42e RÉUNION INTERDISCIPLINAIRE DE CHIMIOTHÉRAPIE ANTI-INFECTIEUSE
LUNDI 12 & MARDI 13 DÉCEMBRE 2022
PALAIS DES CONGRÈS • PARIS
RICAI

La tolérance des staphylocoques aux antibiotiques.

Françoise Van Bambeke, PharmD, PhD

Pharmacologie cellulaire et moléculaire
Louvain Drug Research Institute
UCLouvain, Brussels, Belgium

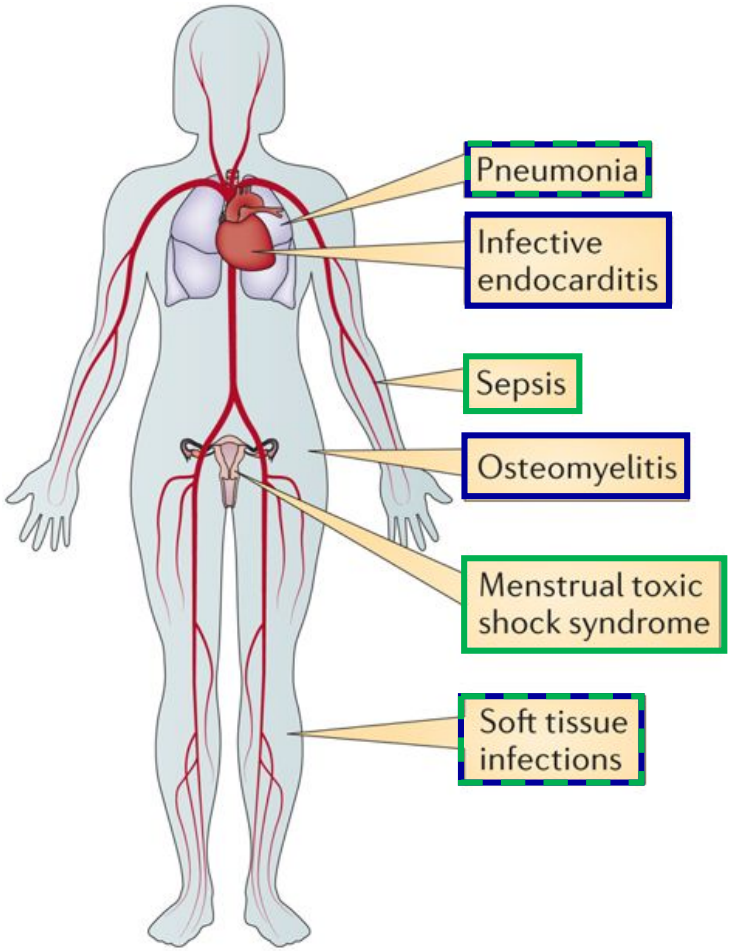
<www.facm.ucl.ac.be>

How do bacteria protect themselves against antibiotics ?

“offensive strategy”: to develop resistance mechanisms

“defensive strategy”: to adopt ‘hidden’ mode of life

Main infections caused by *S. aureus*



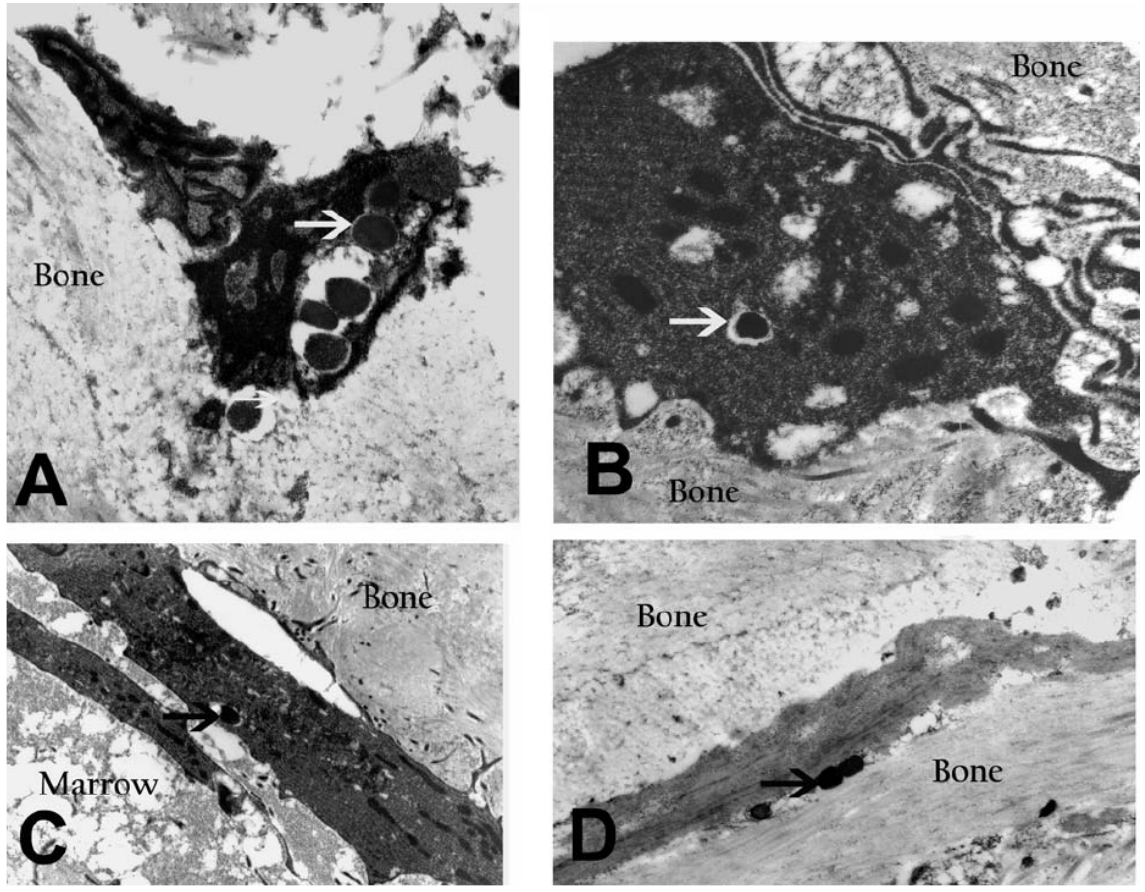
Acute infections

Chronic infections

Salgado-Pabón & Schlievert; Nat Rev Microbiol. 2014; 12:585-91

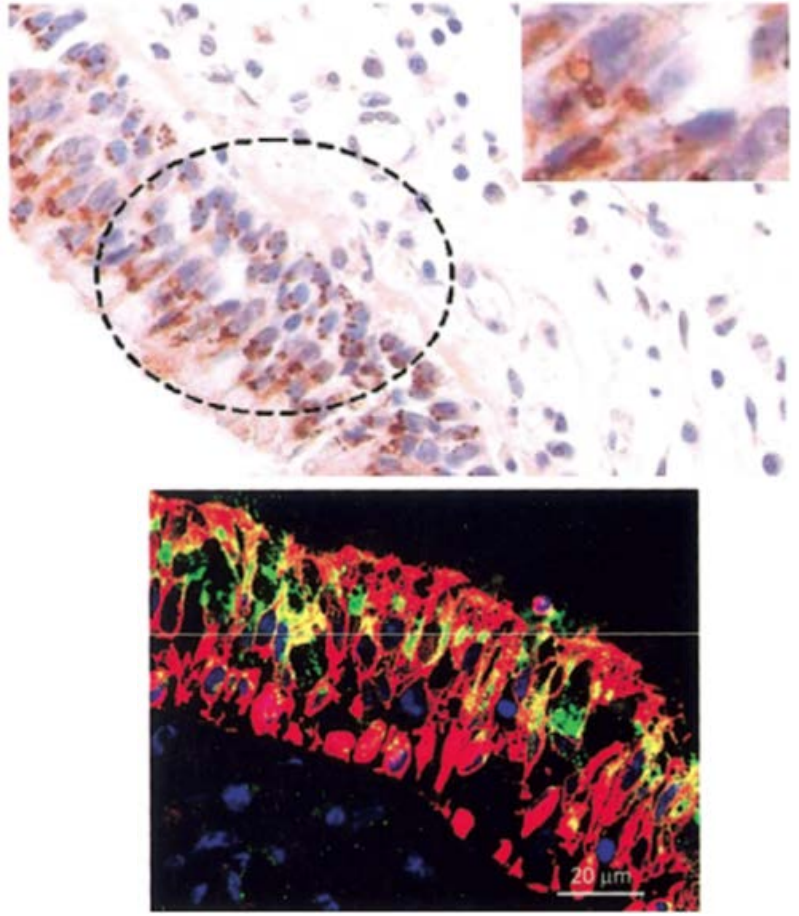
Intracellular survival and persistent infections

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



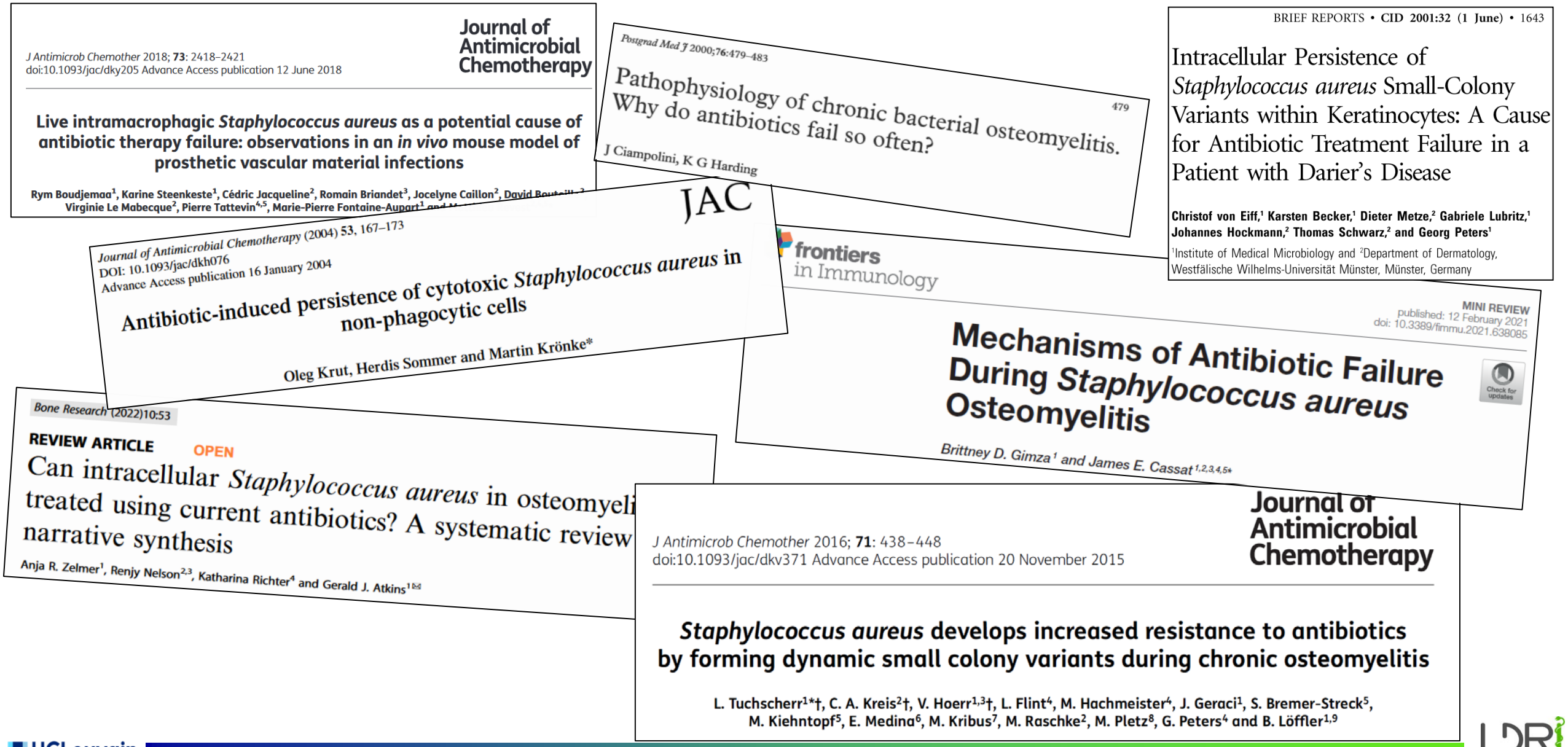
Bosse et al, J Bone Joint Surg Am. 2005; 87: 1343-7

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



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

Antibiotic failure against intracellular *S. aureus*



J Antimicrob Chemother 2018; **73**: 2418–2421
doi:10.1093/jac/dky205 Advance Access publication 12 June 2018

Journal of Antimicrobial Chemotherapy

Live intramacrophagic *Staphylococcus aureus* as a potential cause of antibiotic therapy failure: observations in an *in vivo* mouse model of prosthetic vascular material infections

Rym Boudjemaa[1], Karine Steenkeste[1], Cédric Jacqueline[2], Romain Briandet[3], Jocelyne Caillon[2], David Boutoille[2], Virginie Le Mabecque[2], Pierre Tattevin[4,5], Marie-Pierre Fontaine-Aupart[1] and M...

Postgrad Med J 2000;76:479–483

Pathophysiology of chronic bacterial osteomyelitis. Why do antibiotics fail so often?

