



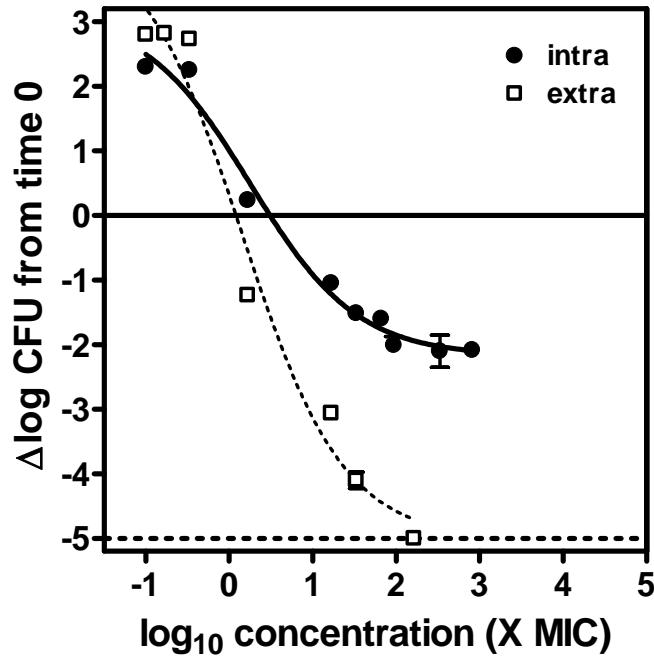
Biofilms: bacterial hibernation



Bauer, Siala et al, AAC 2013

Specific lifestyles associated with persistent infections

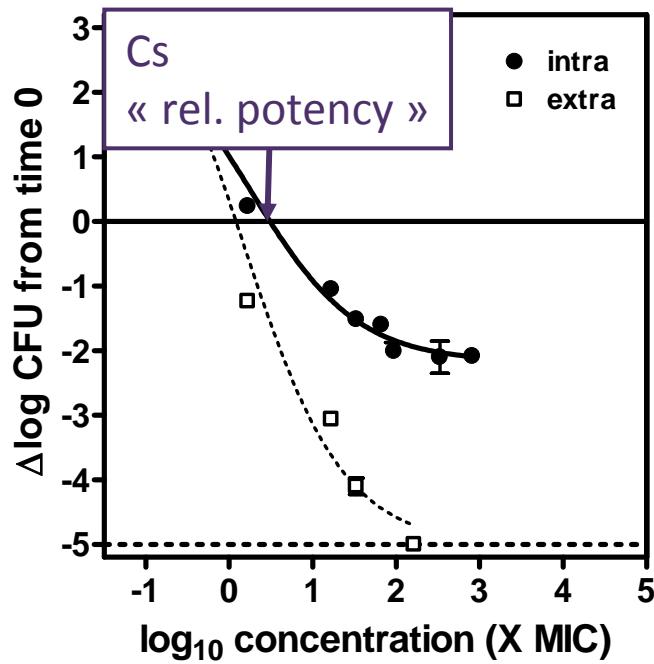
Intracellular infection: activity of the fluoroquinolone moxifloxacin on *S. aureus*



Lemaire et al, JAC 2011

Specific lifestyles associated with persistent infections

Intracellular infection: activity of the fluoroquinolone moxifloxacin on *S. aureus*



Lemaire et al, JAC 2011

Cs ~ intracellular bacteria :

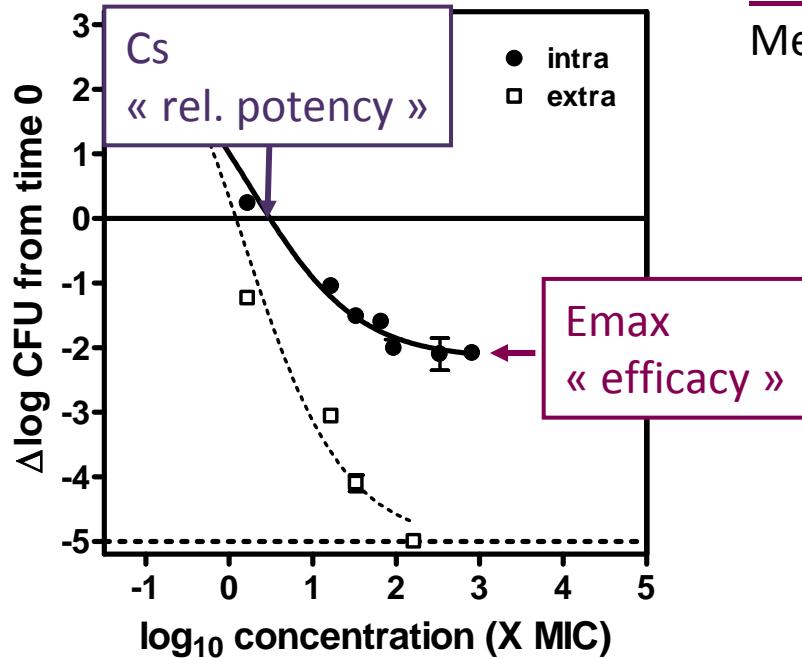
Measure of the « intracellular MIC »

- « Pharmacokinetic-related » parameter:
accumulation in the infected compartment
intracellular bioavailability
- influence of local environment on activity
pH
oxidant species

close to the MIC
even for antibiotics accumulating in cells

Specific lifestyles associated with persistent infections

Intracellular infection: activity of the fluoroquinolone moxifloxacin on *S. aureus*



Emax ~ intracellular bacteria:

