



Antimicrobial activity against intra-osteoblastic *S. aureus*: *a new therapeutic concept for bone and joint infection?*

Florent Valour, Natacha Riffard, Sophie Trouillet-Assant, Jean-Philippe Rasigade,

Christian Chidiac, François Vandenesch, Tristan Ferry and Frédéric Laurent

on behalf of the **Lyon Bone and Joint Infection Study group**

frederic.laurent@univ-lyon1.fr



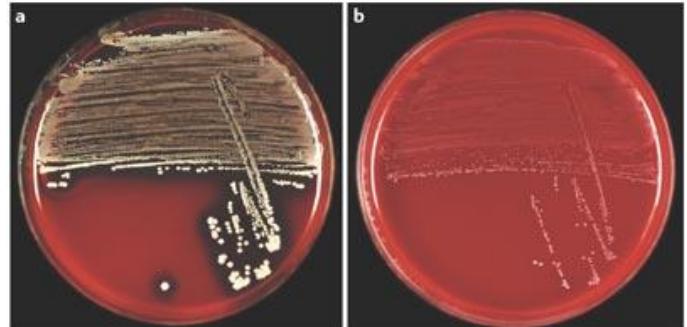
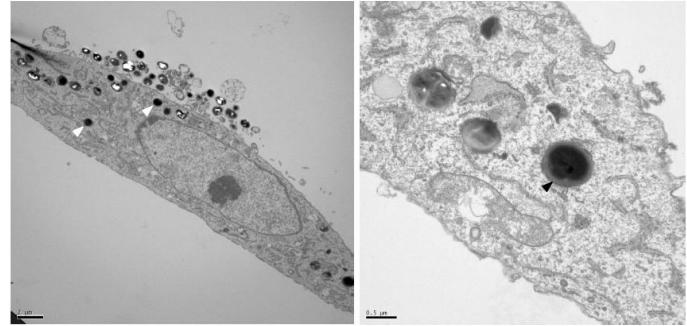
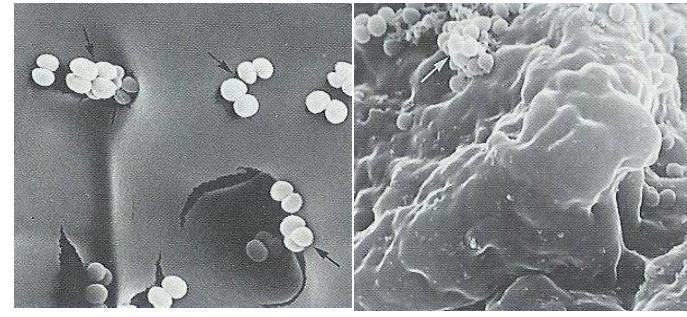
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Background

- *S. aureus* BJI
 - Prolonged antimicrobial therapy: 6 weeks - 6 months
 - High chronicization rate
 - Biofilm formation
 - Invasion and persistence within bone cells
 - Small colony variants



Hall-Stoodley et al. Cell Microbiol 2009 – Bosse et al. J Bone Joint Surg 2005

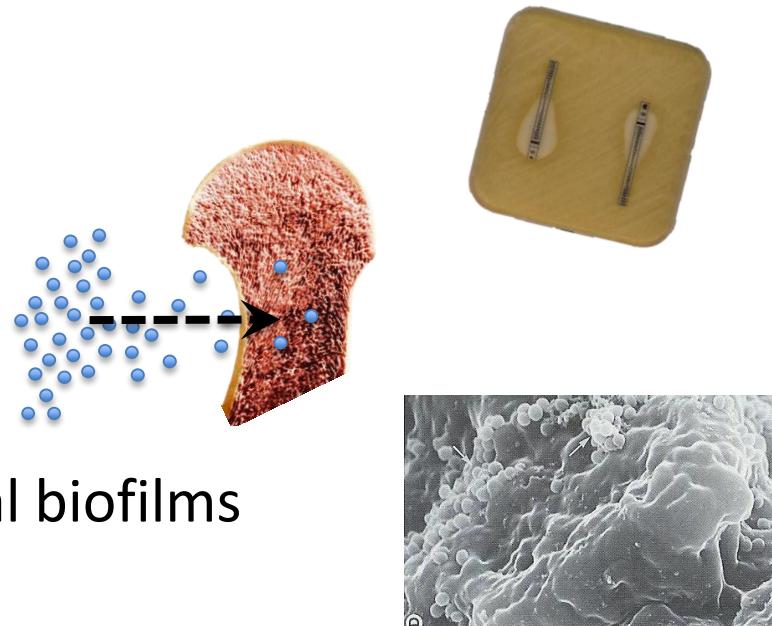
Proctor et al. Nature Rev Microbiol 2006

