15th ISCVID Symposium, University of Lausanne, 2- 4 June 2019
Session III: Experimental and clinical pathogenesis

S08 - Lag phase in bacteria directly derived from patients - implications for therapy in IE

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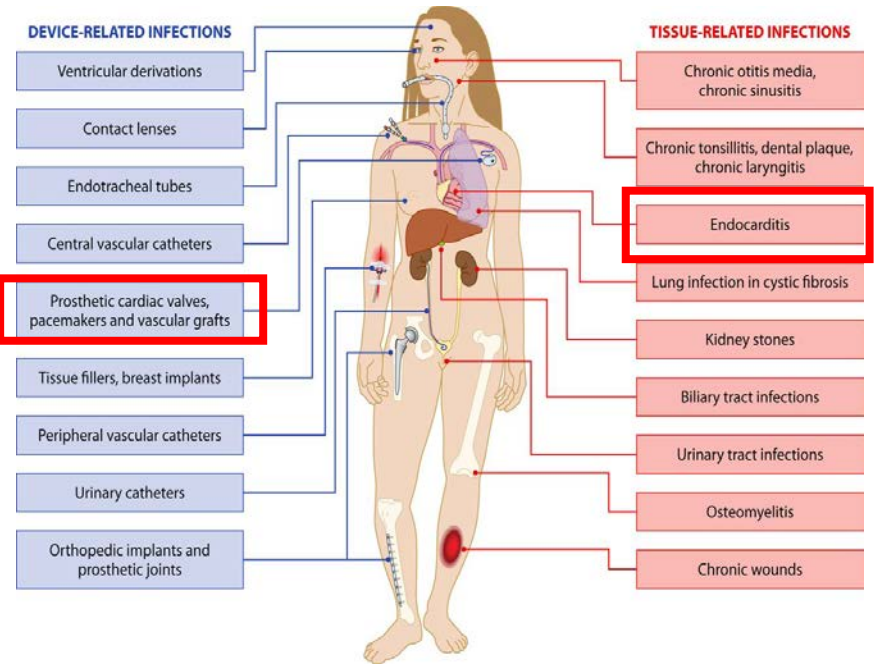
Nothing to disclose

No COI

Staphylococcus ssp. Endocarditis



Antibiotics: Long treatment duration, i.v.

- **How long is long enough?**
  - **Endocarditis: >4 weeks**
    - 1943: Mortality 100% -now with antibiotics 30%

Surgery: Scars- morbidity -mortality

- **Removal of infected tissue -foreign body**

» **Therapy of *Staphylococcus aureus* Bacteremia Associated with a Removable Focus of Infection,**
PAUL B. IANNINI, M.D.; KENT CROSSLEY, M.D. 1976