Measure of killing capacity

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

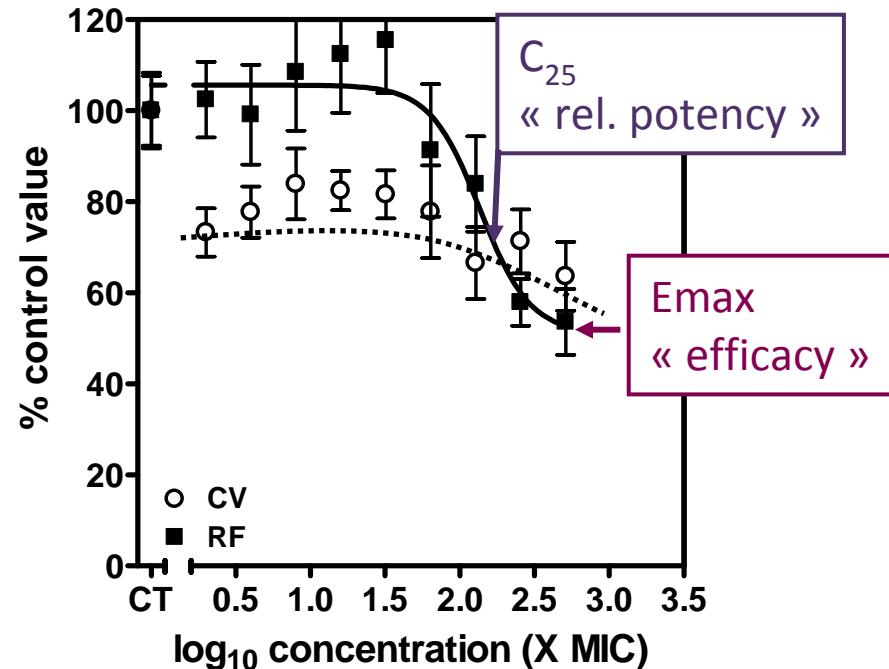
Lower than extracellularly, suggesting poor bacterial responsiveness and/or antibiotic expression of activity

Lemaire et al, JAC 2011

Specific lifestyles associated with persistent infections

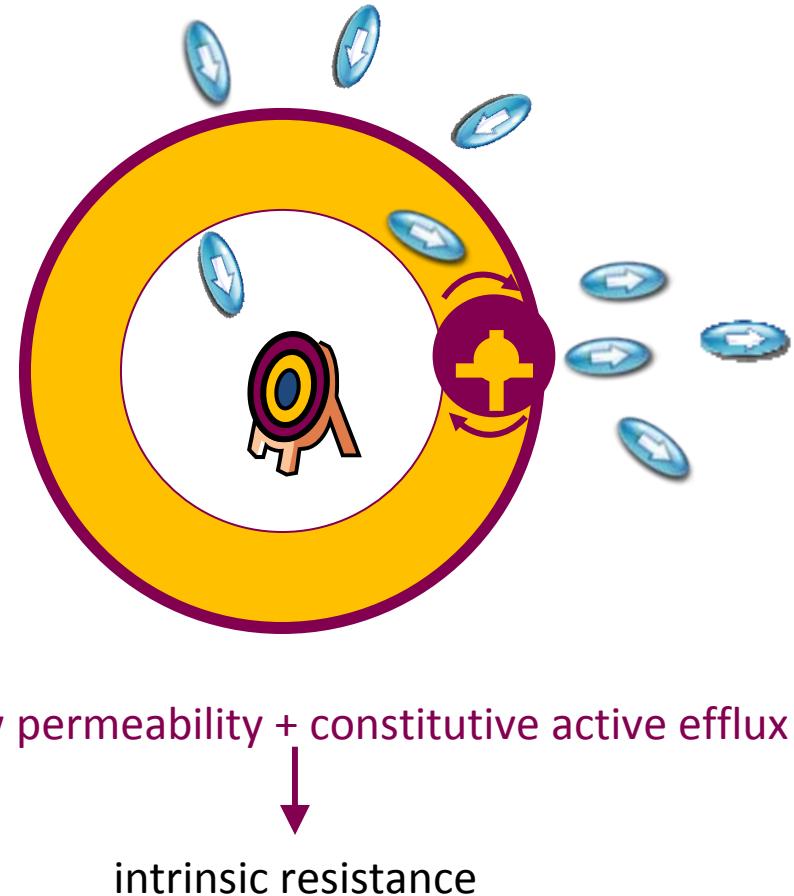
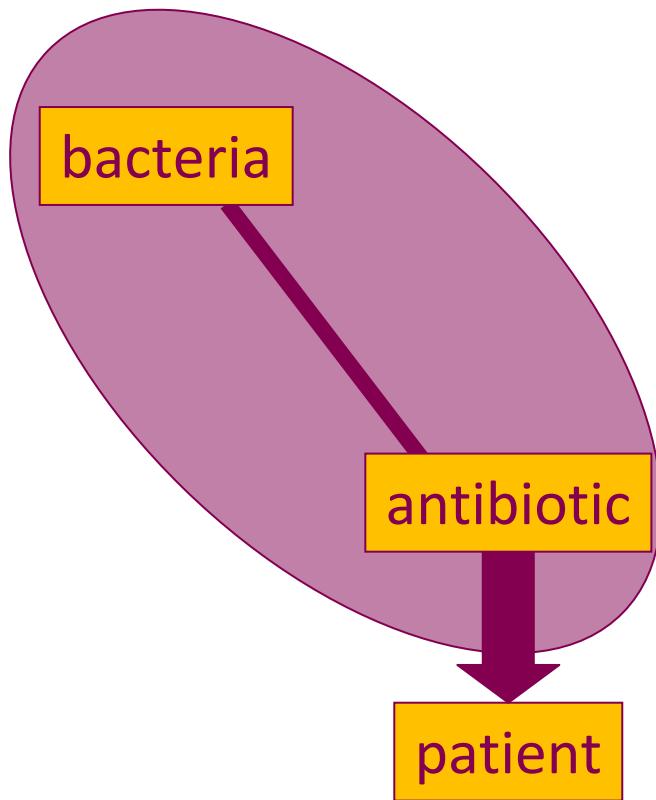
Biofilm: activity of the fluoroquinolone moxifloxacin on *S. aureus*