Rasigade et al. Plos One 2012 – Valour et al. Plos One 2012

Background

- Rational for the choice of antimicrobial therapy strategies in *S. aureus* BJI

- Intrinsic antibacterial activity



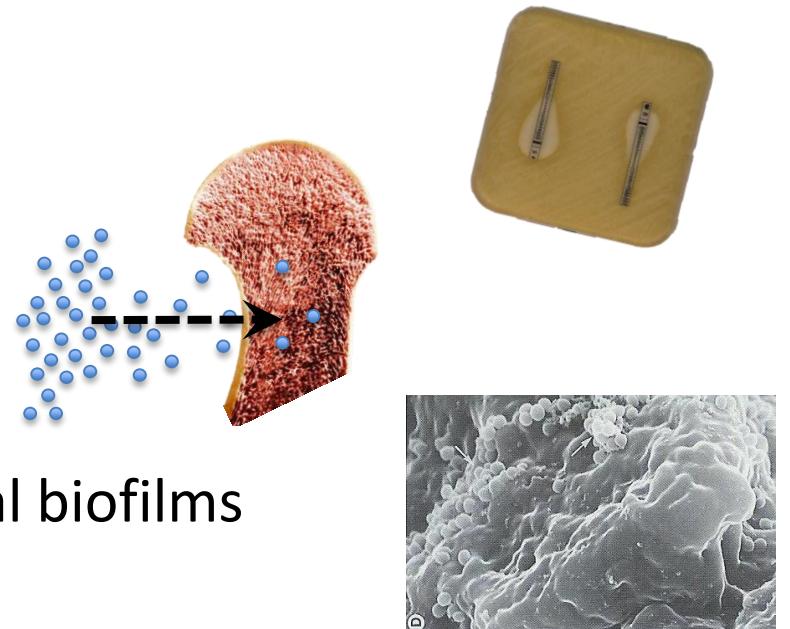
- Bone penetration

- Activity against staphylococcal biofilms

Background

- Rational for the choice of antimicrobial therapy strategies in *S. aureus* BJI

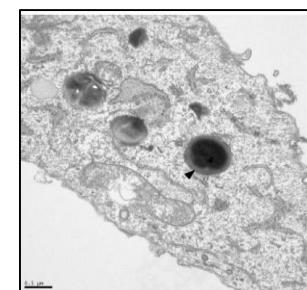
- Intrinsic antibacterial activity



- Bone penetration

- Activity against staphylococcal biofilms

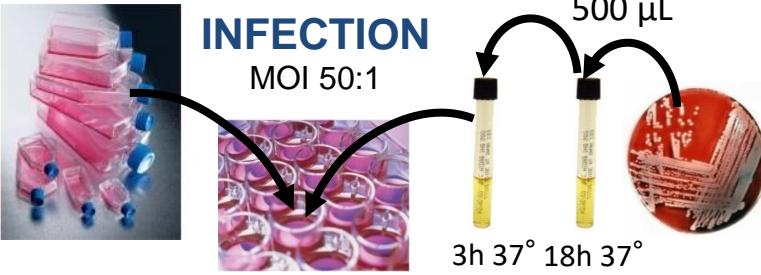
- **Intracellular staphylococcal reservoir ?
Emergence of SCV ?**



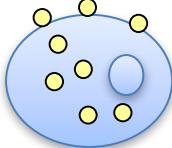
Study objective

- Evaluation of the intra-osteoblastic activity of the main antimicrobials used for staphylococcal BJI in an *ex vivo* model of osteoblast infection
- Assessment of their impact on the emergence of intracellular SCV

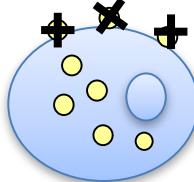
Methods



INVASION (2h)

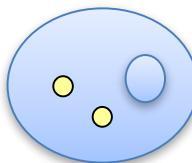


Lysostaphine
200 $\mu\text{g/mL}$

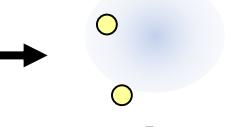


Tested antibiotic – 24h

- . C_{bone}
- . C_{min} = C_{bone}/3
- . C_{max} = C_{bone} x 3



Cell lysis
(osmotic shock)