479

J Ciampolini, K G Harding

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

Intracellular Persistence of *Staphylococcus aureus* Small-Colony Variants within Keratinocytes: A Cause for Antibiotic Treatment Failure in a Patient with Darier's Disease

Christof von Eiff,[1] Karsten Becker,[1] Dieter Metze,[2] Gabriele Lubritz,[1] Johannes Hockmann,[2] Thomas Schwarz,[2] and Georg Peters[1]

[1]Institute of Medical Microbiology and [2]Department of Dermatology, Westfälische Wilhelms-Universität Münster, Münster, Germany

JAC

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

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

Oleg Krut, Herdis Sommer and Martin Krönke*

frontiers in Immunology

MINI REVIEW
published: 12 February 2021
doi: 10.3389/fimmu.2021.638085

Mechanisms of Antibiotic Failure During *Staphylococcus aureus* Osteomyelitis

Brittney D. Gimza[1] and James E. Cassat[1,2,3,4,5]*

Bone Research (2022)10:53

REVIEW ARTICLE OPEN

Can intracellular *Staphylococcus aureus* in osteomyeli... treated using current antibiotics? A systematic review ... narrative synthesis

Anja R. Zelmer[1], Renjy Nelson[2,3], Katharina Richter[4] and Gerald J. Atkins[1]✉

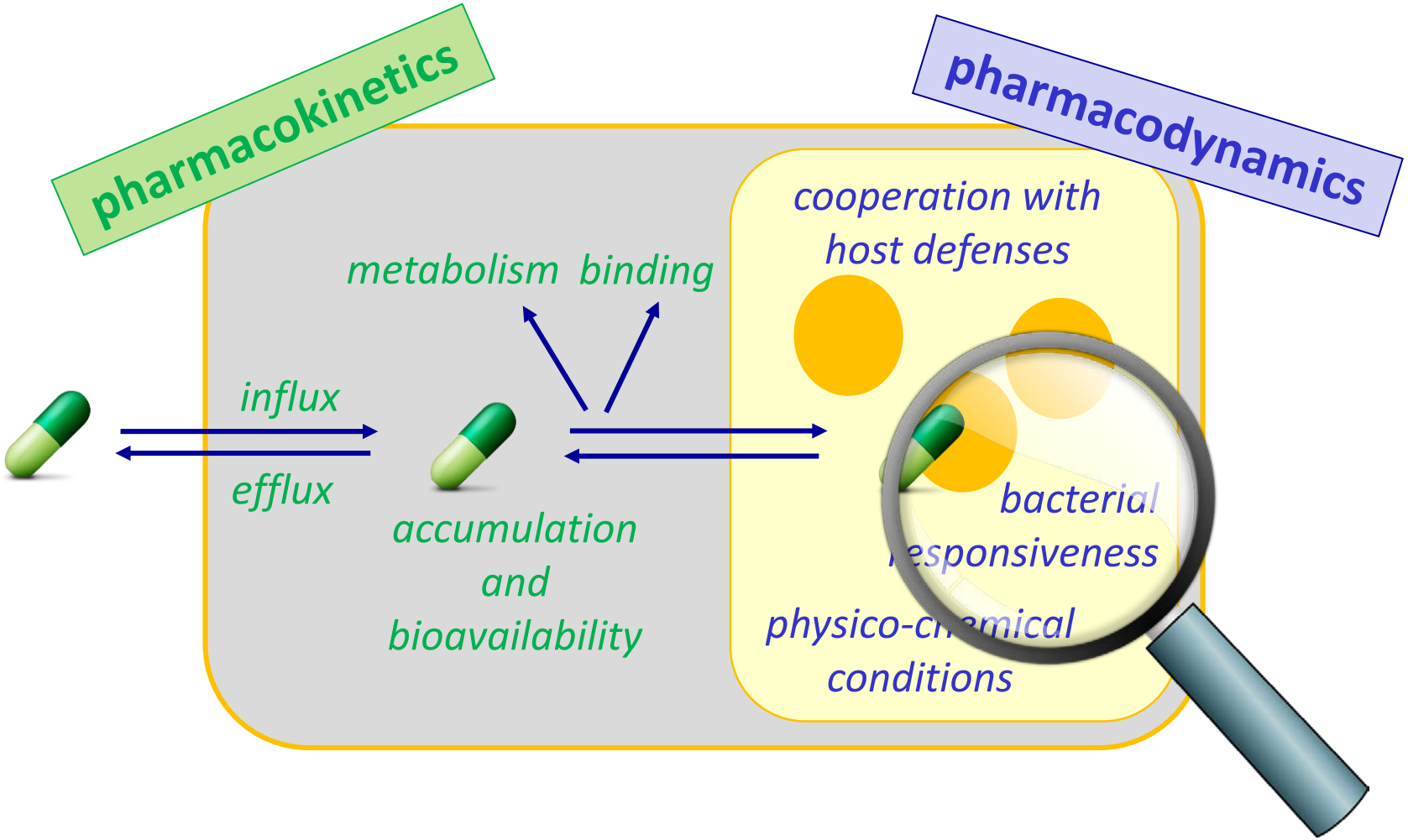
J Antimicrob Chemother 2016; **71**: 438–448
doi:10.1093/jac/dkv371 Advance Access publication 20 November 2015

Journal of Antimicrobial Chemotherapy

***Staphylococcus aureus* develops increased resistance to antibiotics by forming dynamic small colony variants during chronic osteomyelitis**

L. Tuchscherr[1]*†, C. A. Kreis[2]†, V. Hoerr[1,3]†, L. Flint[4], M. Hachmeister[4], J. Geraci[1], S. Bremer-Streck[5], M. Kiehntopf[5], E. Medina[6], M. Kribus[7], M. Raschke[2], M. Pletz[8], G. Peters[4] and B. Löffler[1,9]

PK/PD parameters and intracellular activity



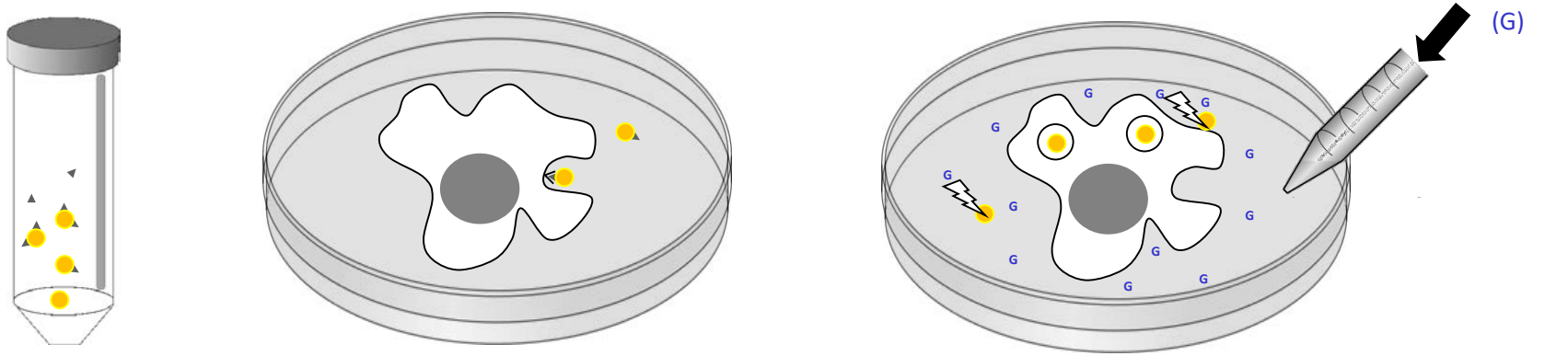
Carryn et al, Infect Dis Clin North Am 2003; 17:615-34

In vitro model of intracellular infection

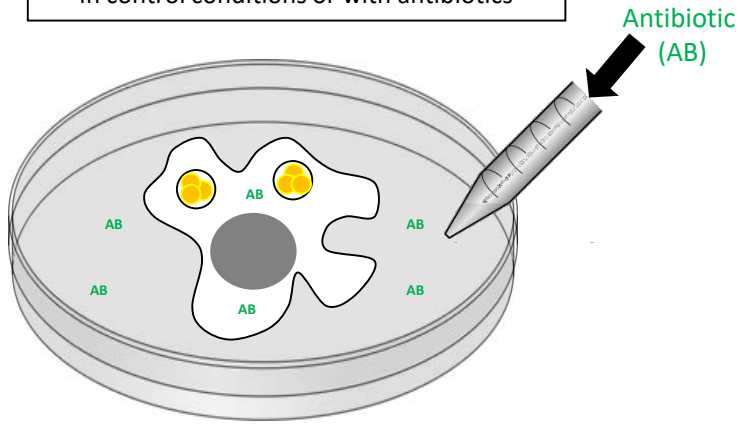
Opsonization
Incubation
with 10 % human serum
in culture medium

Phagocytosis
Incubation of bacteria with
cells at an appropriate MOI

Extracellular wash
Elimination of non phagocytosed bacteria
by incubation with 50-100 X MIC gentamicin;
elimination of gentamicin by washing



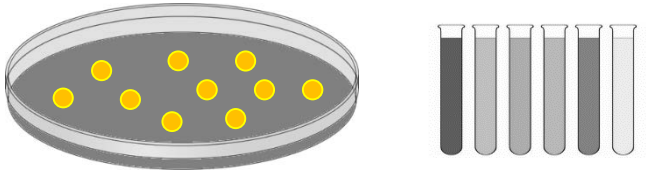
Intracellular infection
Incubation over time
in control conditions or with antibiotics



Determination of residual intracellular inoculum
Determination of cfu and protein content in cell lysates

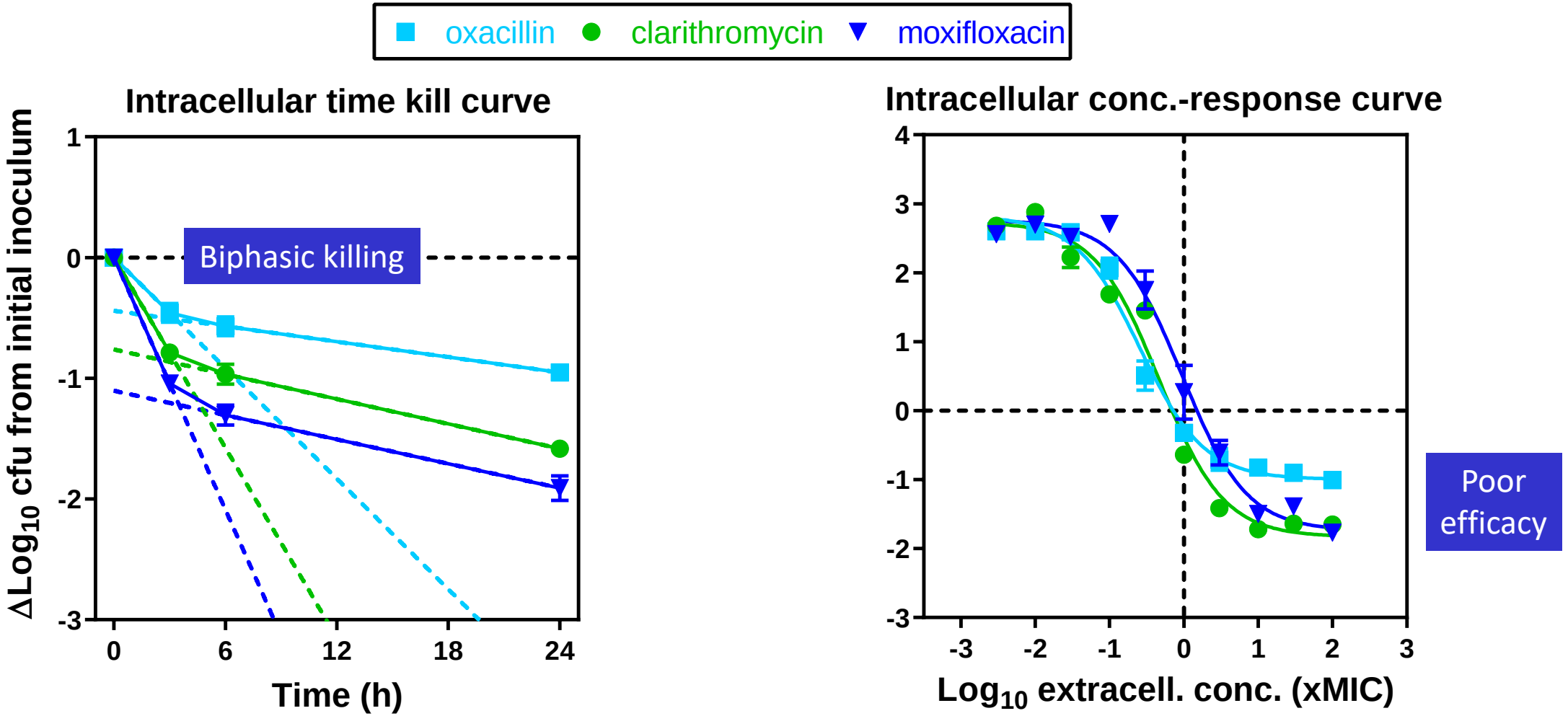
cfu

proteins



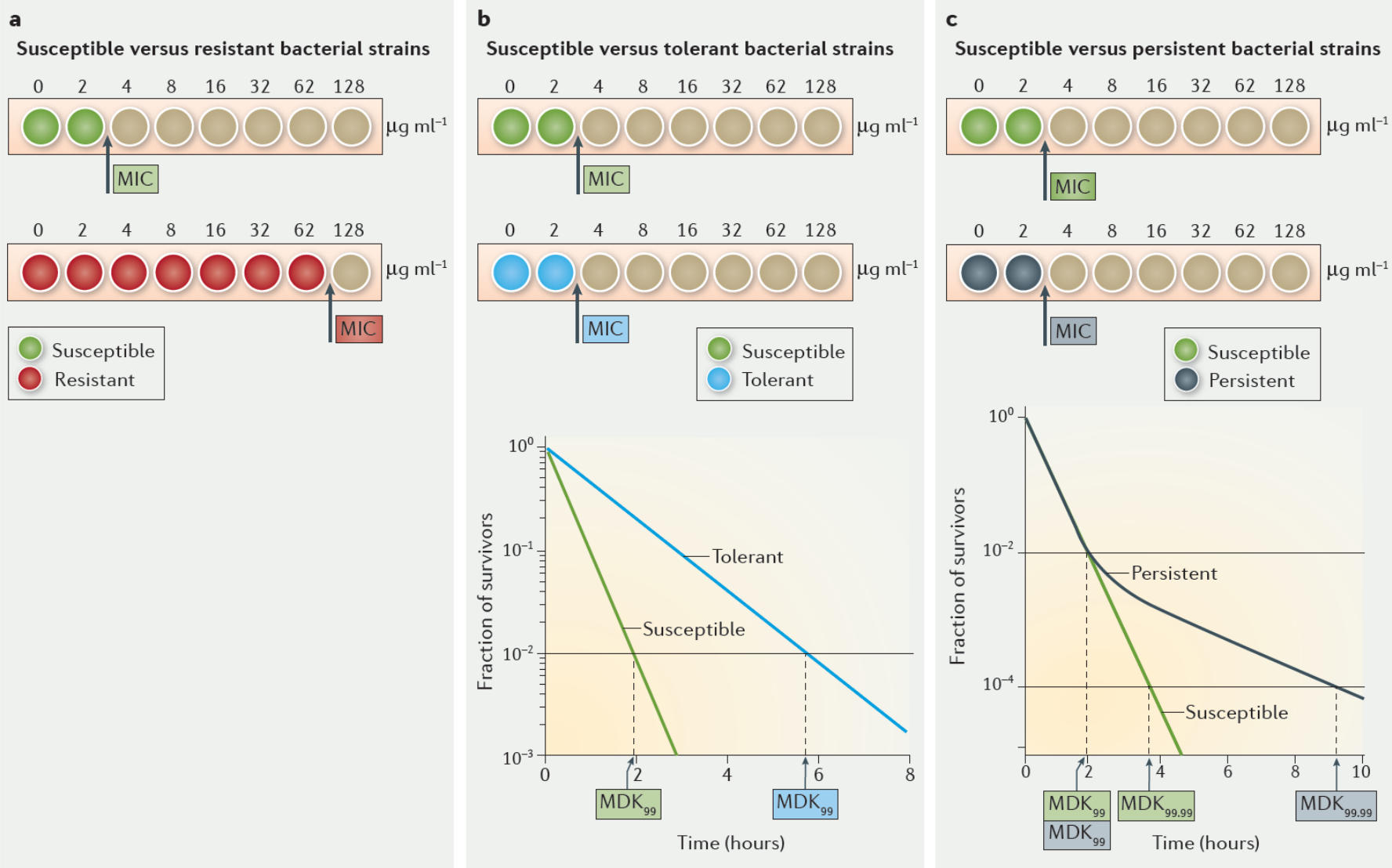
Peyrusson et al, Methods Mol Biol. 2021; 2357:239-251.