Pharmacodynamic profile similar to that observed intracellularly, suggesting similar defeating factors



Bauer, Siala et al, AAC 2013

Active efflux and intrinsic/acquired resistance to antibiotics



Active efflux and intrinsic/acquired resistance to antibiotics

Azithromycin is inactive on *P. aeruginosa* ...

medium	strain	MIC (mg/L)
CA-MHB	PAO1	512

Active efflux and intrinsic/acquired resistance to antibiotics

Azithromycin is inactive on *P. aeruginosa* ...

medium	strain	MIC (mg/L)
CA-MHB	PAO1	512

... but is successfully used in cystic fibrosis patients

J Antimicrob Chemother 2011; **66**: 968–978
doi:10.1093/jac/dkr040 Advance Access publication 2 March 2011

Effectiveness and safety of macrolides in cystic fibrosis patients: a meta-analysis and systematic review

Yun Cai¹, Dong Chai¹, Rui Wang^{1*}, Nan Bai¹, Bei-Bei Liang¹ and Youning Liu²

Conclusions: Long-term use of azithromycin can improve lung function, especially for *P. aeruginosa*-colonized CF patients. There was no evidence of increased adverse events with azithromycin. More data are needed to verify the best azithromycin regimen and to evaluate other macrolides in CF patients.

Active efflux and intrinsic/acquired resistance to antibiotics

Azithromycin becomes active on *P. aeruginosa* when efflux systems are inactivated

medium	strain	MIC (mg/L)
CA-MHB	PAO1	512
	PAO1 Δ (<i>mexAB-oprM, mexCD-oprJ, mexJK, mexXY, mexEF-oprN</i>)	8

Buyck et al, CID 2012

Active efflux and intrinsic/acquired resistance to antibiotics

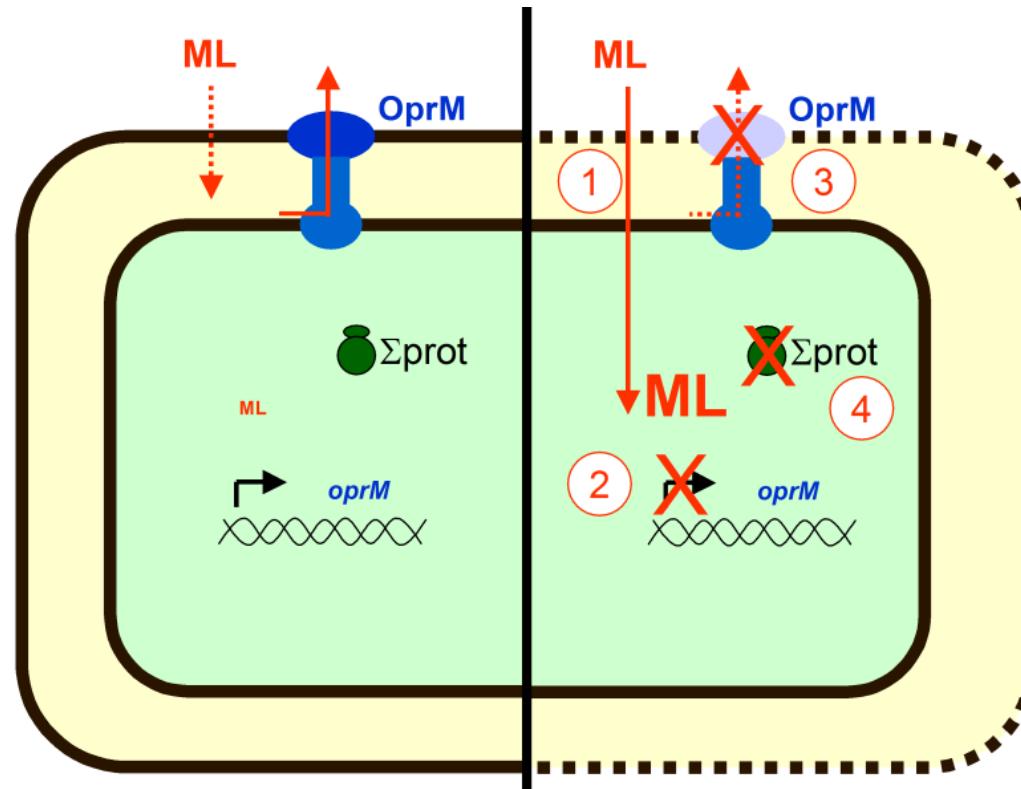
Azithromycin becomes active on *P. aeruginosa* when cultivated in biological fluids

medium	strain	MIC (mg/L)
CA-MHB	PAO1	512
	PAO1 Δ (<i>mexAB-oprM, mexCD-oprJ, mexJK, mexXY, mexEF-oprN</i>)	8
Bronchoalveolar lavage	PAO1	16
CA-MHB/serum 50:50		8