Quantification of
intracellular bacteria
by plating cell lysates

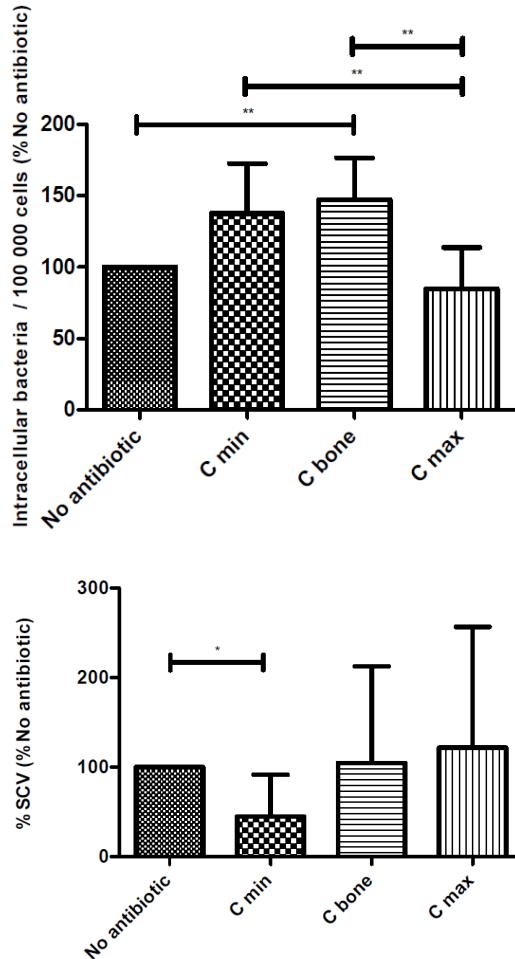


Quantification of
intracellular
phenotype switching

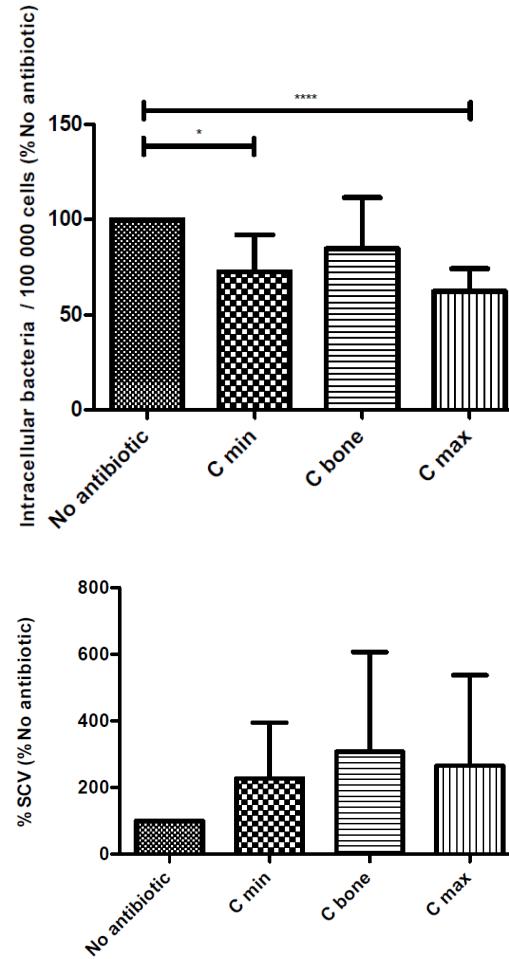
SCV = area <5% of the
median area of all colonies