Intracellular activity of antibiotics



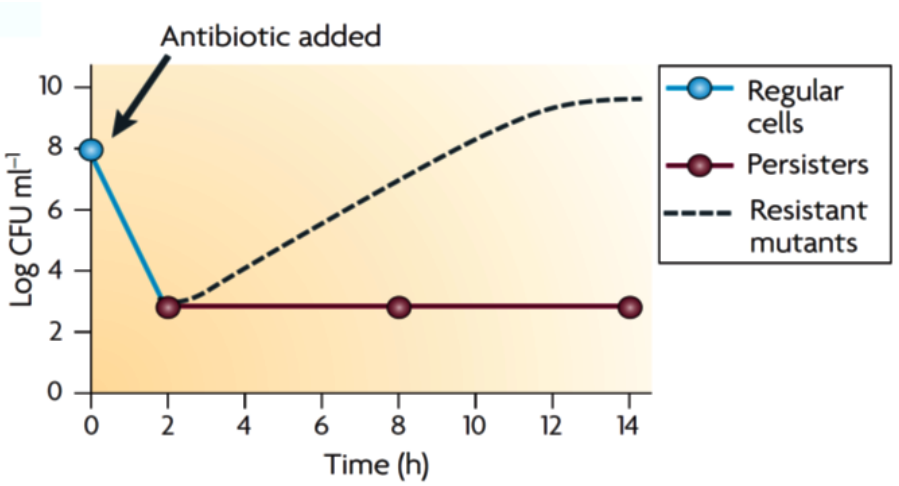
Peyrusson et al, Methods Mol Biol. 2021; 2357:239-251.

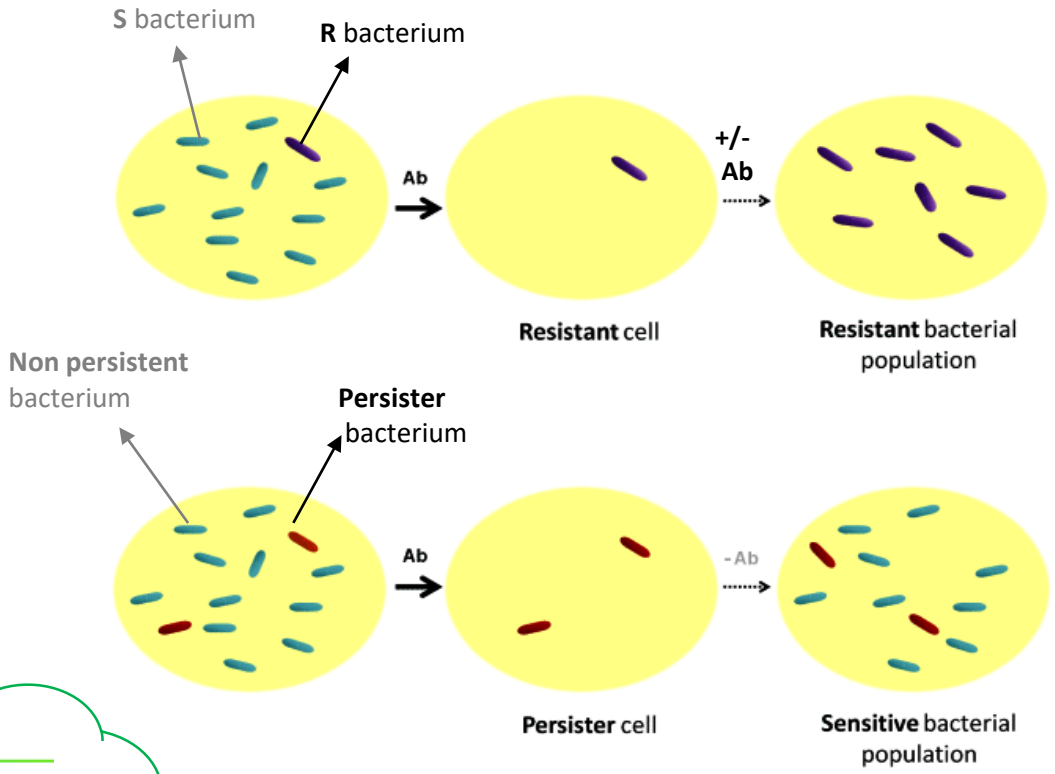
Poor response to antibiotics: resistance, tolerance, or persistence ?



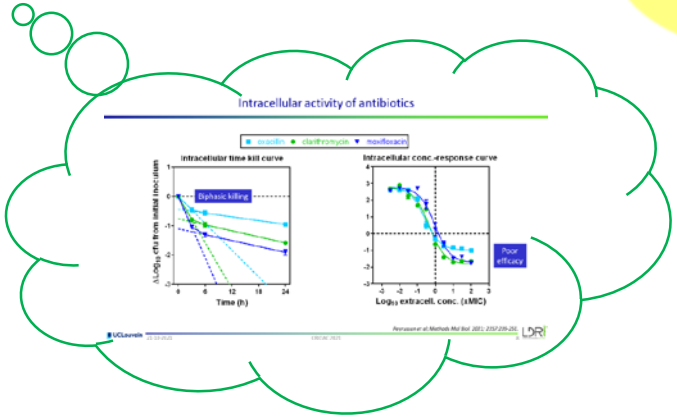
Brauner et al, Nat Rev Microbiol. 2016; 14:320-30.

Persisters and antibiotics





- ✓ Biphasic kill curve in the presence of antibiotics
- ✓ Survival at bactericidal concentrations of antibiotics
- ✓ Low impact of antibiotic concentration (if >>> MIC) on persistence level

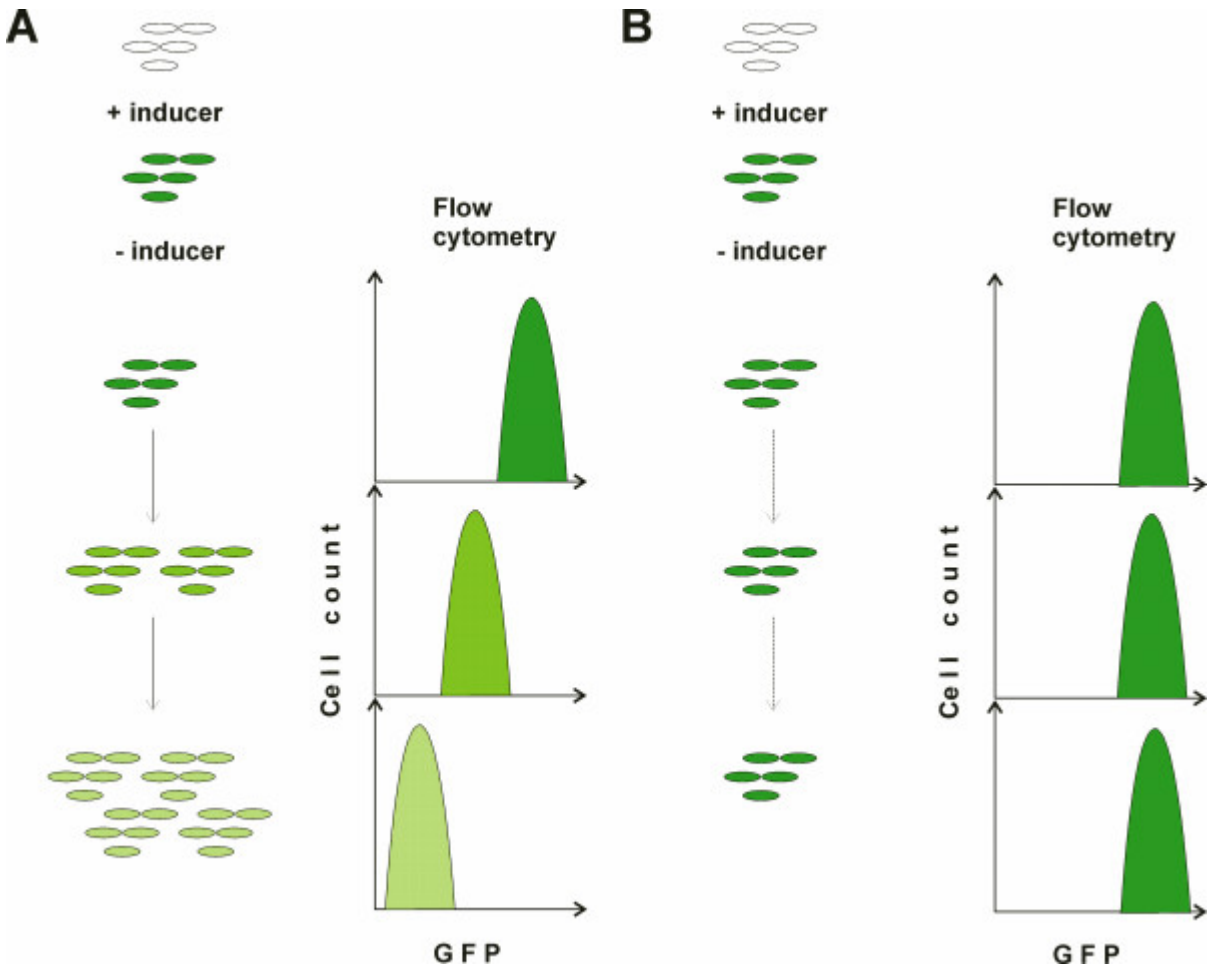


- ? No replication in the presence of antibiotics
- ? Phenotype reversible after withdrawal of the antibiotic

Balaban et al, Nat. Rev. Microbiol. 2019; 17:441-448

https://www.biw.kuleuven.be/dtp/cmpg/spi/research.aspx

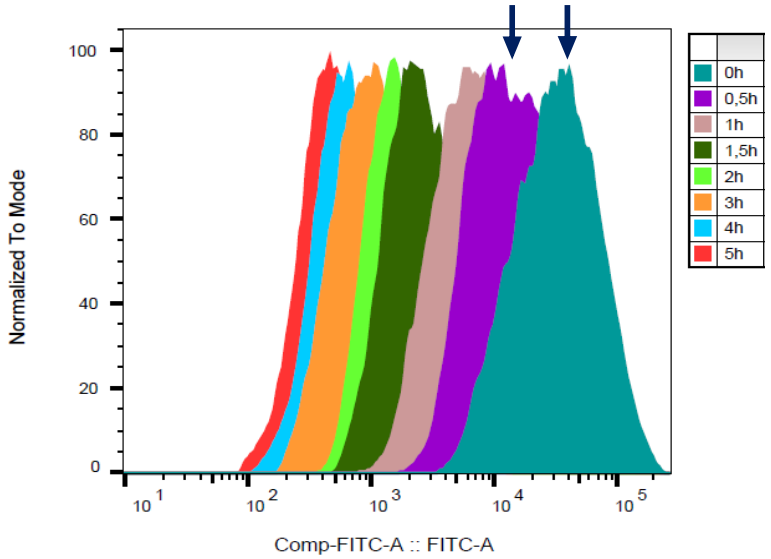
Following bacterial multiplication at the single cell level and in real time



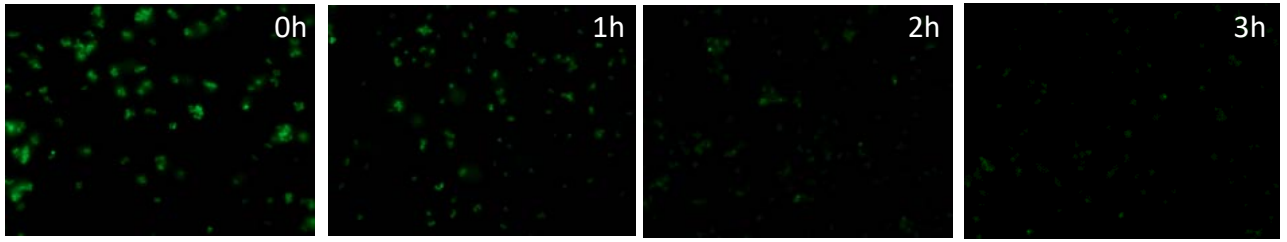
Roostalu et al, BMC Microbiol. 2008; 8:68.