Buyck et al, CID 2012

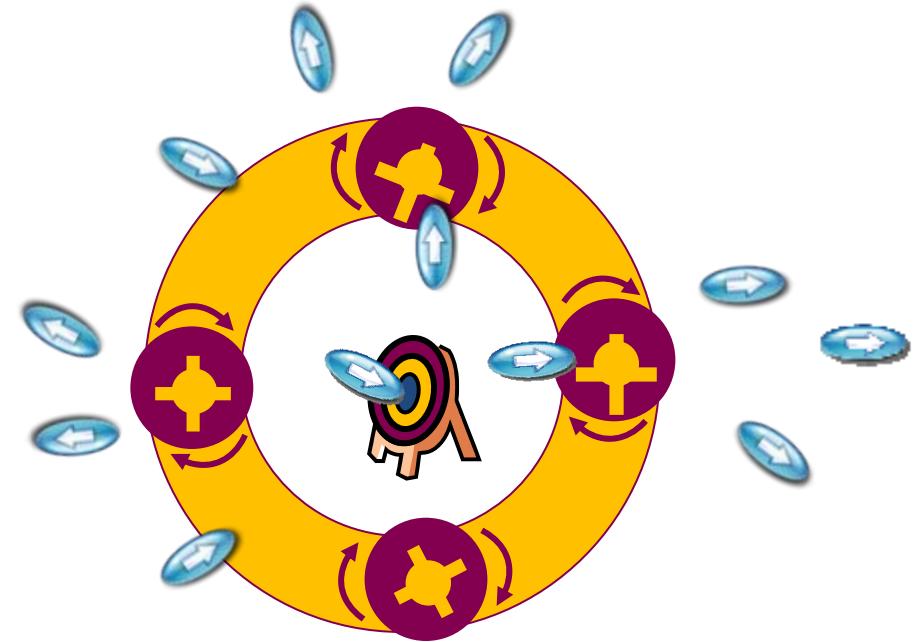
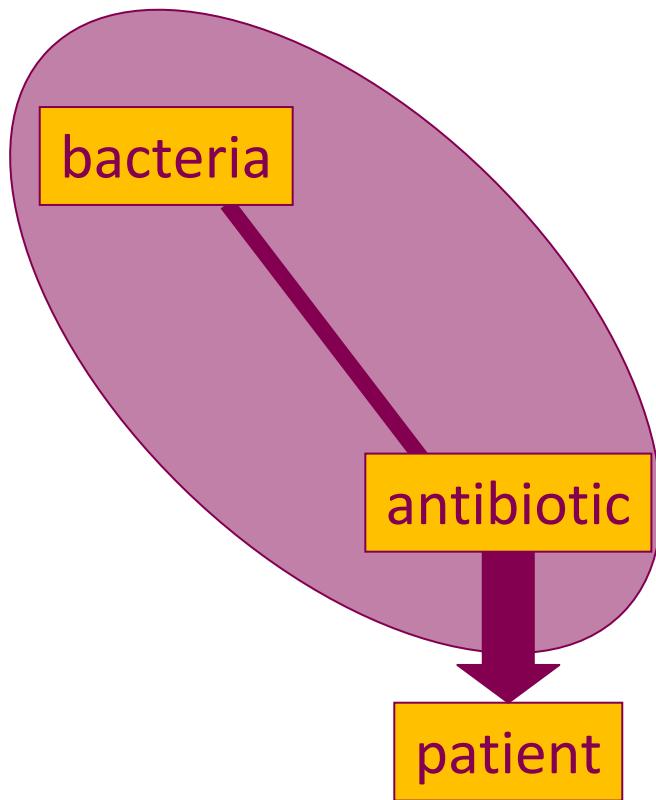
Active efflux and intrinsic/acquired resistance to antibiotics

Azithromycin does express its activity on *P. aeruginosa* in biological fluids by repressing the expression of its efflux transporters



Buyck et al, CID 2012

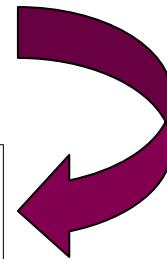
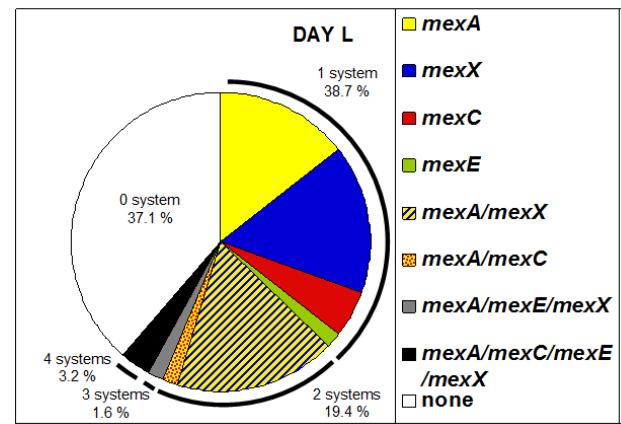
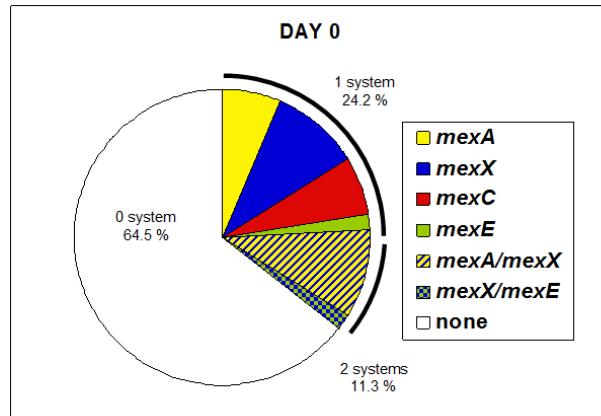
Active efflux and intrinsic/acquired resistance to antibiotics



- reduced concentration at the target site
- risk of suboptimal dosage
- risk of selection of resistance (target mutations)