Results

Vancomycin

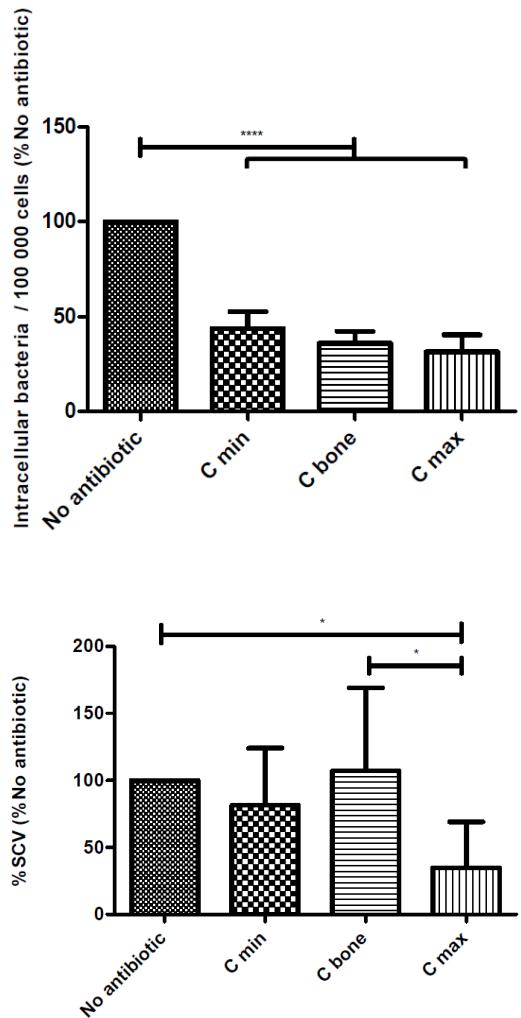


Teicoplanin

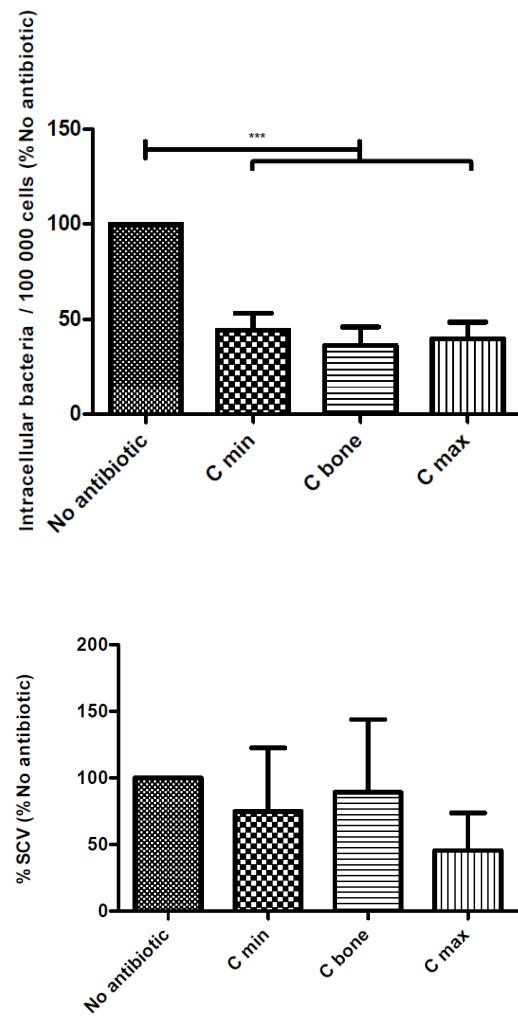


Results

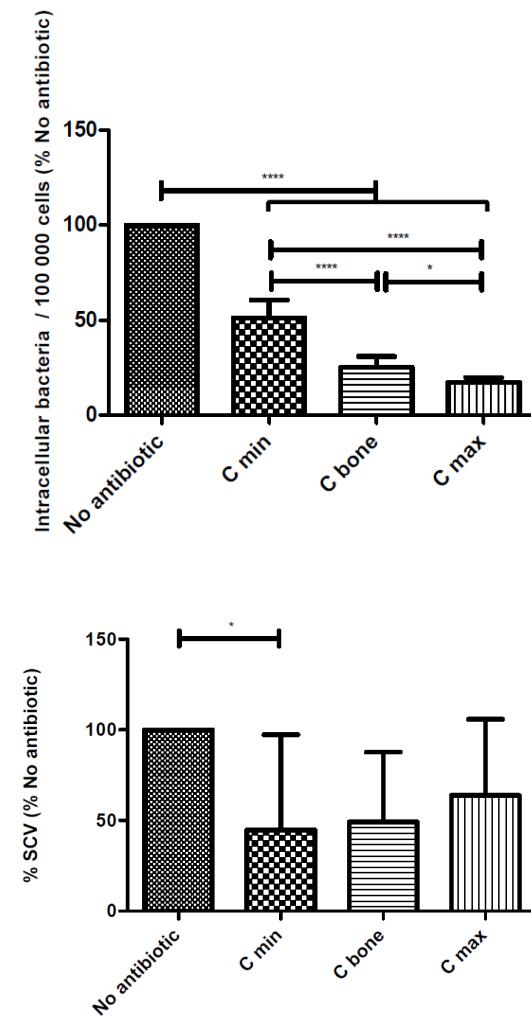
Oxacillin



Rifampin

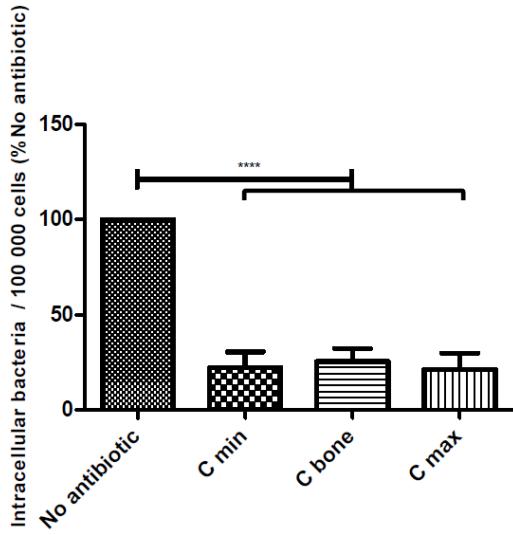


Ofloxacin

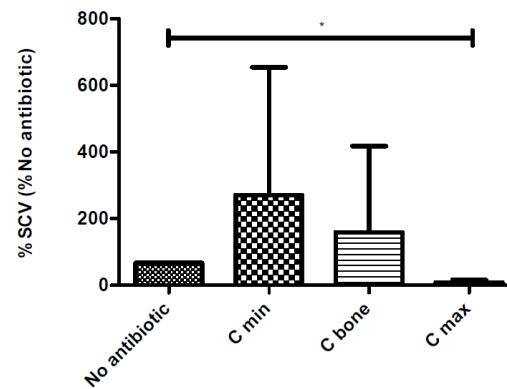
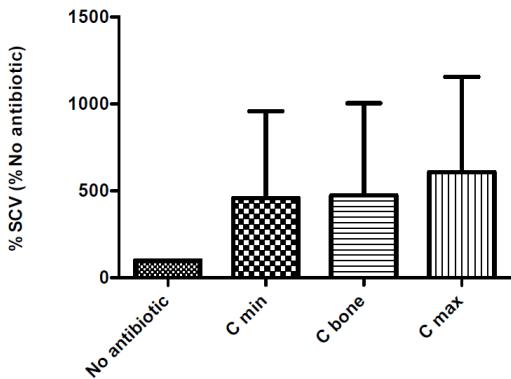
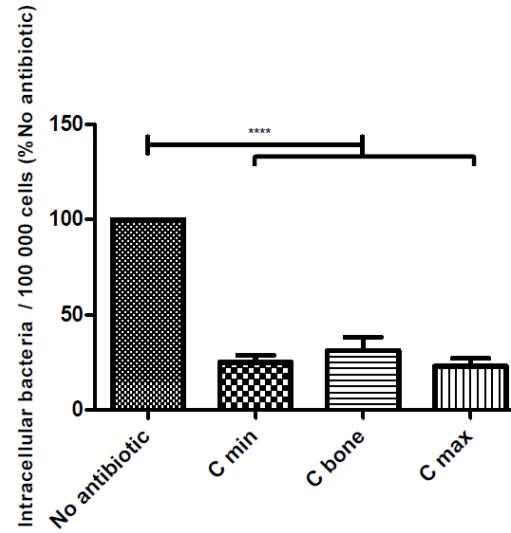