Application to *S. aureus* planktonic cultures

O/N induction
TET 125 ng/mL → Entry into exponential phase → STOP TET → Fluorescence : +1h, +2h, +3h…



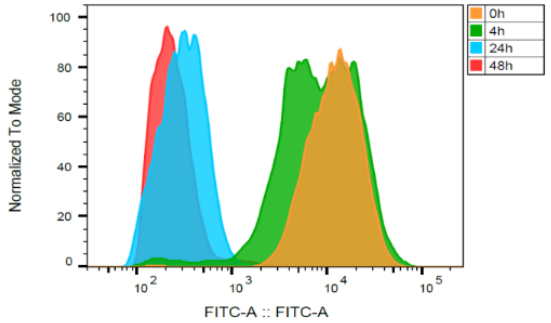
Growth rate
- 28.5 min / generation
- 30.3 min / generation (CFU method)

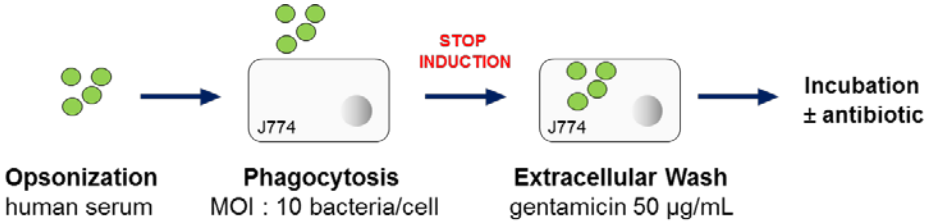


Peyrusson et al, Nat. Comm. 2020; 11:2200

Application to intracellular *S. aureus*

w/o
antibiotic
(gentamicin 5xMIC)





Inoculum actively dividing inside the cell

Peyrusson et al, Nat. Comm. 2020; 11:2200

Application to intracellular *S. aureus*

w/o
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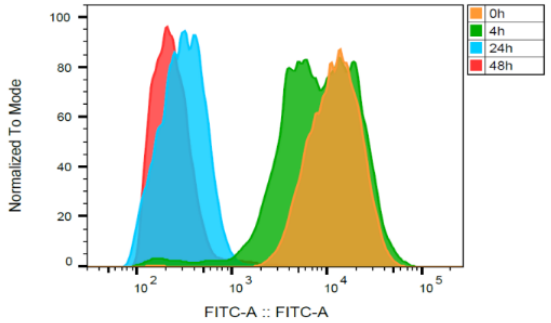
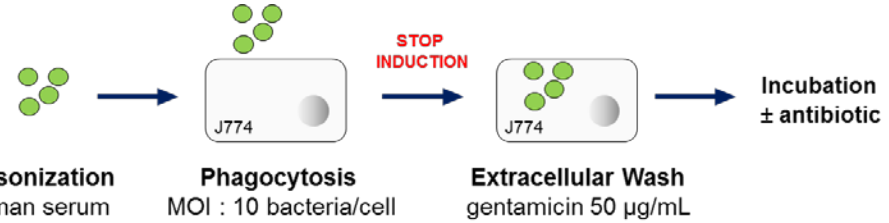
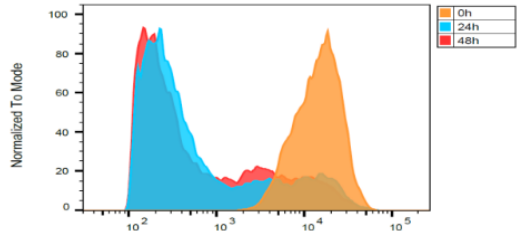




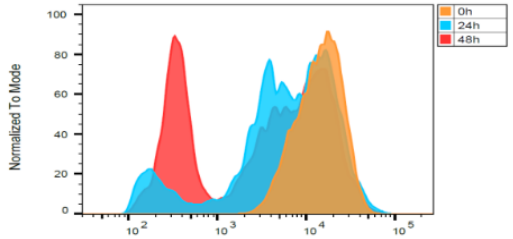
Inoculum actively dividing inside the cell

2 MIC

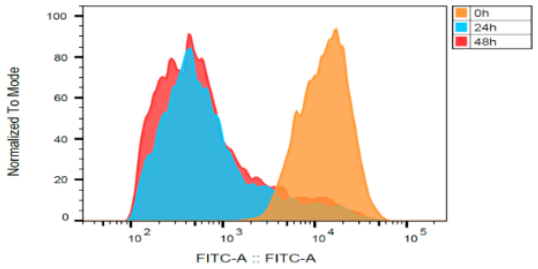
Oxacillin

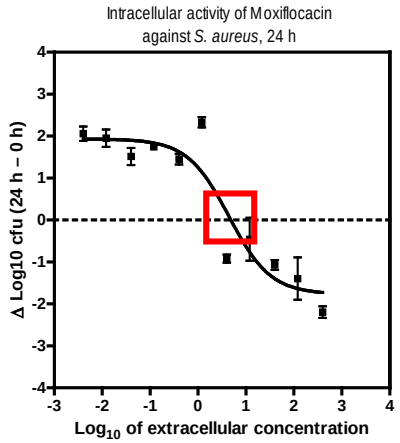


Clarithromycin



Moxifloxacin

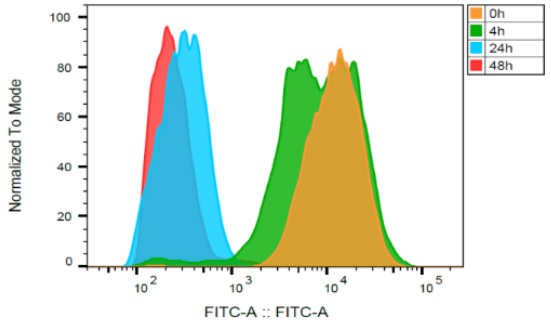


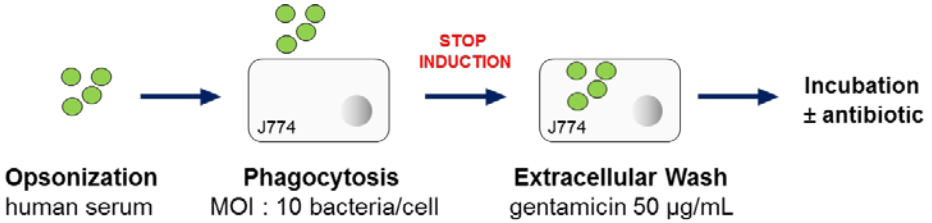


Peyrusson et al, Nat. Comm. 2020; 11:2200

Application to intracellular *S. aureus*

w/o
antibiotic
(gentamicin 5xMIC)





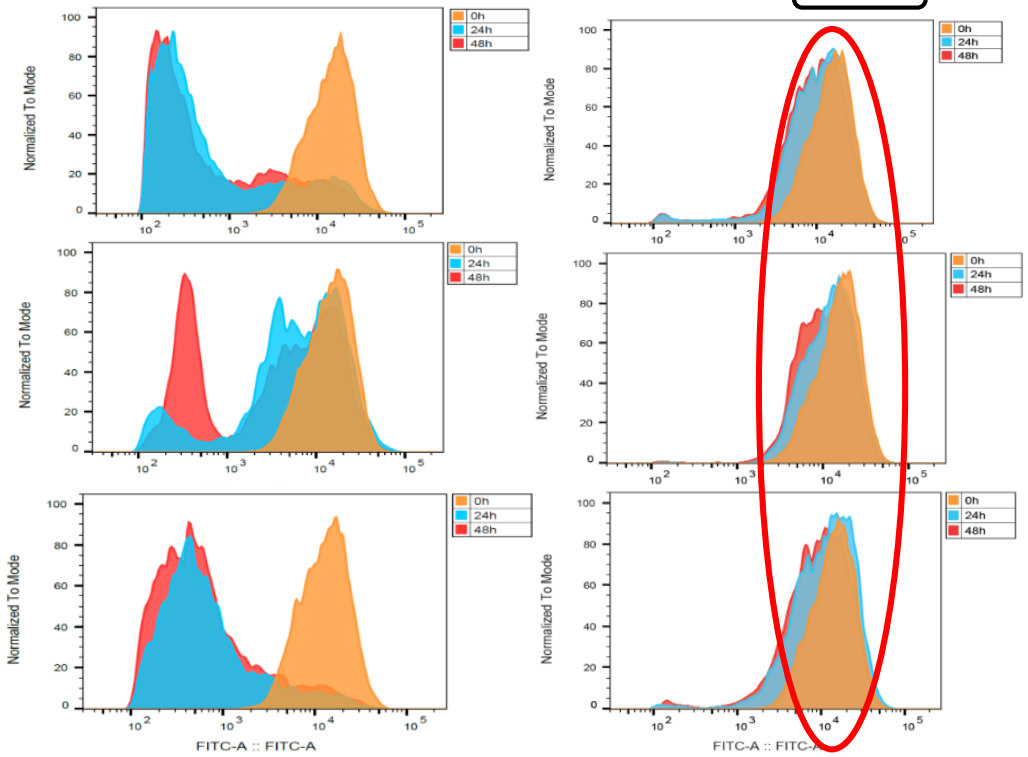
Antibiotic pressure

2 x MIC → 50 x MIC

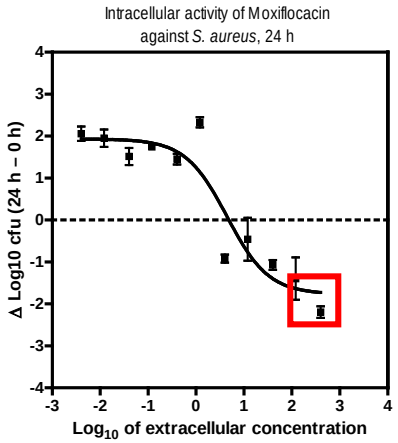
Oxacillin

Clarithromycin

Moxifloxacin



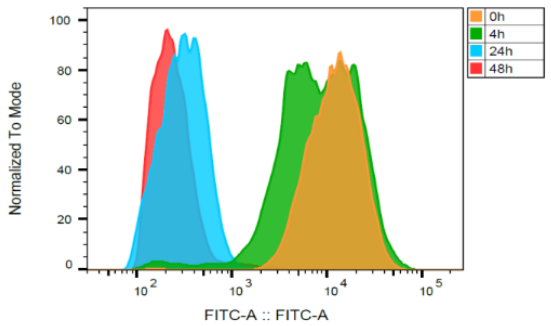
Fully homogenous and non-dividing population

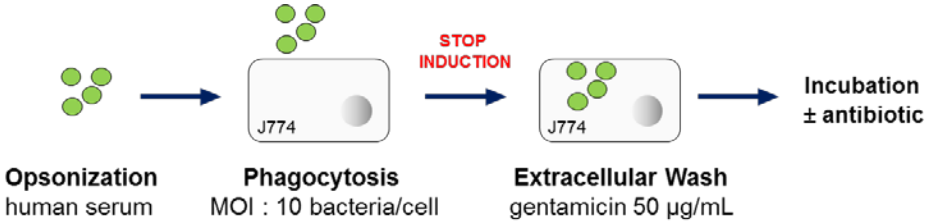


Peyrusson et al., Nat. Comm. 2020; 11:2200

Application to intracellular *S. aureus*

w/o antibiotic (gentamicin 5xMIC)