Active efflux and intrinsic/acquired resistance to antibiotics

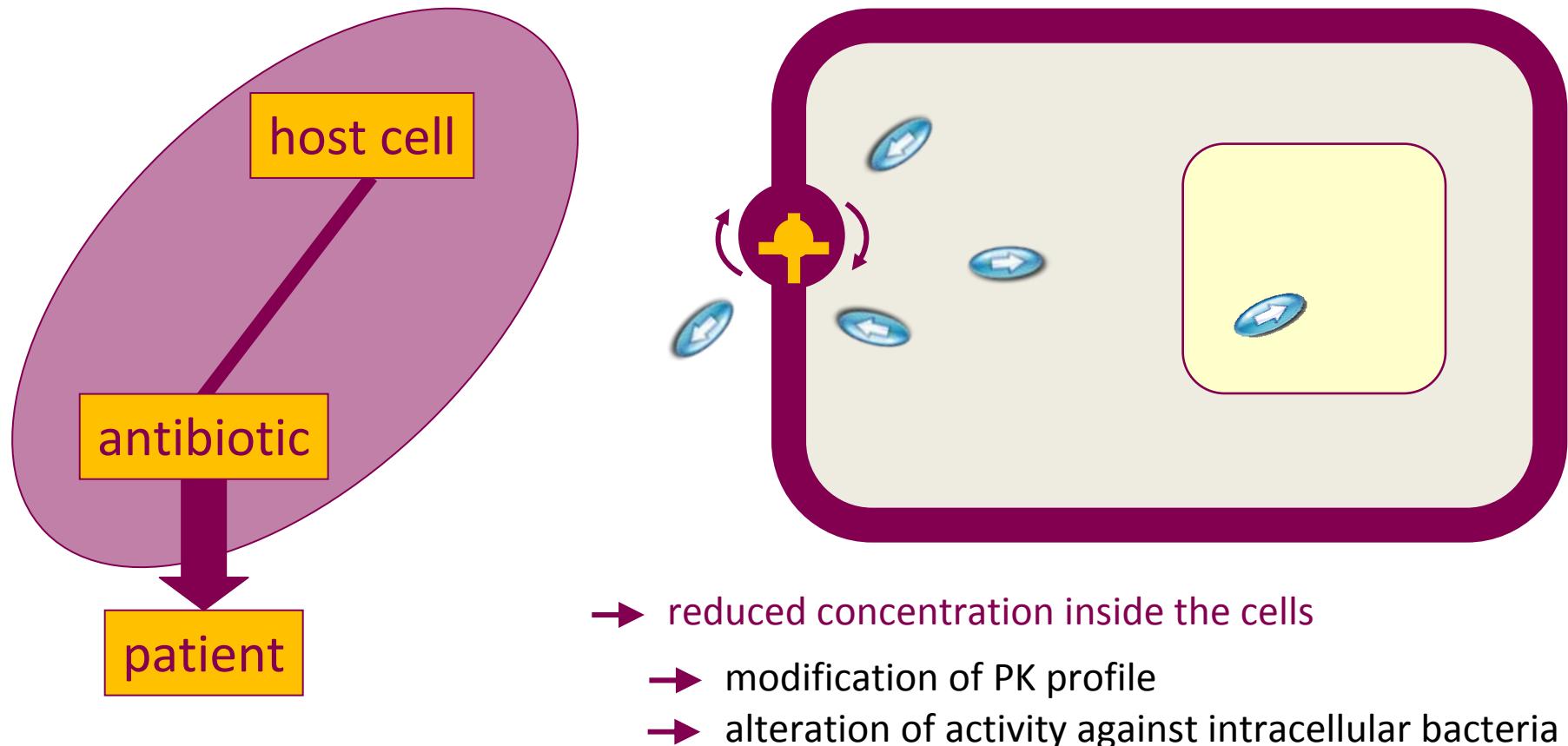
Efflux mechanisms are overexpressed during treatment



Pseudomonas aeruginosa isolated from patients in intensive care units suffering from hospital-acquired pneumonia

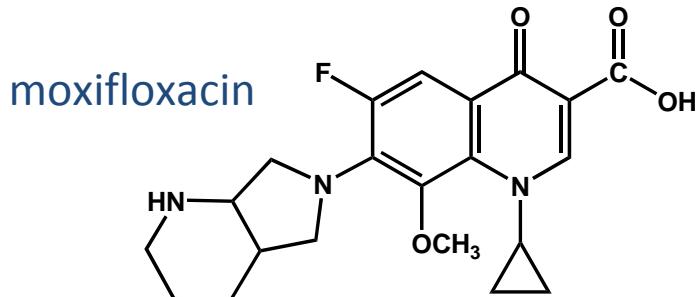
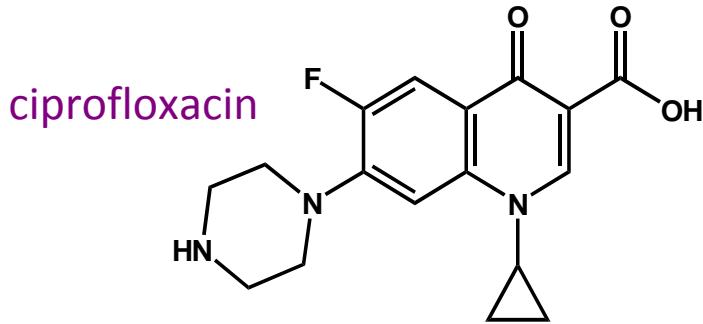
treatment with antibiotics