Results

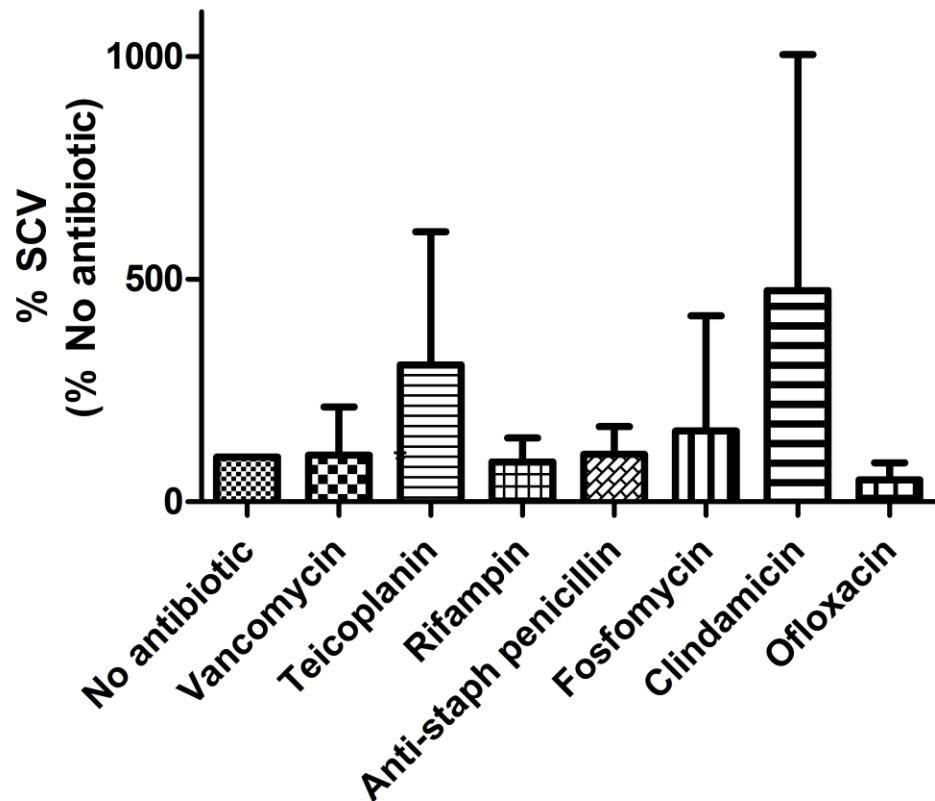
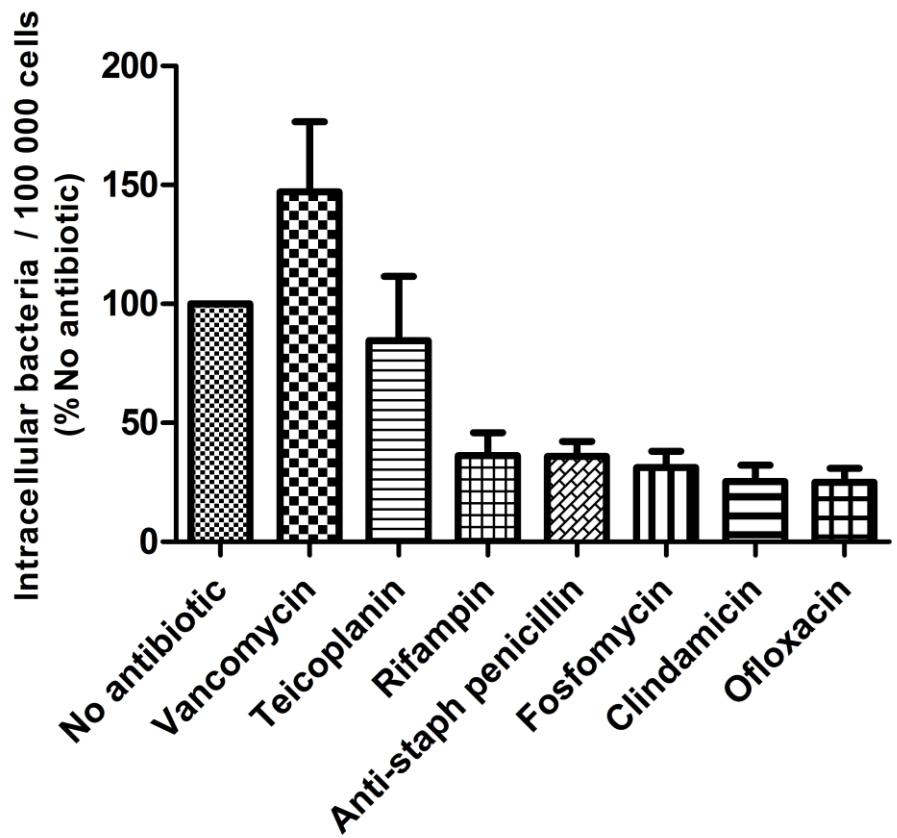
Clindamycin