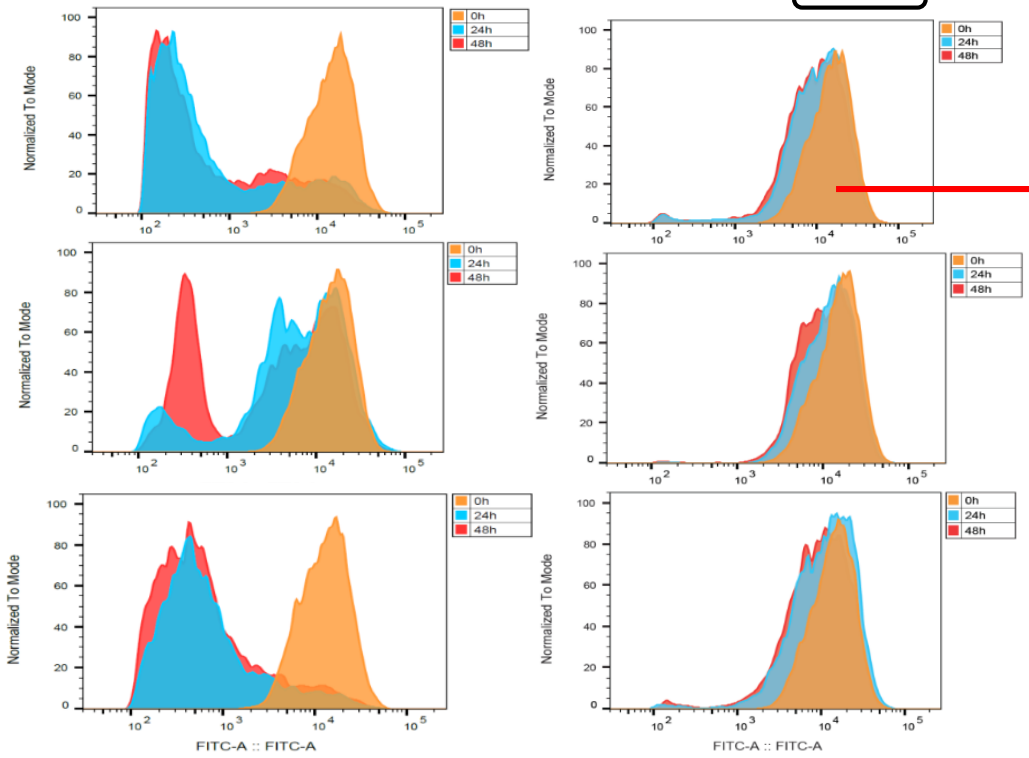
Antibiotic pressure

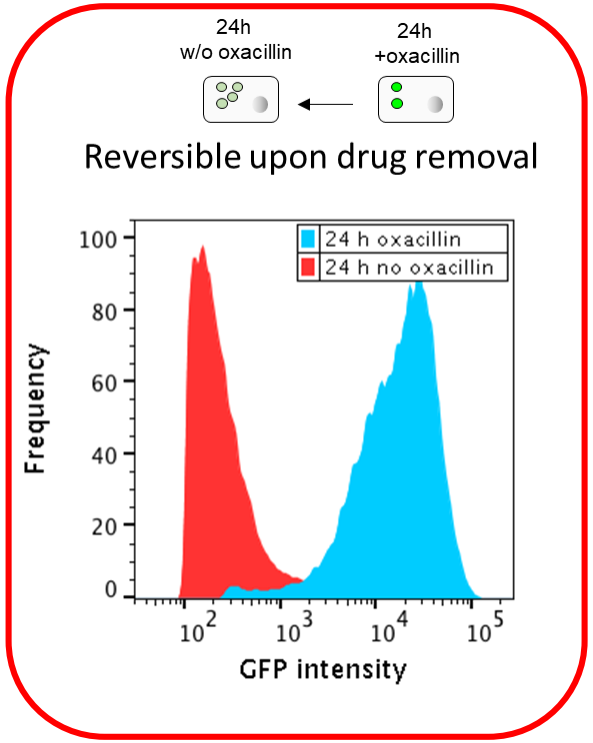
2 x MIC → 50 x MIC

Oxacillin

Clarithromycin

Moxifloxacin

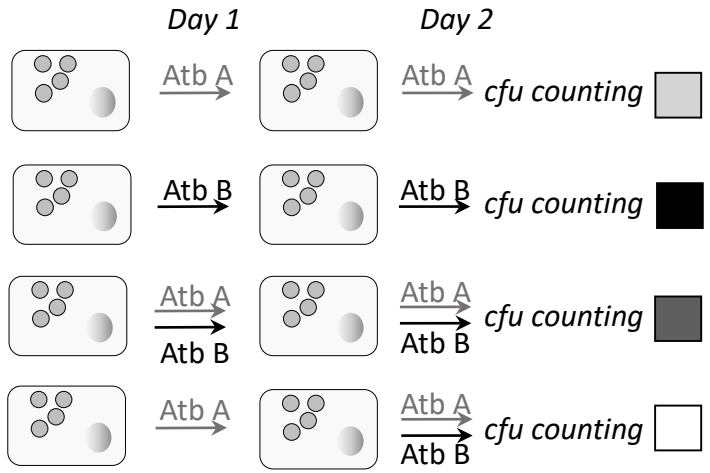




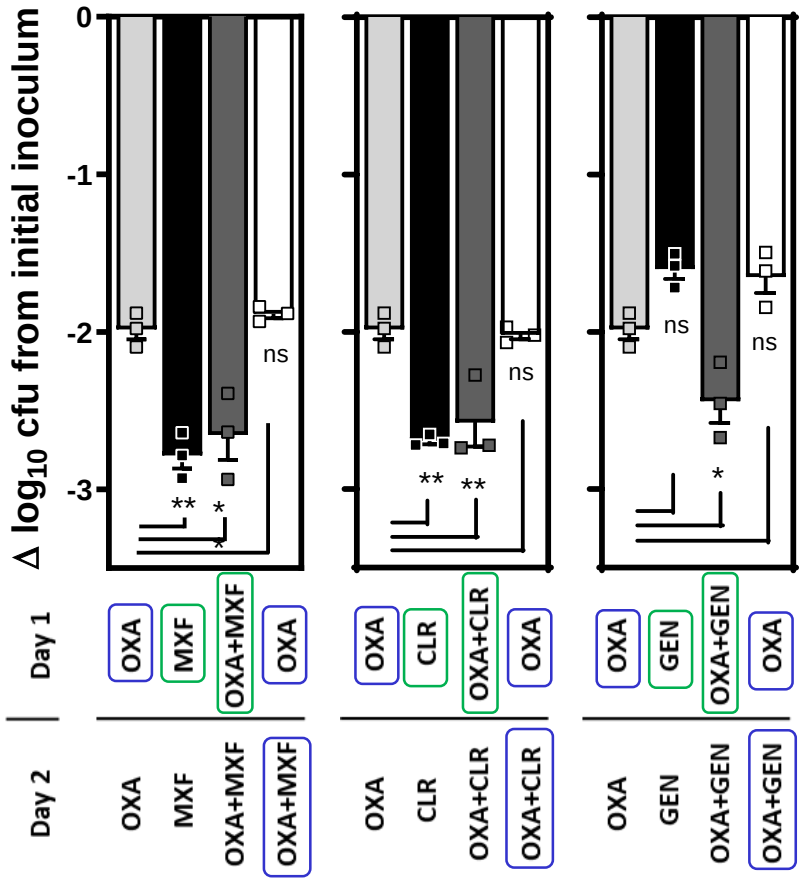
Peyrusson et al., Nat. Comm. 2020; 11:2200

Intracellular activity of antibiotic combinations

Multidrug tolerance

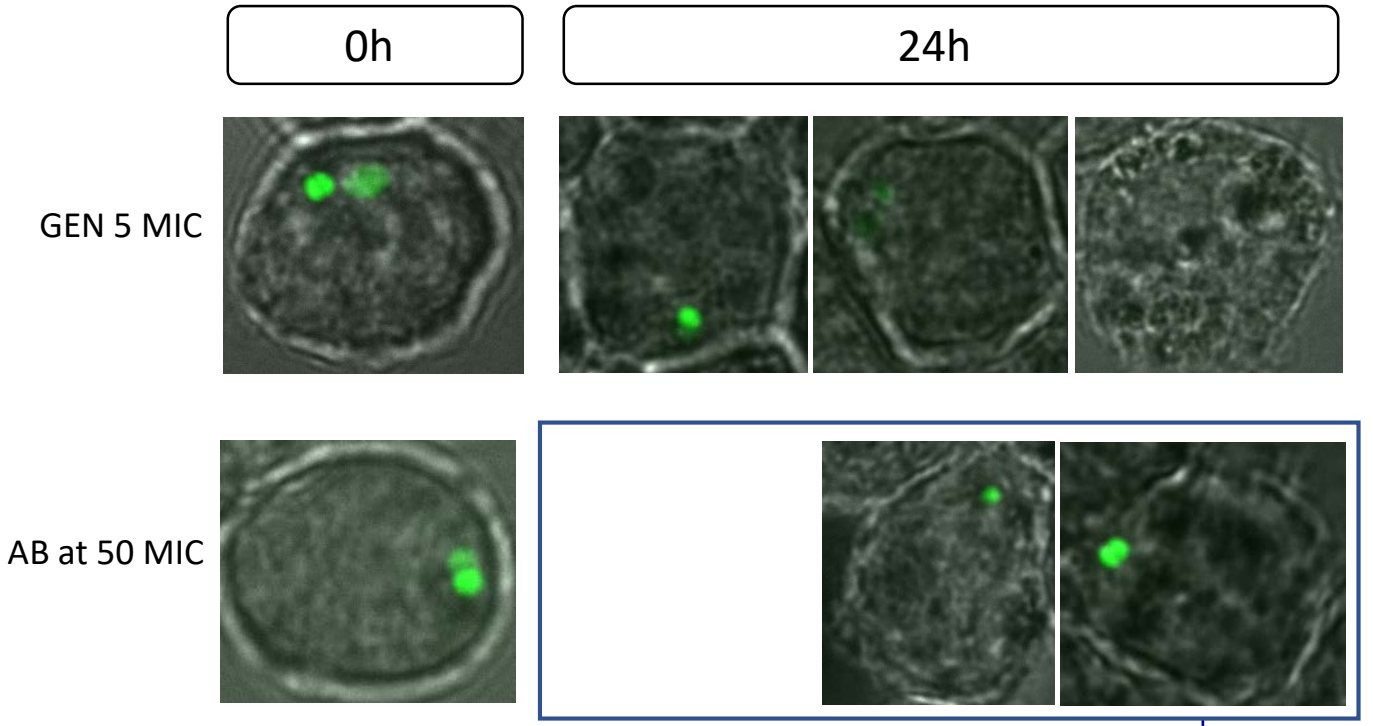


Persister level defined by exposure to the first antibiotic



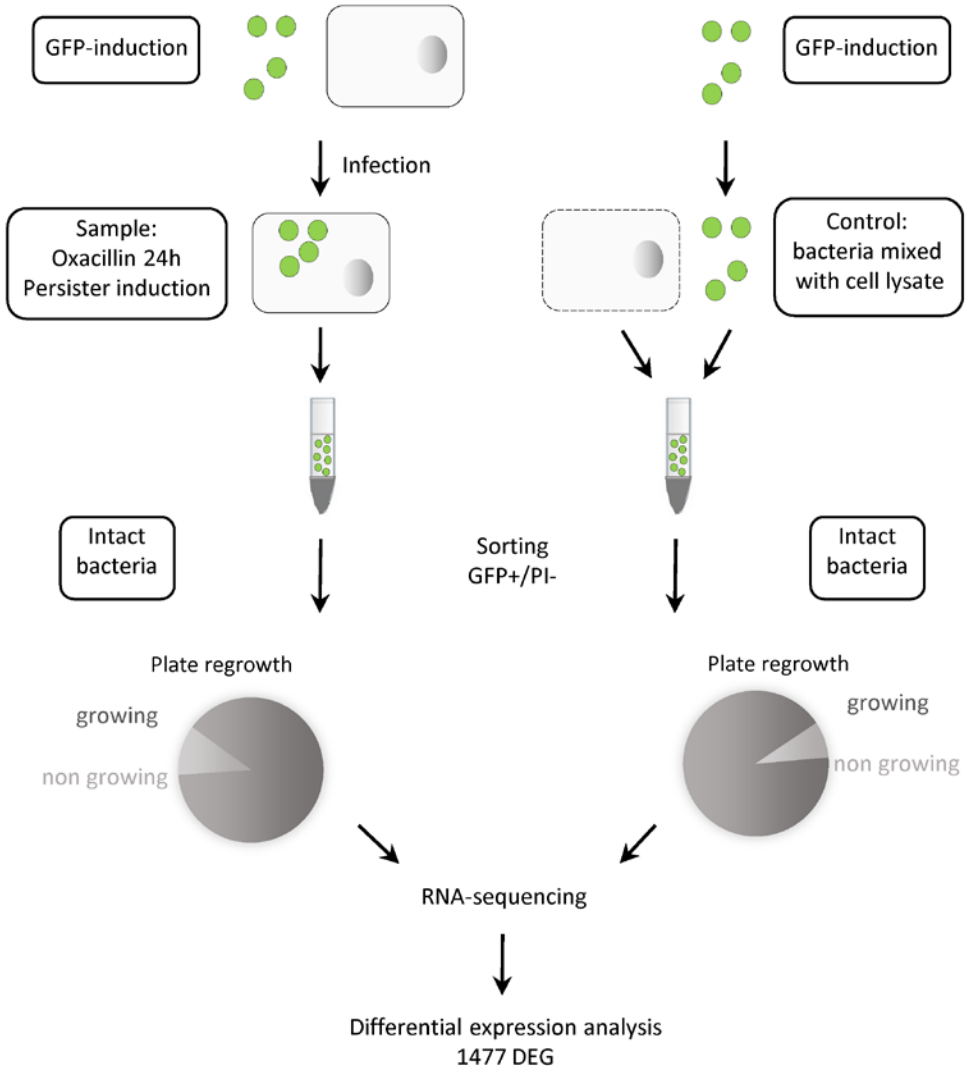
Peyrusson et al, Nat. Comm. 2020; 11:2200

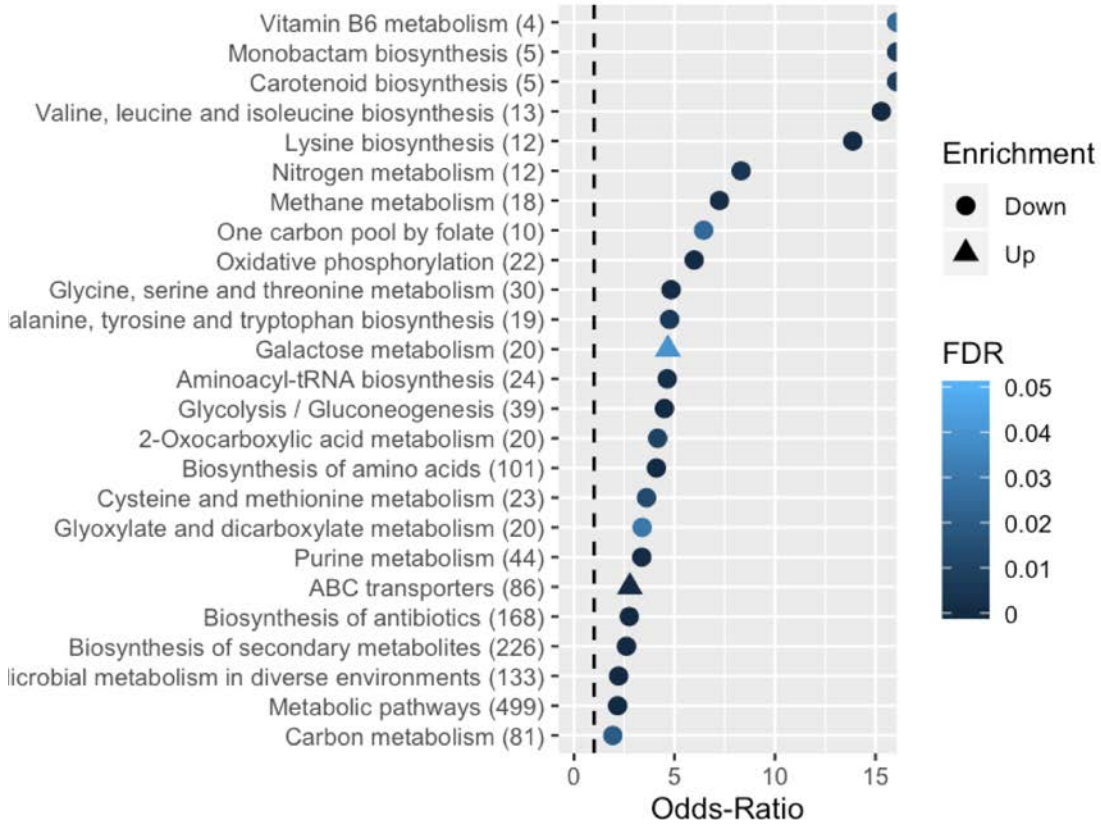
Non dividing bacteria persist inside the cells