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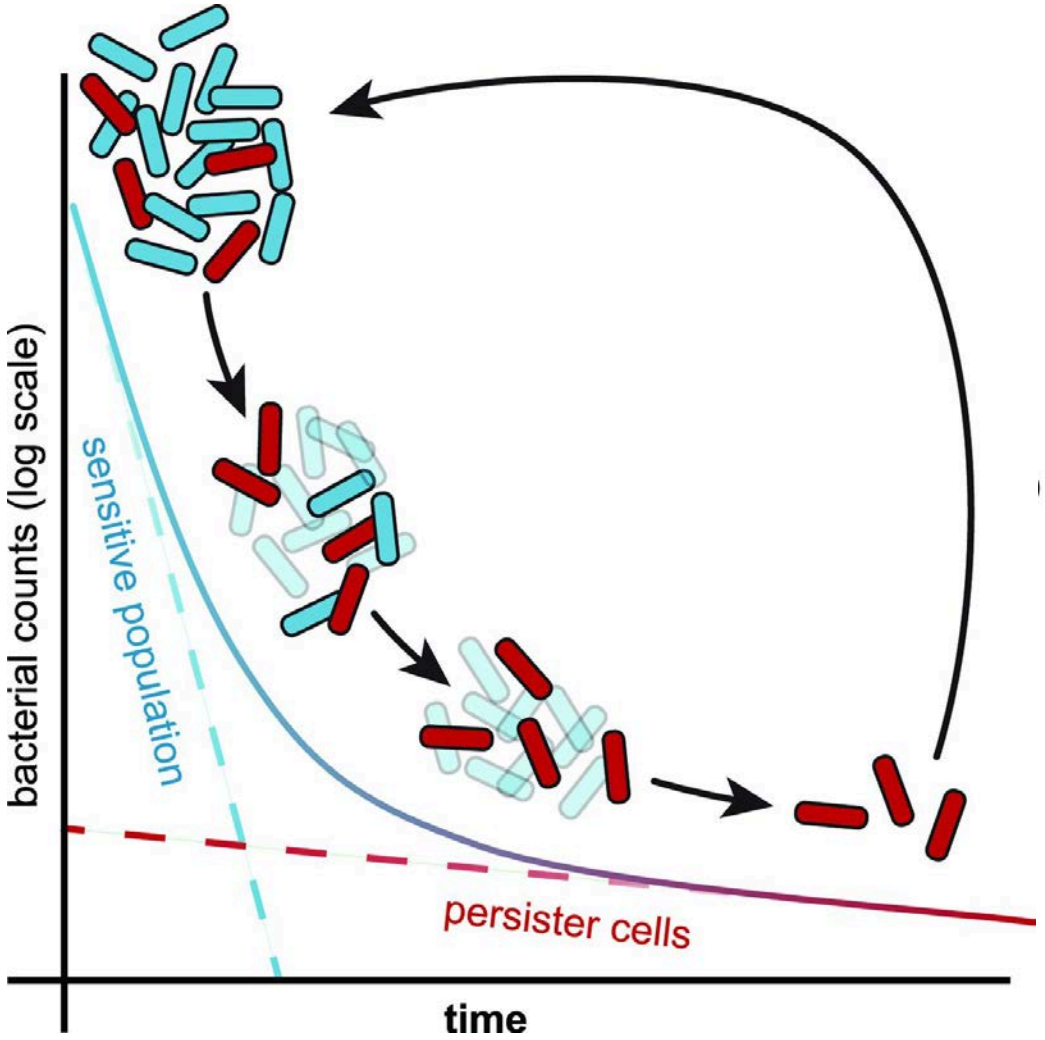
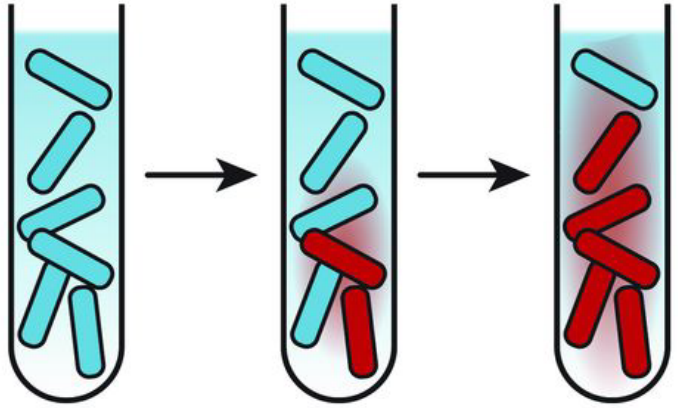
Persisters = metabolically inactive bacteria



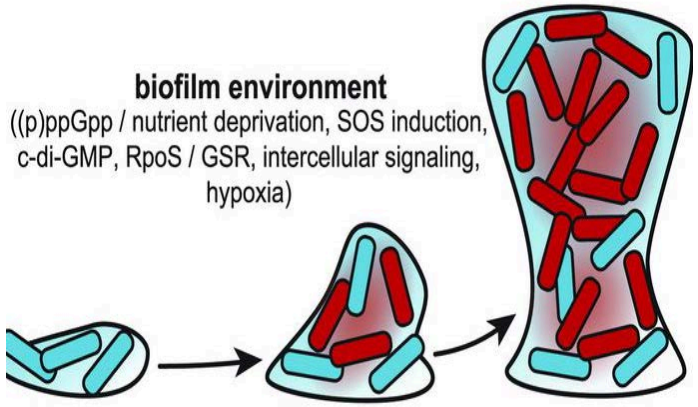
Biphasic killing kinetics of bactericidal antibiotic treatment.