Active efflux and modulation of cellular pharmacokinetics of antibiotics

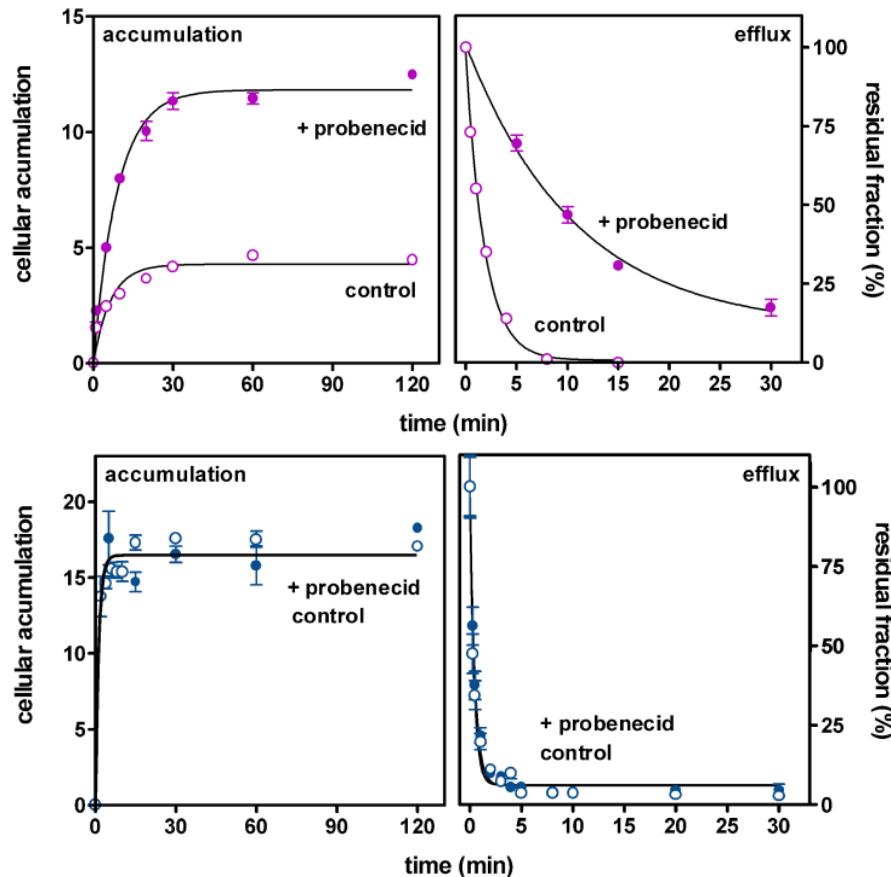


Active efflux and modulation of cellular pharmacokinetics of antibiotics

Differential recognition of fluoroquinolones by macrophage efflux transporters



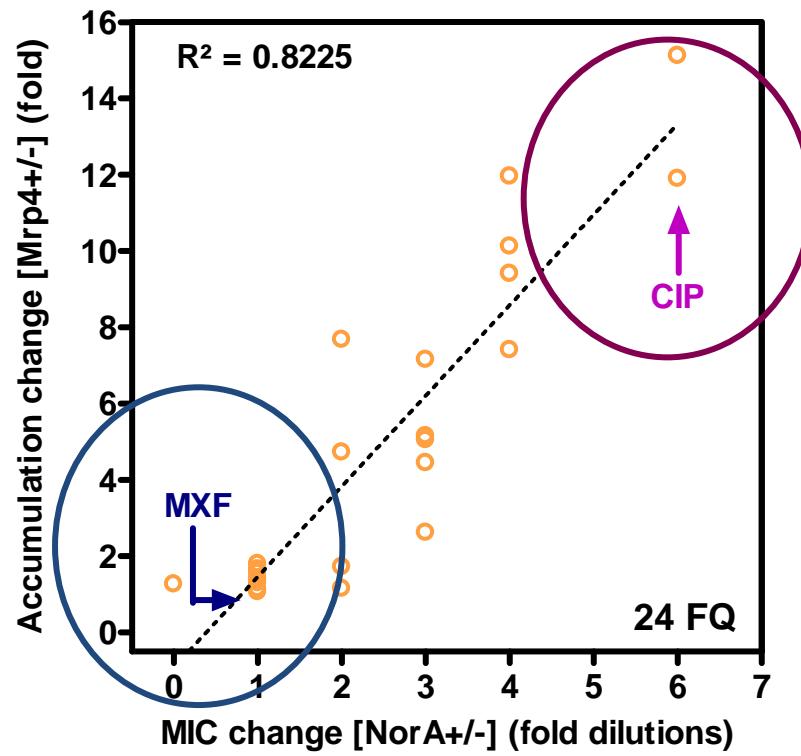
Michot et al., AAC 2004 & 2005



Active efflux and modulation of cellular pharmacokinetics of antibiotics

But similarity in recognition of fluoroquinolones by macrophage and bacterial efflux transporters

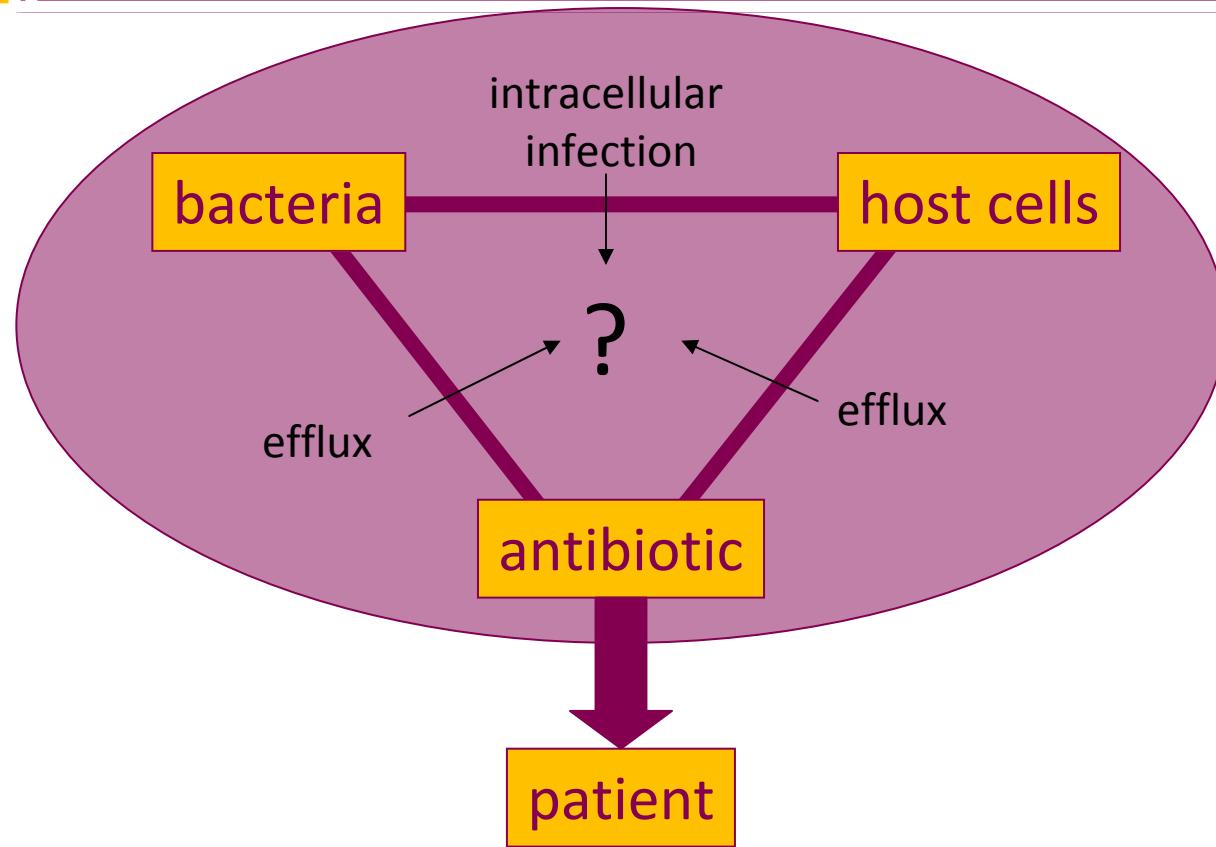
Poor substrates of efflux systems from macrophages and Gram-(+) bacteria