Peyrusson et al, Nat. Comm. 2020; 11:2200

Transcriptomic analysis: global view

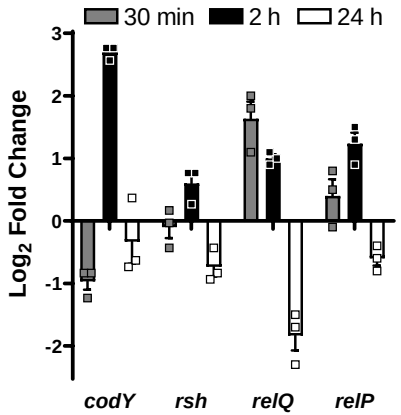


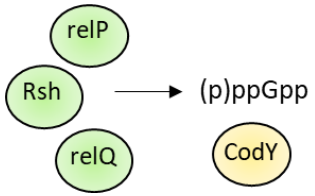


Peyrusson et al, Nat. Comm. 2020; 11:2200

Transcriptomic analysis: more details

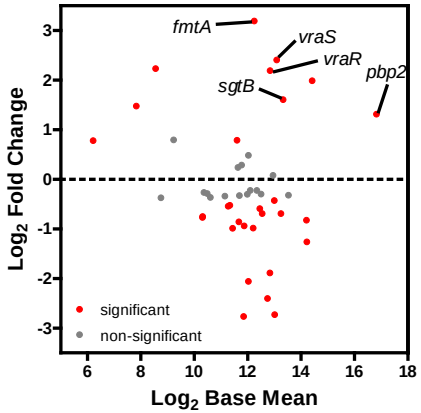
Stringent response

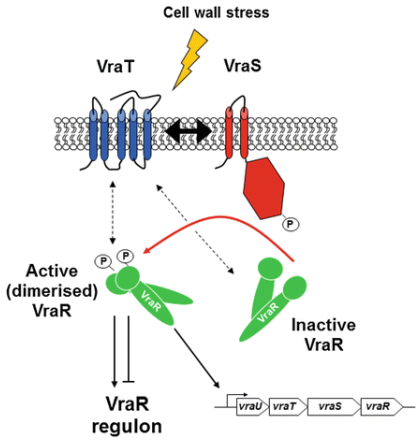




Shutdown energy consuming processes

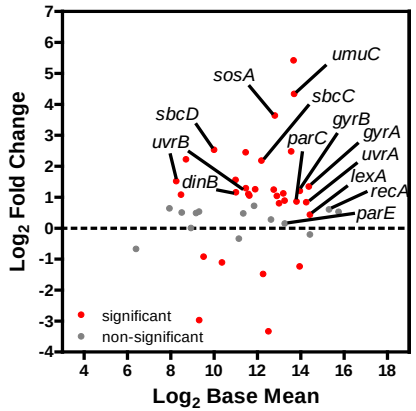
Cell Wall Stress response

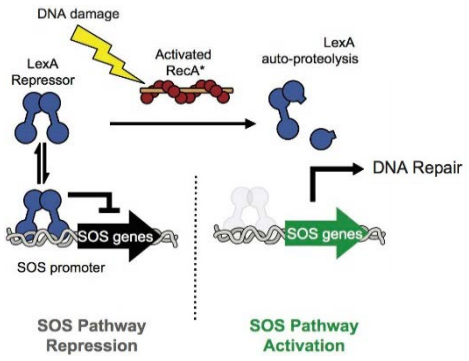




Cell wall maintenance **tolerance to β-lactams**

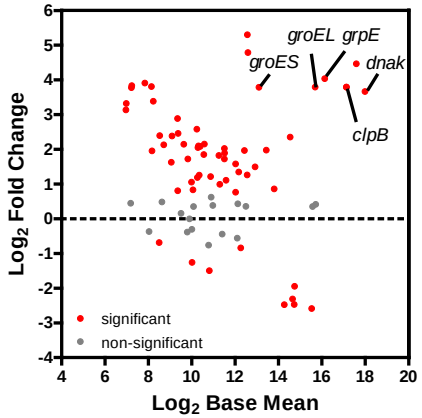
SOS response

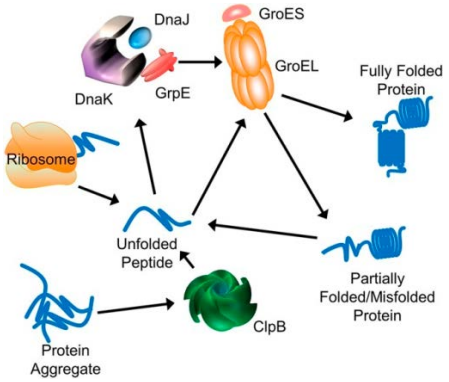




DNA repair **tolerance to quinolones/ β-lactams**

Heat shock response



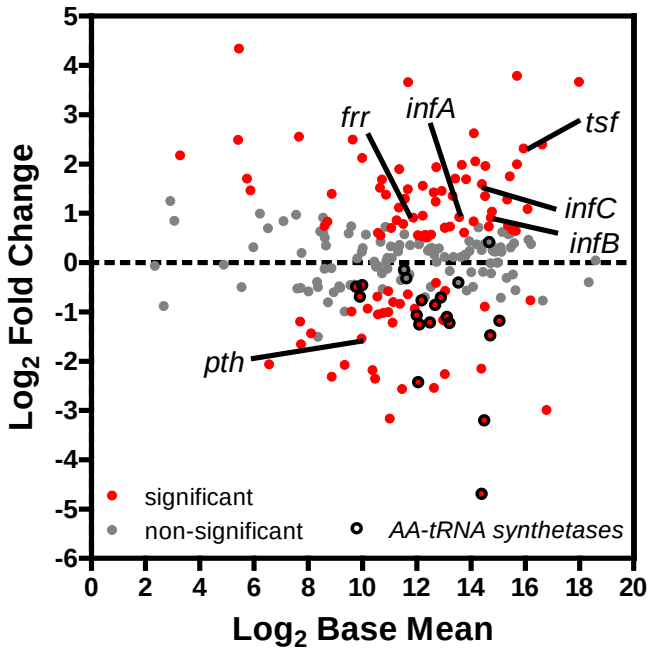


Chaperones system **tolerance to aminoglycosides /macrolides/β-lactams**

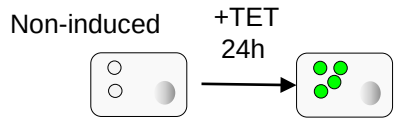
Peyrusson et al, Nat. Comm. 2020; 11:2200

Transcriptomic analysis: more details

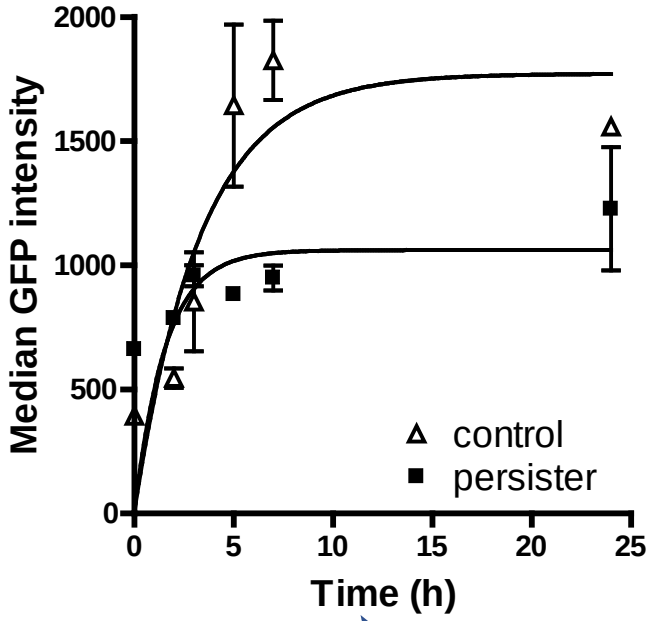
dysregulated but active **protein synthesis**



Protein machinery activated
AA-tRNA synthetases silenced

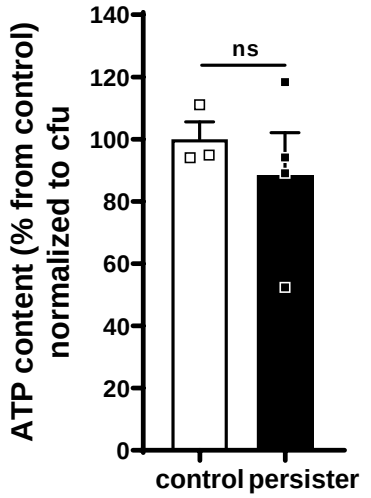


Translation rate



Reduced but still active
de novo protein synthesis

ATP content



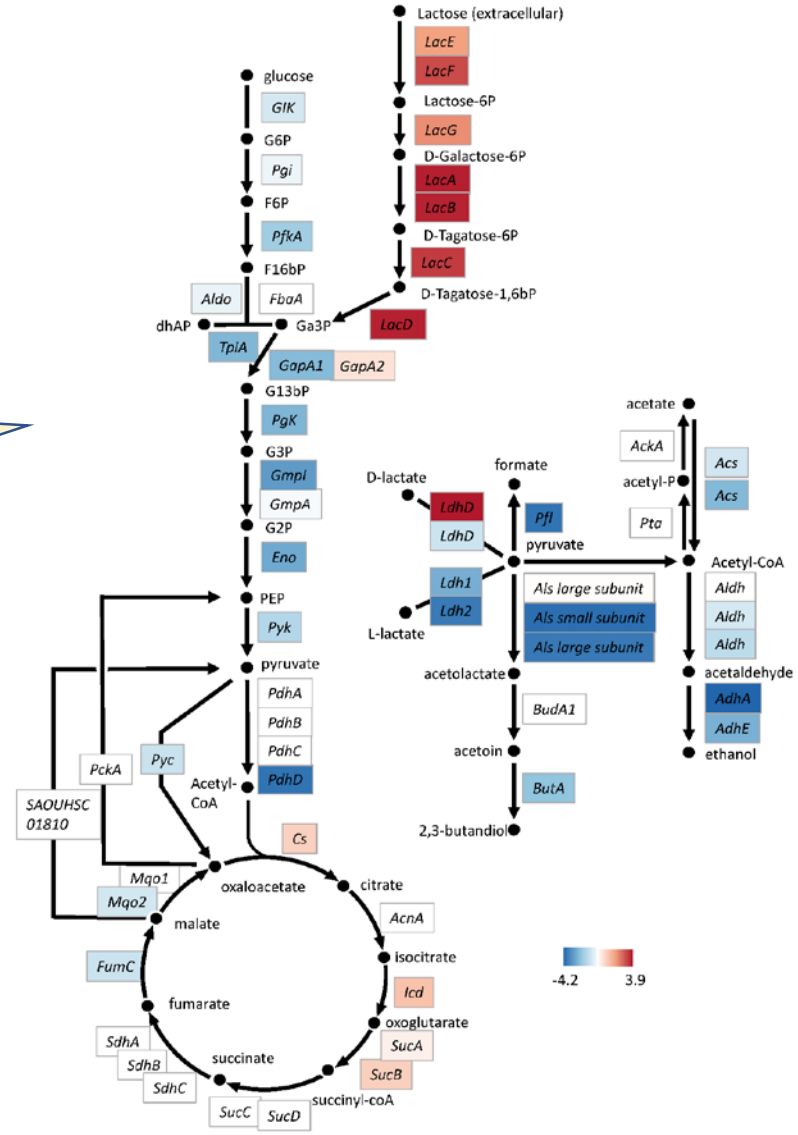
ATP level
maintained

Peyrusson et al, Nat. Comm. 2020; 11:2200

Transcriptomic analysis: more details

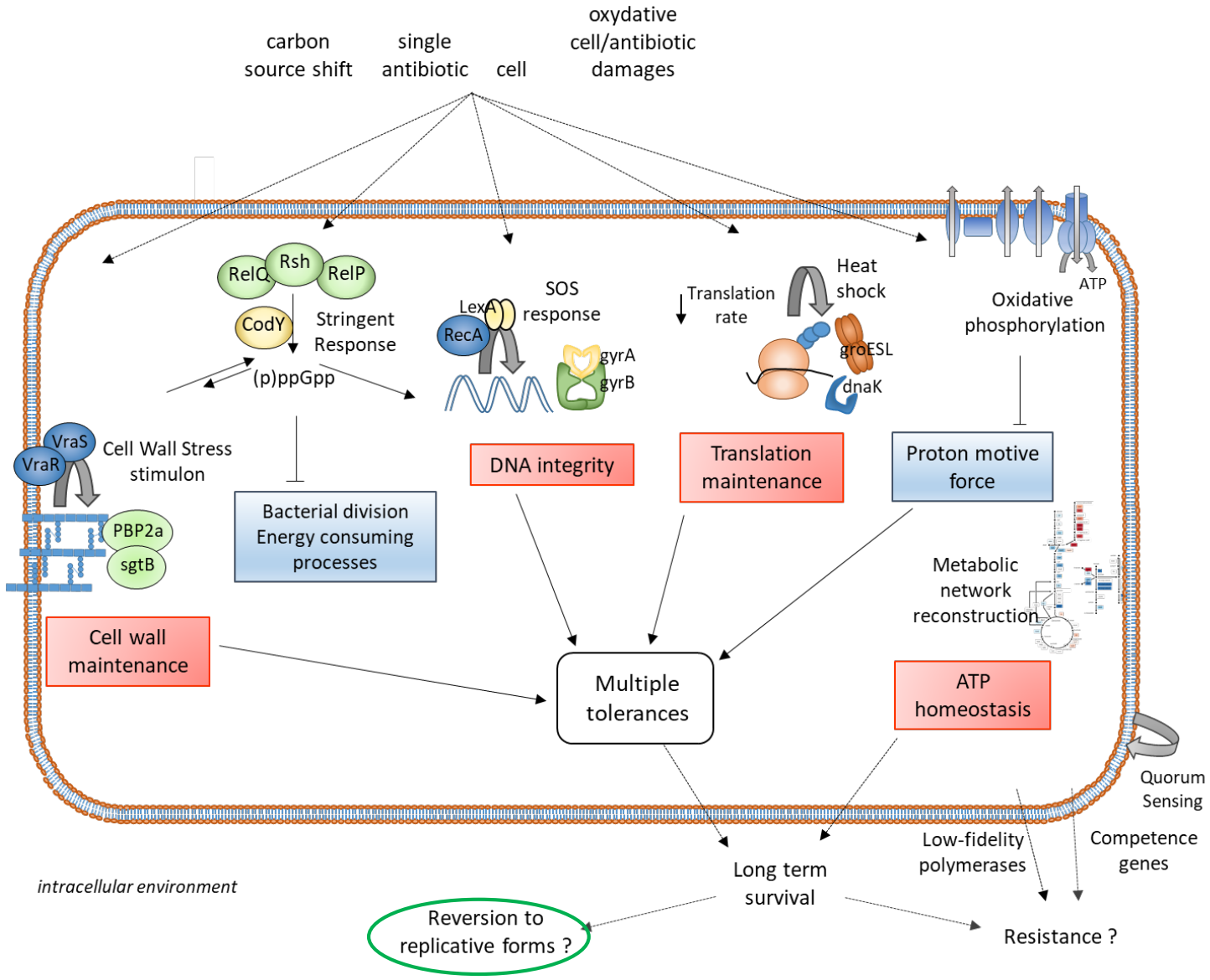
Reorientation of **central metabolic flux**