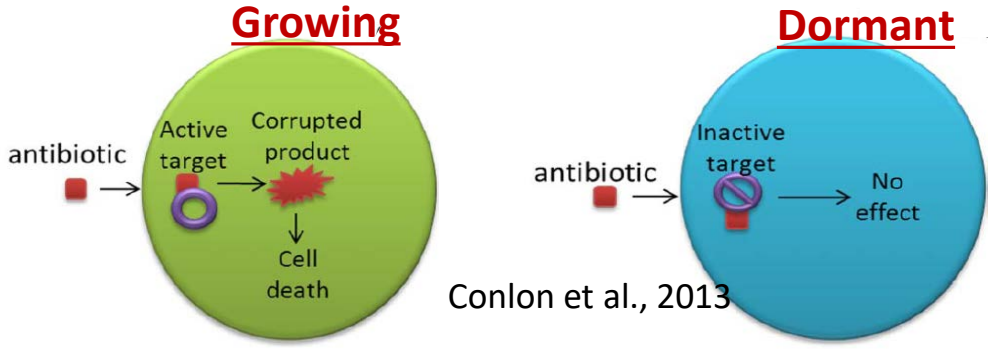
Alexander Harms et al. Science 2016;354:aaf4268



stationary phase

((p)ppGpp / nutrient deprivation, RpoS / GSR)



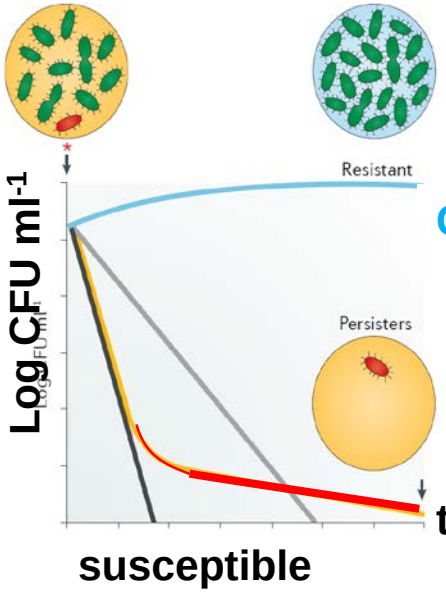


Conlon et al., 2013

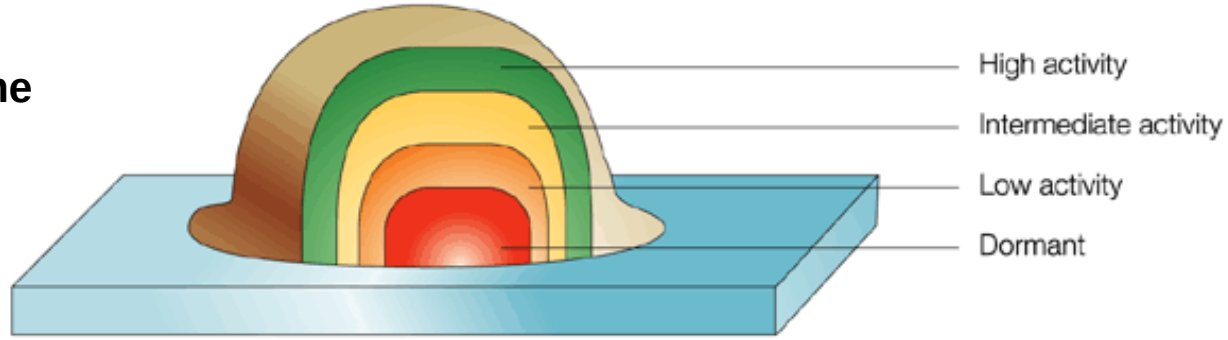
E.coli, Tbc, *S.aureus,* Salmonella ssp.