Substrates of efflux systems from macrophages and Gram-(+) bacteria

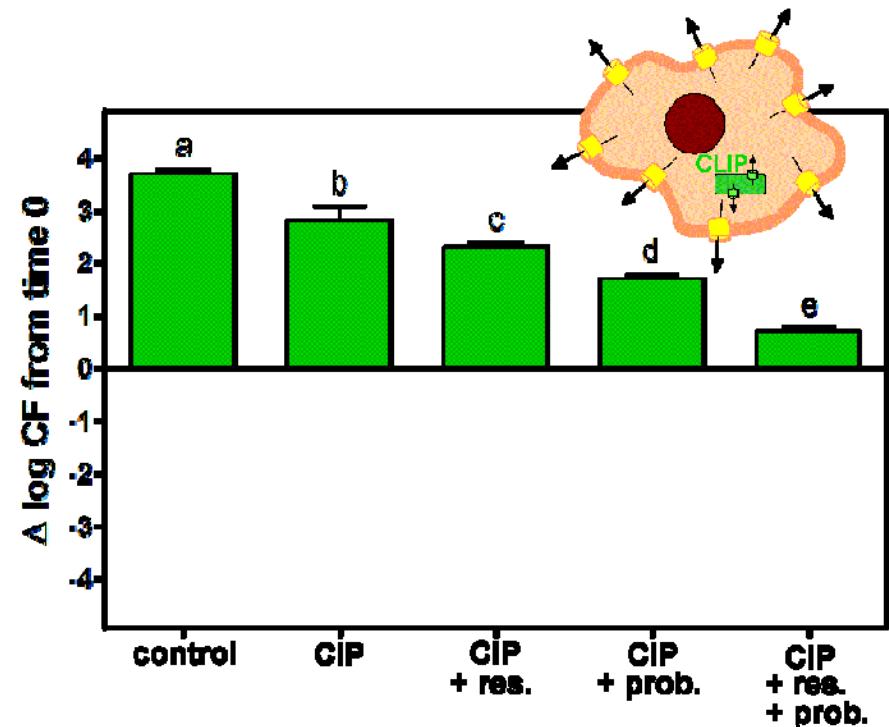
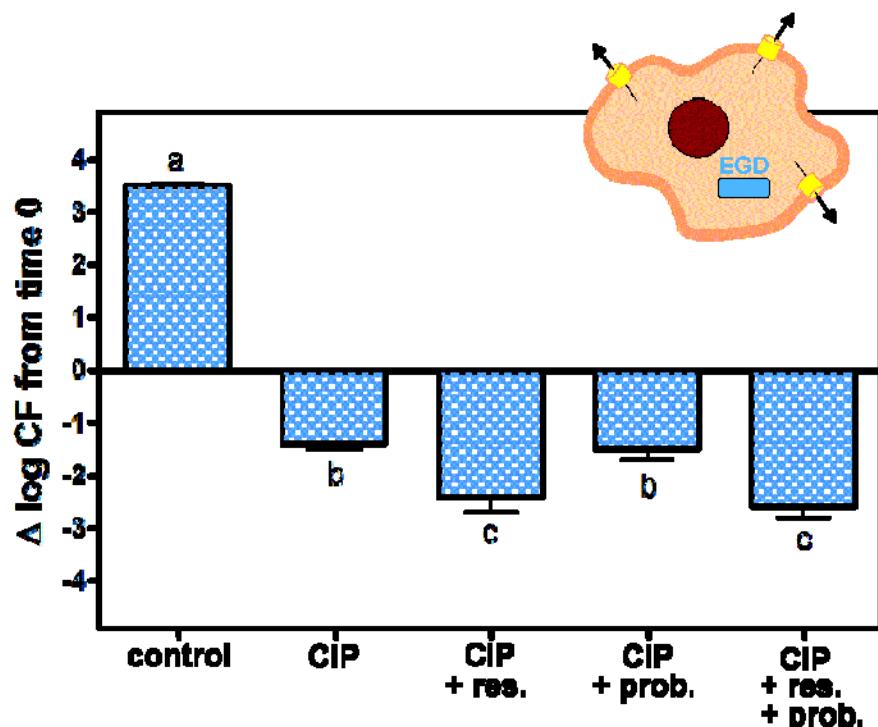
Dupont et al., ECCMID 2012