- carbon source shift between glucose and lactose
- Respiration resembling that observed in anaerobiosis.
- oxidative phosphorylation repressed to the benefit of D-lactate fermentation



Peyrusson et al, Nat. Comm. 2020; 11:2200

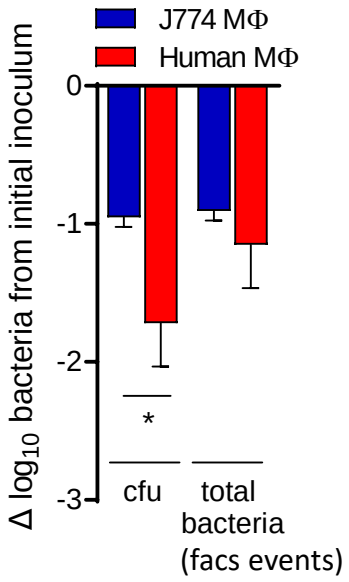
Intracellular persisters: a global view

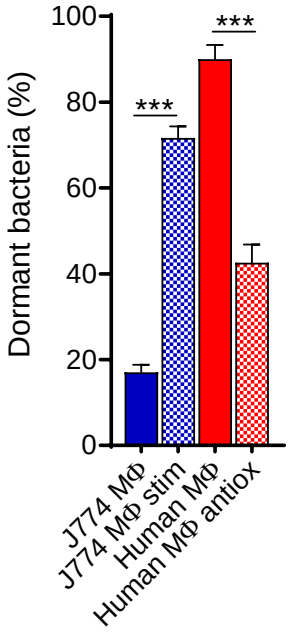


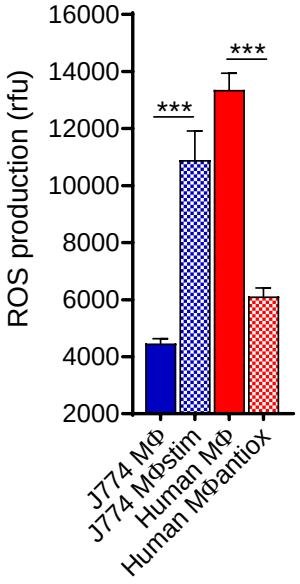
Peyrusson et al, Nat. Comm. 2020; 11:2200

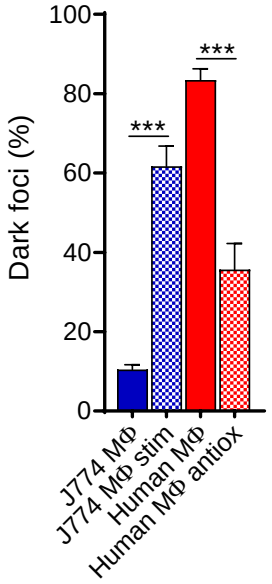
Deepness of dormancy

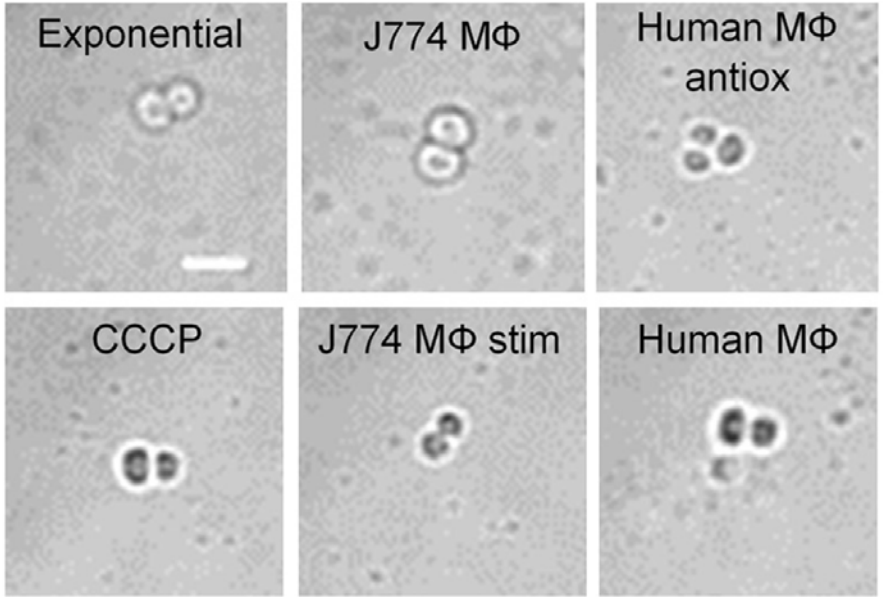
Bacteria counts vs colony forming units











Dormancy level depends on the cell type (oxidative stress level)

Dormant bacteria show protein aggregates

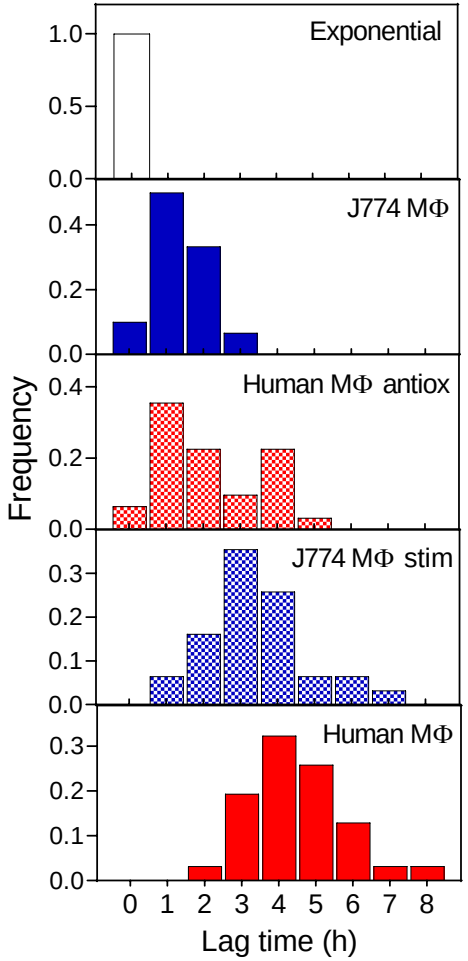
J774 MΘ stim by INFγ and LPS
Human MΘ antiox: treated by butylated hydroxyanisole (BHA)

Peyrusson et al, Microbiology Spectrum 2022; 10(1):e0231321

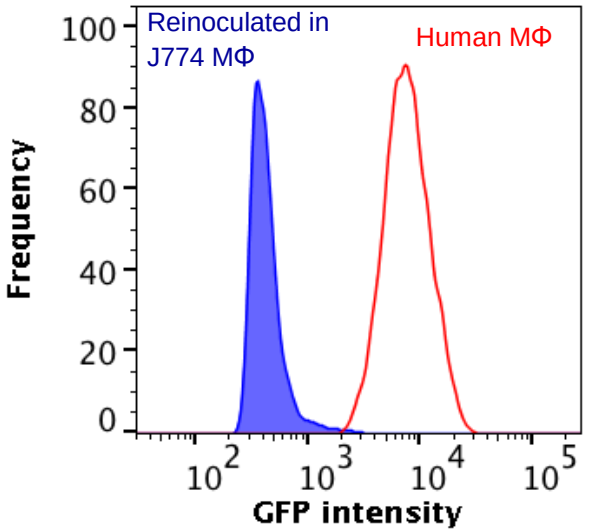
Awakening of dormant forms

Awakening of intracellular bacteria upon drug removal

In liquid culture



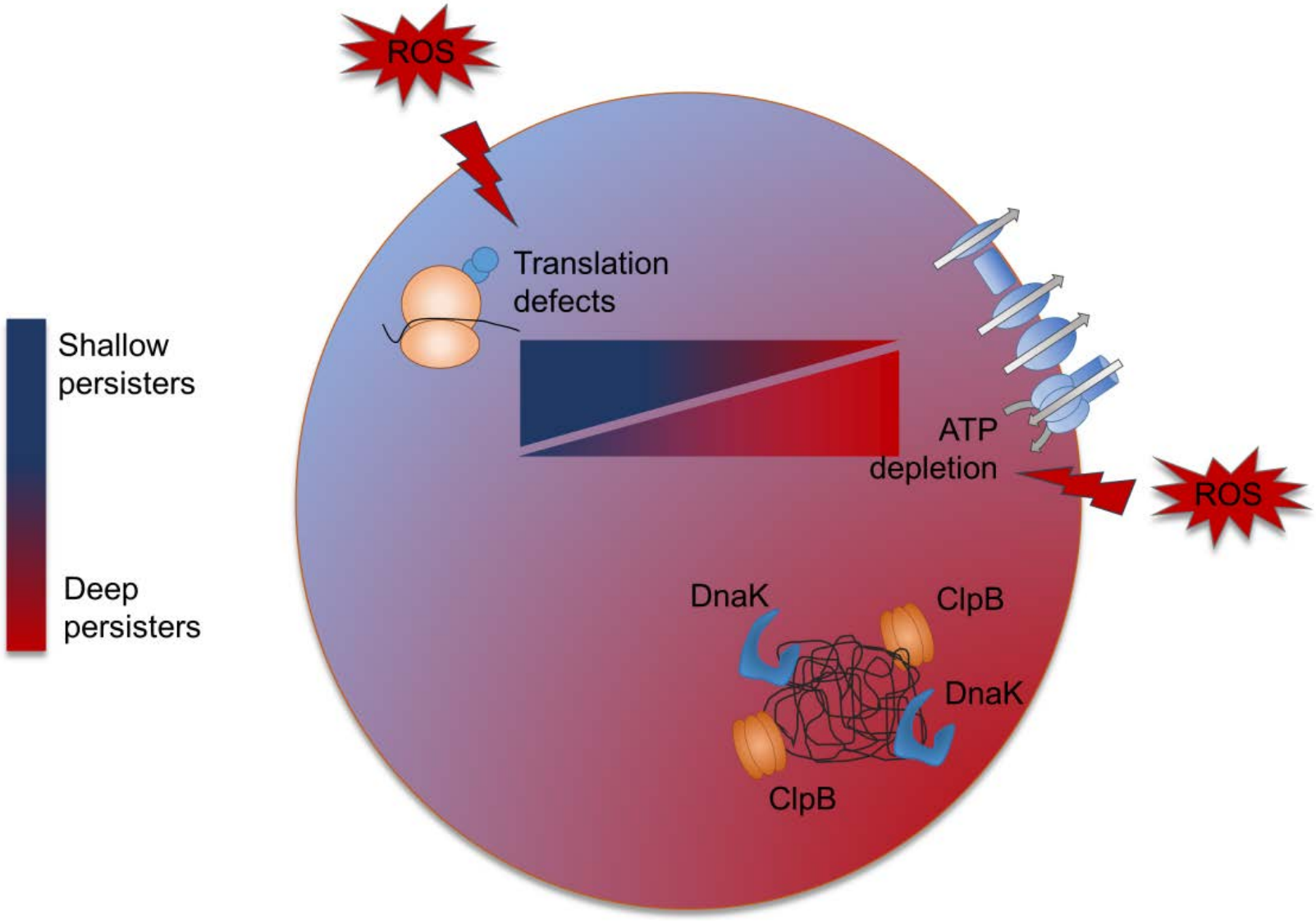
Intracellularly



Awakening time is longer in oxidative cells

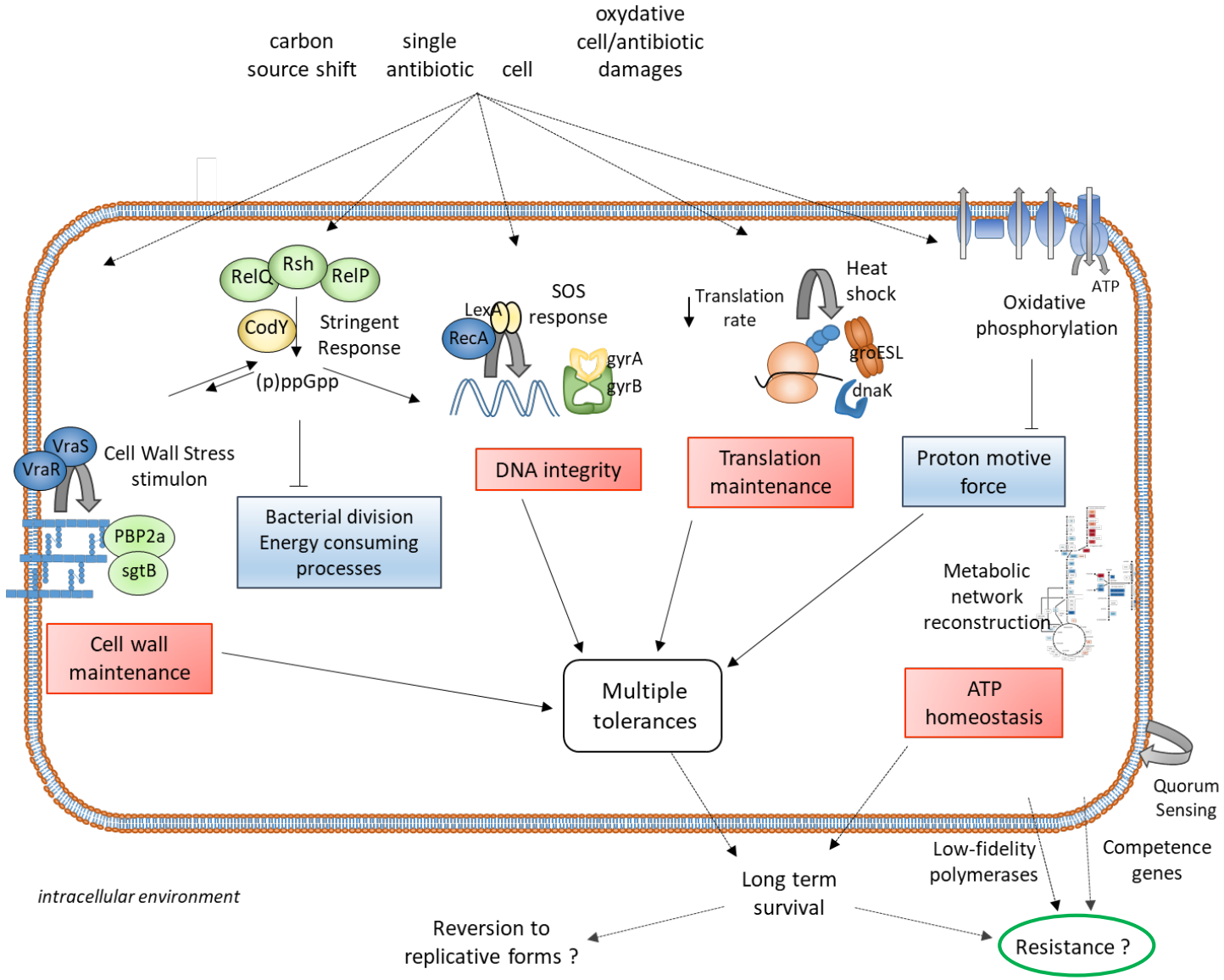
Peyrusson et al, Microbiology Spectrum 2022; 10(1):e0231321