Stressors: reactive oxygen species (ROS), lack in nutrients, low pH, antibiotics

Persisters = metabolically inactive bacteria



Genetic Resistant



Phenotypic Resistance

Persistenz, Toleranz

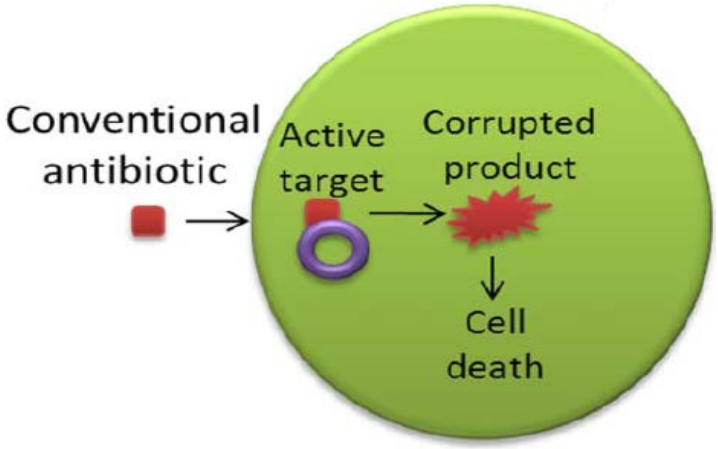
Natural Resistance

outer Membrane Gram-

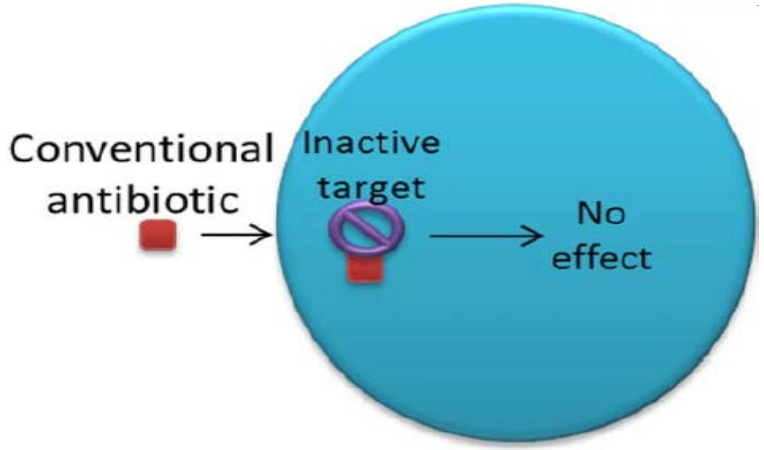
Genetic Resistance

Mutations
Effluxpumps upregulated
Modified enzymes