Studying anti-infective pharmacology to improve antibiotic treatment



Cooperation between prokaryotic and eukaryotic efflux systems

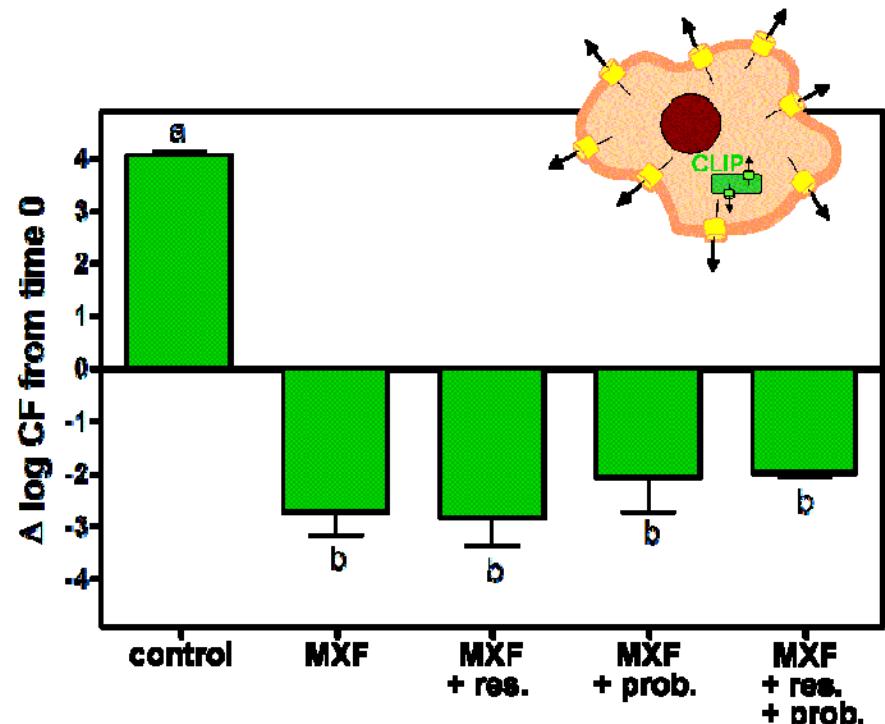
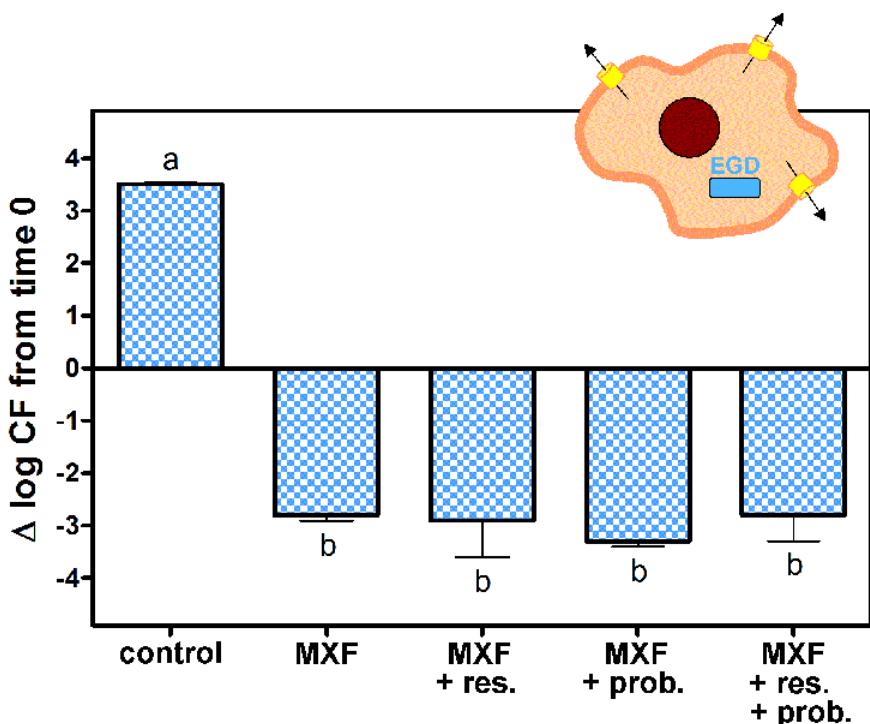
Bacterial and eukaryotic efflux pumps cooperate to make intracellular *Listeria monocytogenes* resistant to ciprofloxacin



Lismond et al., AAC 2008

Cooperation between prokaryotic and eukaryotic efflux systems

Moxifloxacin intracellular activity is not affected by eukaryotic / bacterial efflux pumps



Lismond et al., AAC 2008

Clinical implications of this work

1. Expression of activity is highly dependent on the environment

- Growth medium can influence bacterial physiology
- Intracellular bacteria / biofilms are refractory to antibiotics

→ importance of testing antibiotic activity in relevant media/models

2. Screening tests in routine laboratories may need to be revisited

- Susceptibility changes during treatment
- Low level resistance mechanisms (efflux) often escape detection in routine but are clinically meaningful

→ interest of basing dosages on PK/PD approaches and MIC determinations

→ importance of developing new diagnostic tools

3. Pertinent in vitro models may be helpful in preclinical evaluation of new drugs

- Intracellular survival is considered as determinant in recurrence / persistence
- Biofilms are associated to about 80 % of infections

→ in vitro screening may help selecting drug candidates

Acknowledgments

Financial support to this work



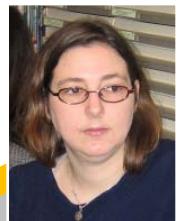
Wallonie



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Acknowledgments

Coworkers over the years in the team - efflux



Laetitia Avrain



Julien Buyck



Nancy Caceres



Hussein Chalhoub



Farid El Garch



Ann Lismond



Coralie Vallet



Mickaël Riou



Hariri Mustafa



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Béatrice Marquez

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Coworkers over the years in the team – intracellular infections & biofilms



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Anantharajah



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Barcia



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Baudoux



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Carryn



Laetitia
Garcia



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Youssef
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Julia
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Basseres



Wafi
Siala



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Marie-Claire
Cambier



Charlotte
Misson



Virginie
Mohymont



Pierre
Muller



Katia
Santos



Martial
Vergauwen

The two successive « heads »
of the team



Paul
Tulkens

Marie-Paule
Mingeot