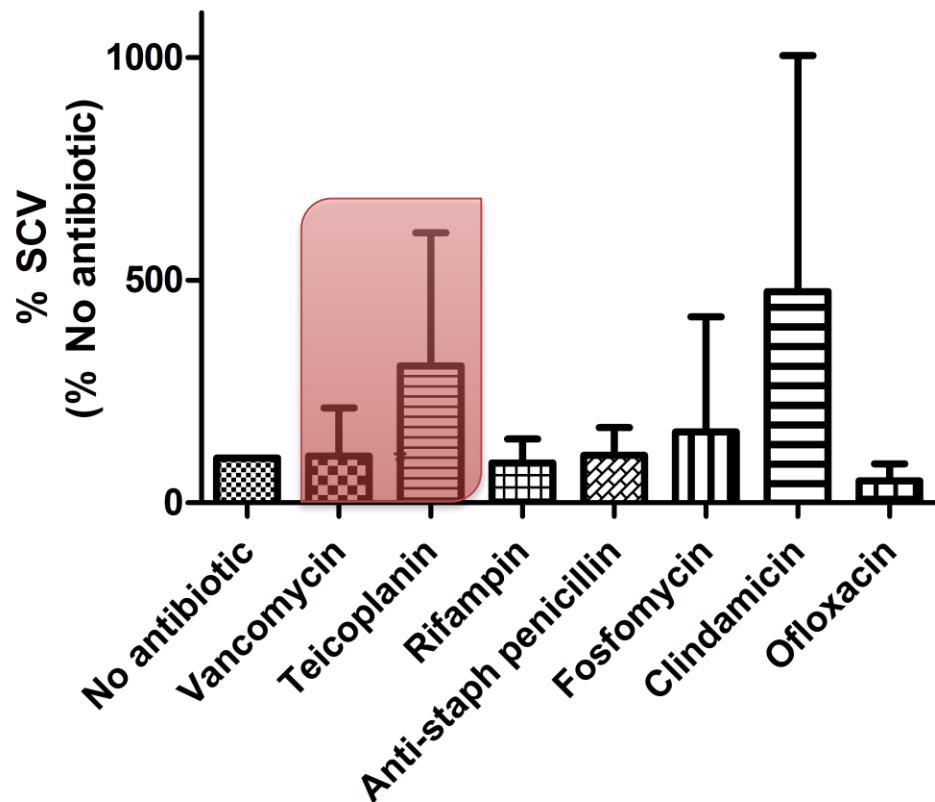
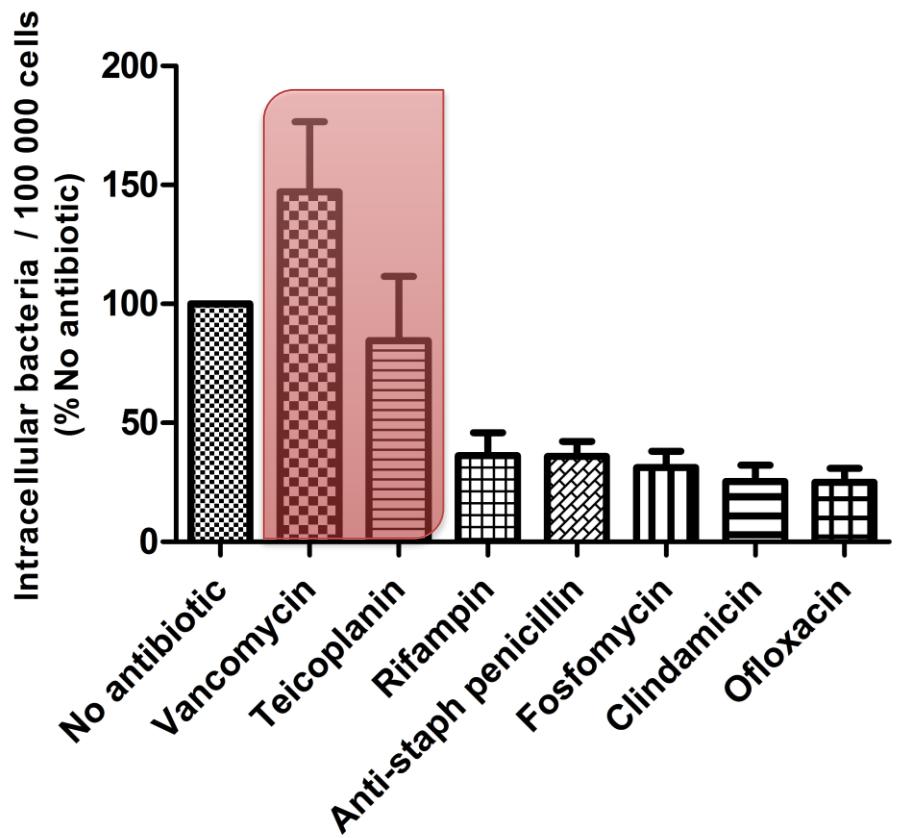
Fosfomycin



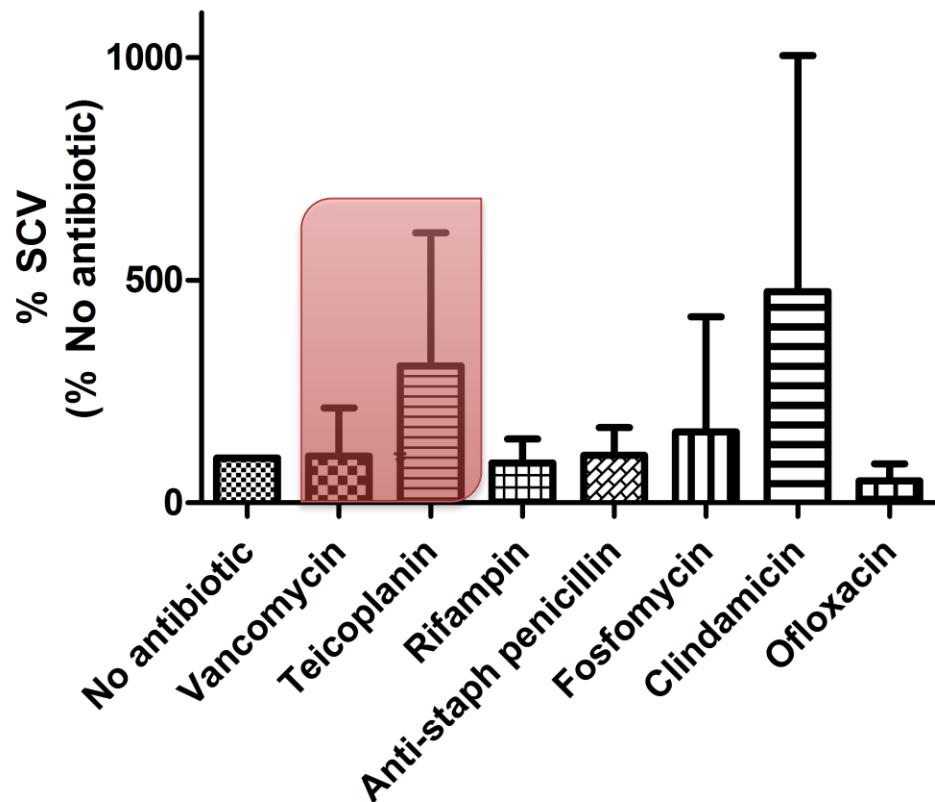
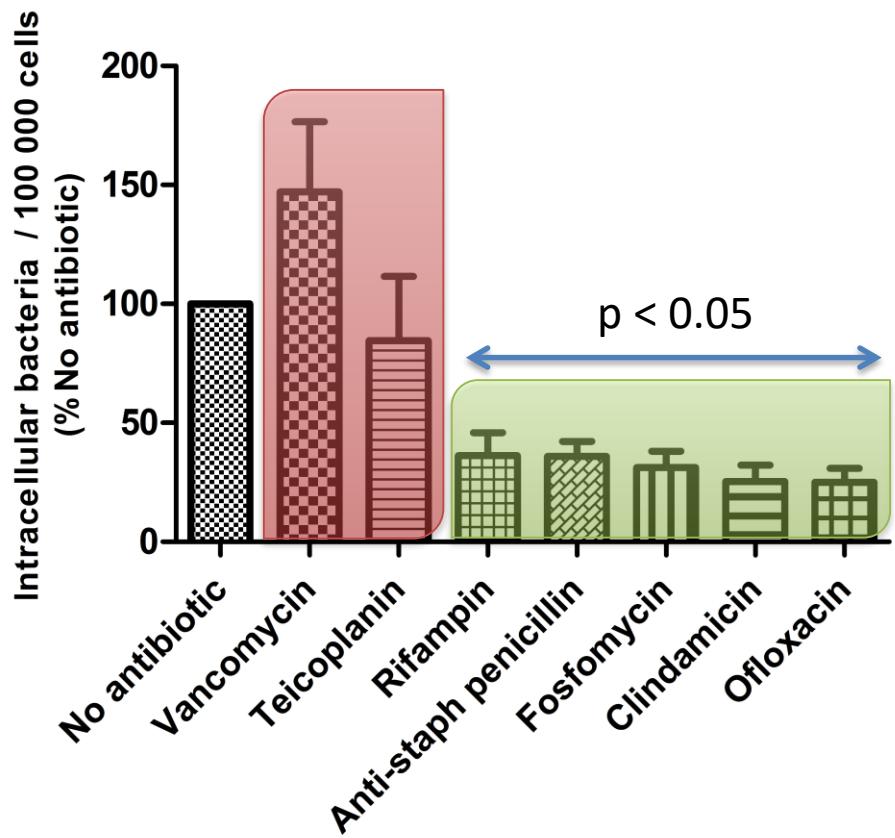
Results