Deepness of dormancy : a balance between translation defects and ROS



Peyrusson et al, Microbiology Spectrum 2022; 10(1):e0231321

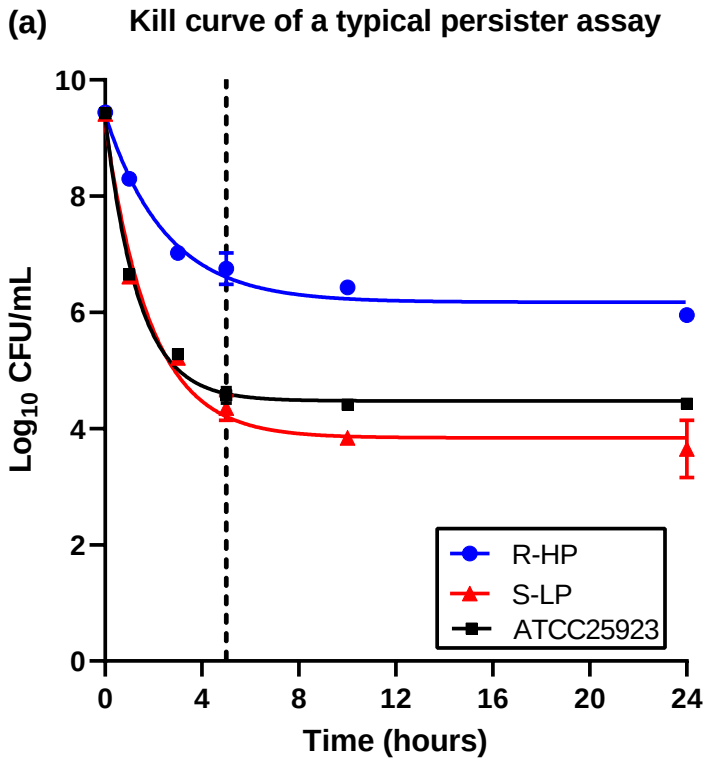
Intracellular persisters: a global view

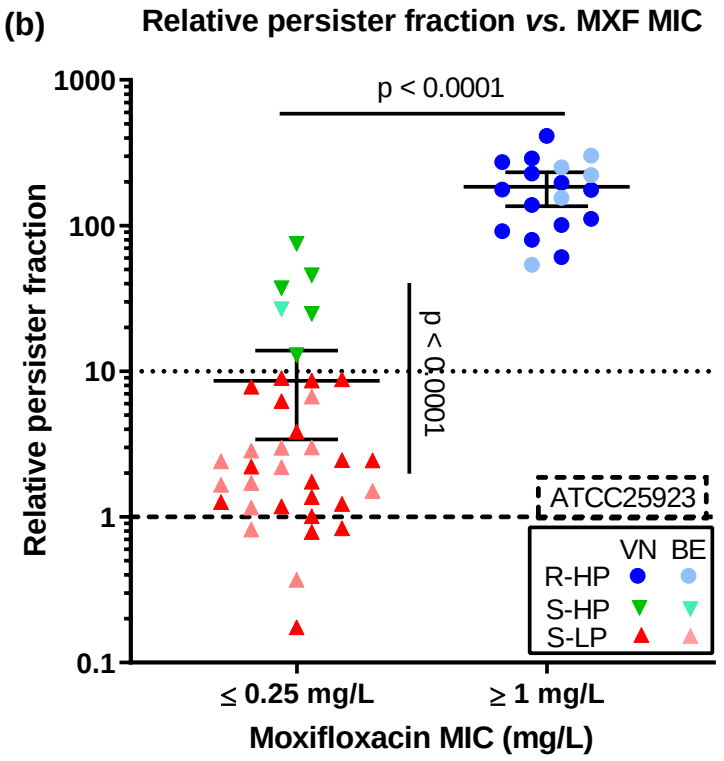


Peyrusson et al, Nat. Comm. 2020; 11:2200

What about clinical isolates ?

Relative persister fraction to the fluoroquinolone moxifloxacin in a collection of clinical isolates





Resistant; High persister

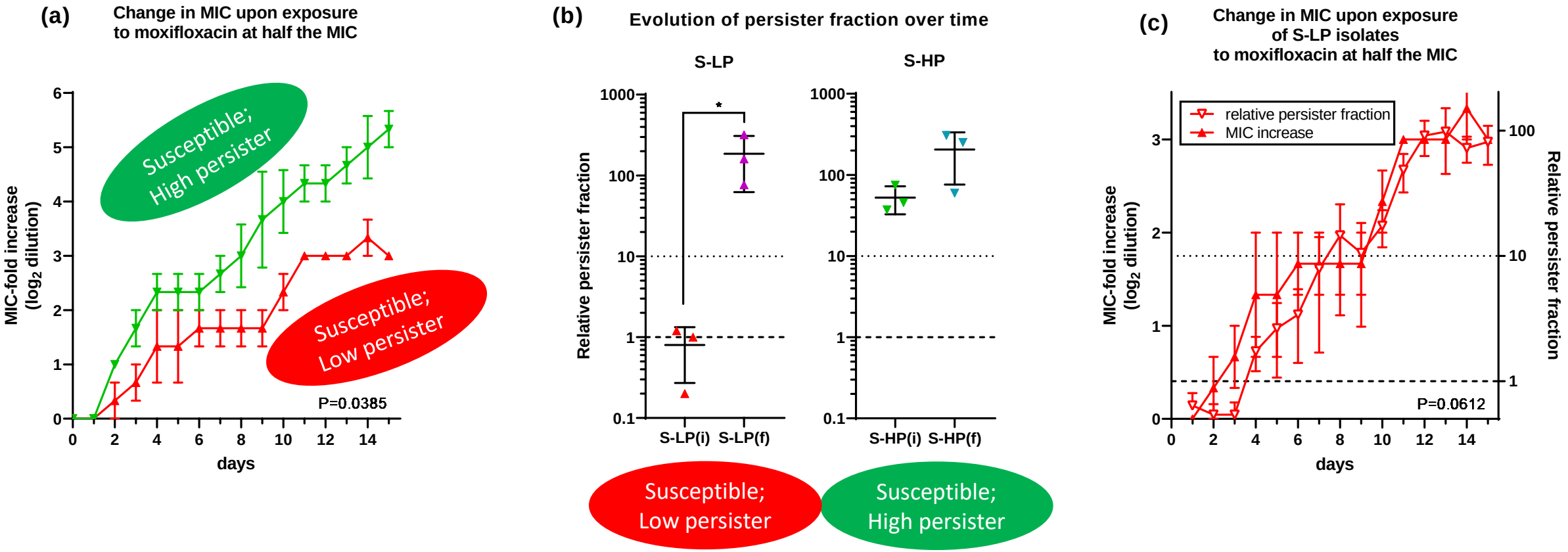
Susceptible; High persister

Susceptible; Low persister

- All resistant isolates have a high relative persister fraction
- Most susceptible isolates have a low relative persister fraction

Nguyen et al, Front. Microbiol 2020; 11:587364

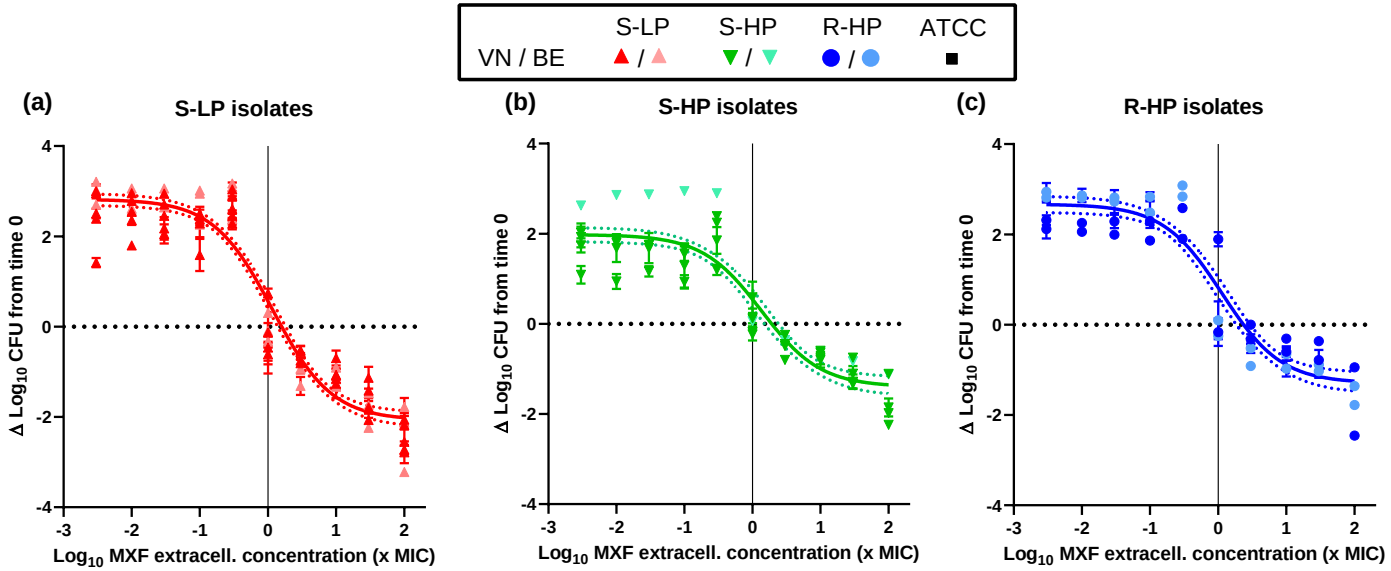
Does persistence prepare for resistance?

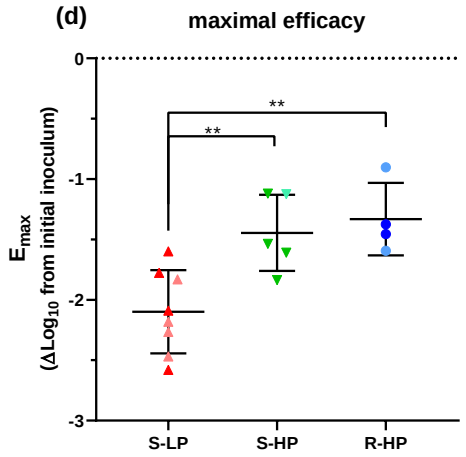


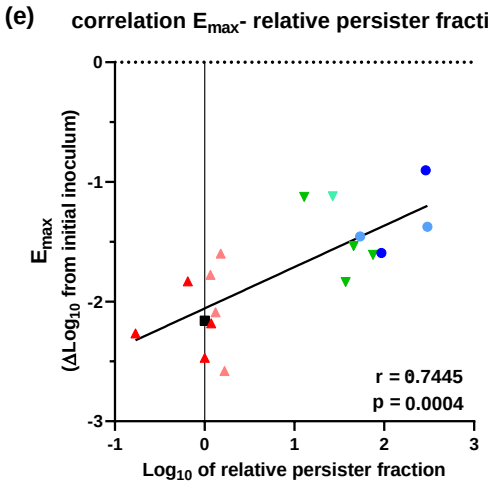
- Persistence accelerates the selection of resistance
- Persistence and resistance are acquired together

Nguyen et al, Front. Microbiol 2020; 11:587364

Persister character to predict intracellular tolerance







Isolates with higher relative persister fraction are less responsive to moxifloxacin intracellularly

Nguyen et al, Front. Microbiol 2020; 11:587364

Intracellular persisters to antibiotics: clinical implications

D. Parkins

- Intracellular bacteria can remain ‘dormant’ inside eukaryotic cells
- Dormancy is favored by stressful conditions (antibiotic pressure, oxidative stress e.g.)

- Dormancy is associated to the activation of a global stress response
  → Dormant bacteria are ‘multidrug tolerant’
  → Exposure to one drug makes bacteria non-responsive to other classes of drugs

- Dormancy is reversible when the stress is relieved
  → A possible reason for recurrence of the infection ?

- If deeply dormant, persisters do no grow on agar plates
  → How to detect them in biological samples ?

- Clinical isolates differ by the fraction of persisters in their populations
  → Is there a link with the risk of clinical failure ?

- Activation of stress in persisters favors selection of resistance
  → Another reason to see resistance increase when dealing with chronic infections ?

Still more questions than answers ….

Acknowledgments

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Vasileios Yfantis

Cartoon: https://kids.frontiersin.org/articles/10.3389/frym.2019.00045