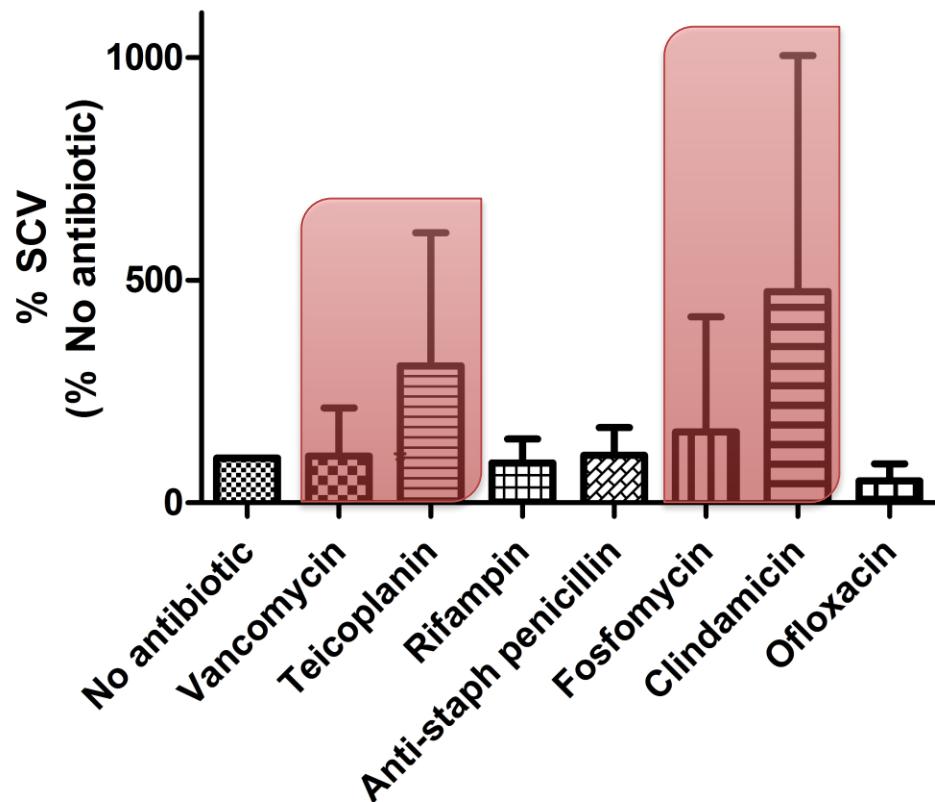
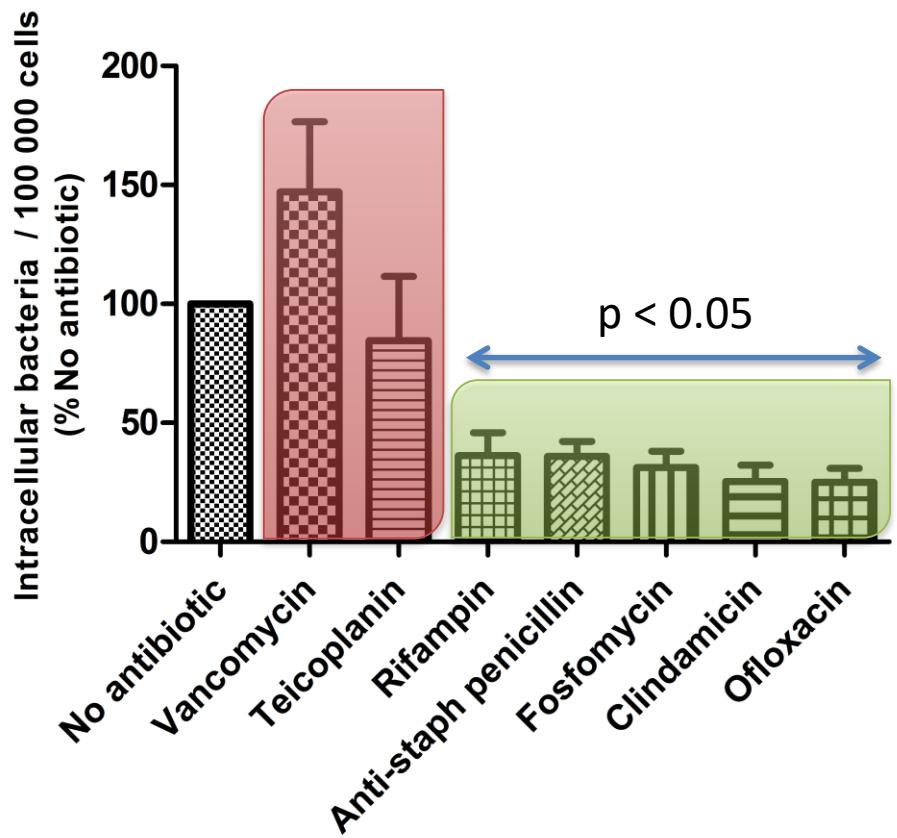
Results



Results



Results



Conclusions

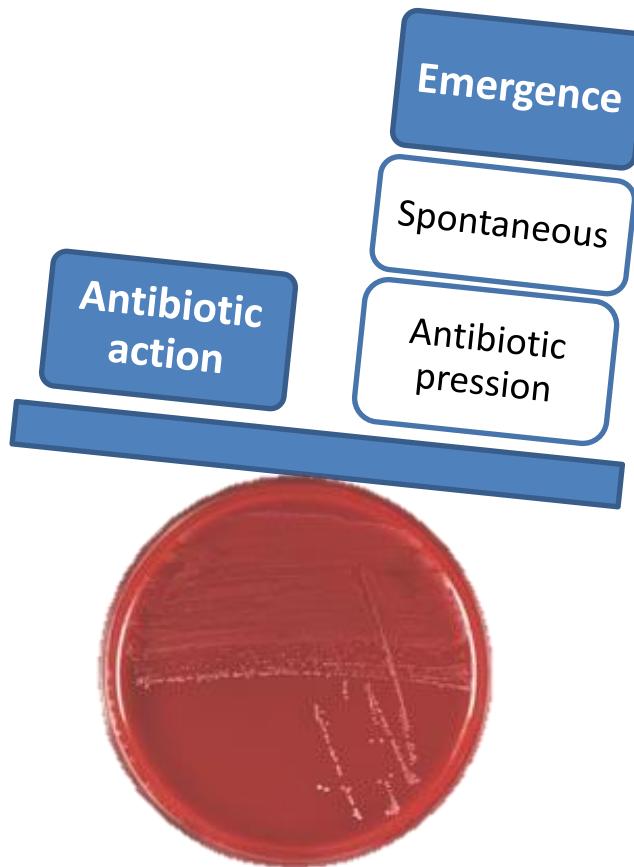
- First evaluation of the intracellular activity of the main antistaphylococcal molecules used in BJI
- Advantages compared to previous studies
 - Osteoblast infection model ≠ monocytes macrophages
 - Bone concentrations ≠ plasmatic concentrations
- Limitations
 - *Ex vivo* model, using only one laboratory strain (HG001)
 - No assessment of intracellular antibiotic concentrations
 - No consideration of antibiotic binding to proteins in fetal calf serum

Conclusions

- **Intracellular activity:** all the tested molecules are active against intracellular *S. aureus*, with the exception of glycopeptides
 - *S. aureus* : endolysosomal, 30% cytoplasmic
 - Antibiotics
 - Intracellular penetration modalities and localization
 - GP : very slow diffusion in endolysosomes
 - RMP, CLINDA, FQ : rapid diffusion in all cellular compartment
 - Importance of local chemical conditions (pH)
 - RMP, oxacillin : optimal conditions

Conclusions

- **Phenotype switching:** difficult interpretation



Conclusions

- Comparison to previous similar study
 - *Kreis et al. BMC Infect dis 2013*
 - Satisfactory intracellular activity of tigecycline and rifampin
 - Used concentrations = C_{bone} x 30 !!
 - *Ellington et al. J Orth Res 2006*
 - Satisfactory intracellular activity of rifampin, clindamycin and macrolides
 - But decreased activity if *S. aureus* intracellular persistence > 12h (bacterial wall modifications)

Conclusions

- Intracellular activity: a potential important parameter for the choice of *S. aureus* BJI treatment strategies
- All tested molecule were intracellularly active, with the exception of glycopeptides
- Only fluoroquinolones allowed
 - An excellent intracellular activity
 - A limitation of intracellular emergence of SCV
- What about « new » molecules?



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Michèle BES
Anissa BOUAZIZ
Marine BUTIN
Christian CHIDIAC
Jérôme ETIENNE
Tristan FERRY
Sacha FLAMMIER
Judith KARSENTY
Hélène MEUGNIER
Jean-Philippe RASIGADE
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