Growing



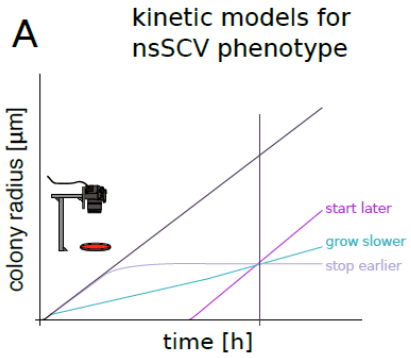
Dormant

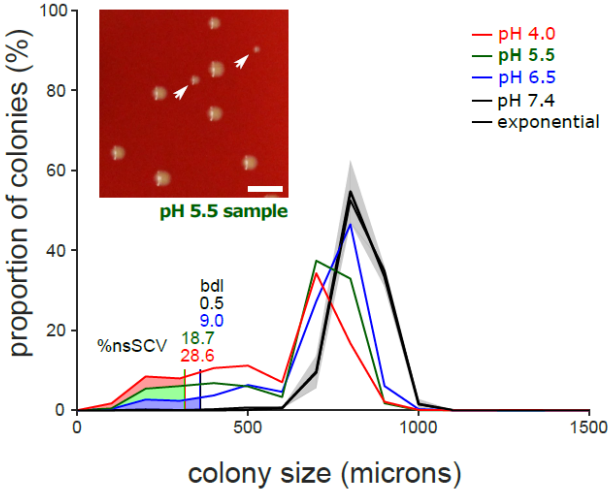


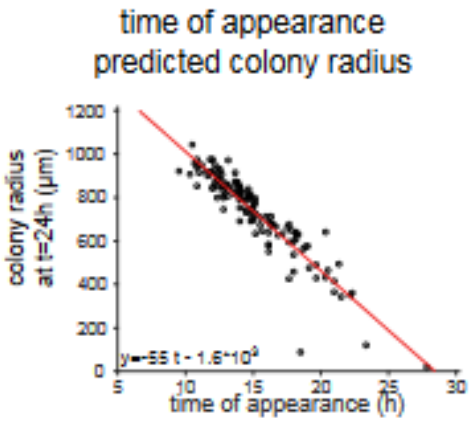
Growth – No Growth

Conlon et al., 2013

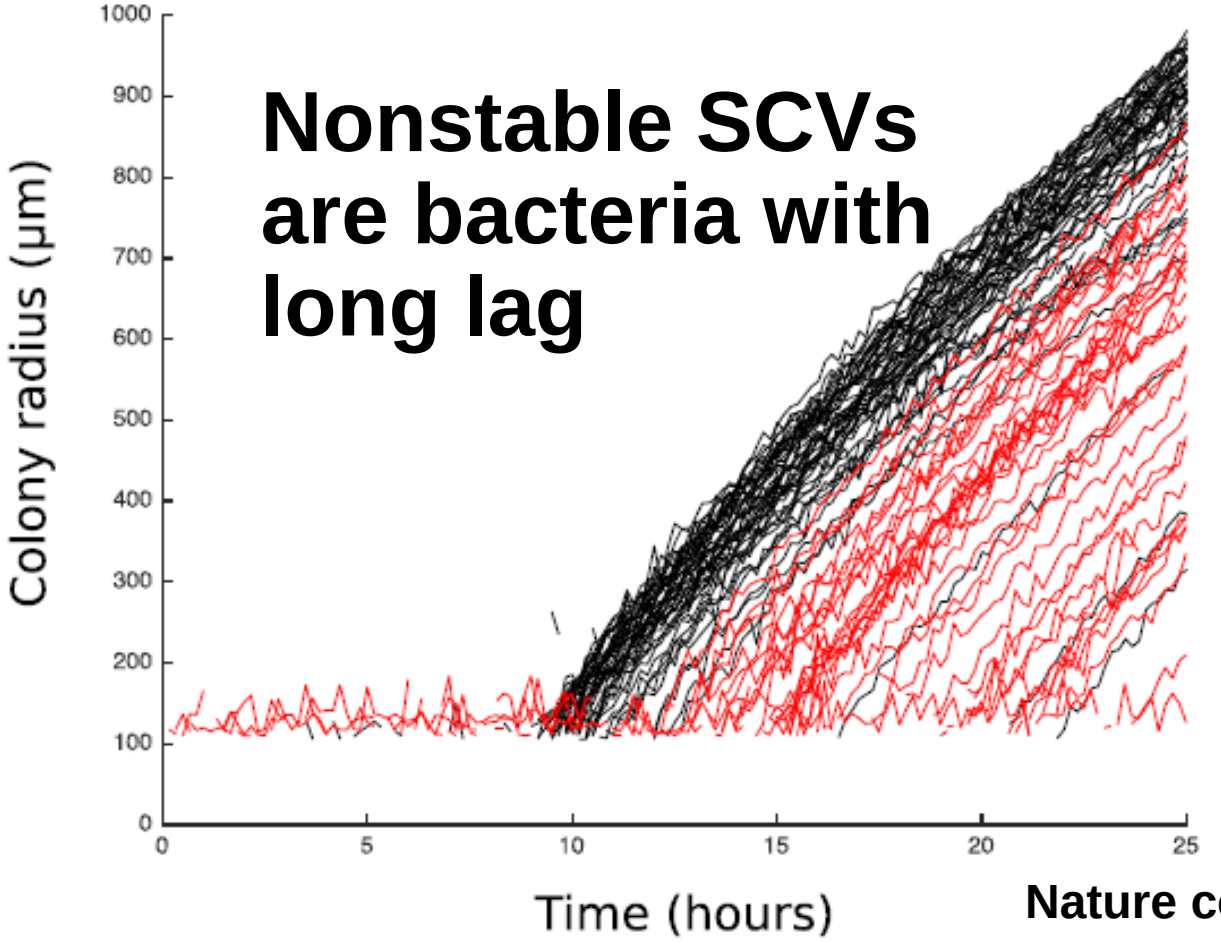
SCV formation is a consequence of a late emergence of colonies







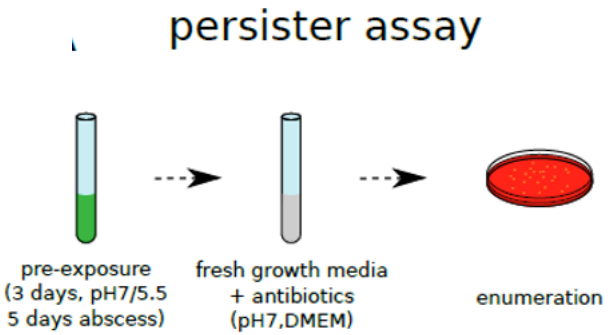
Nonstable SCVs are bacteria with long lag



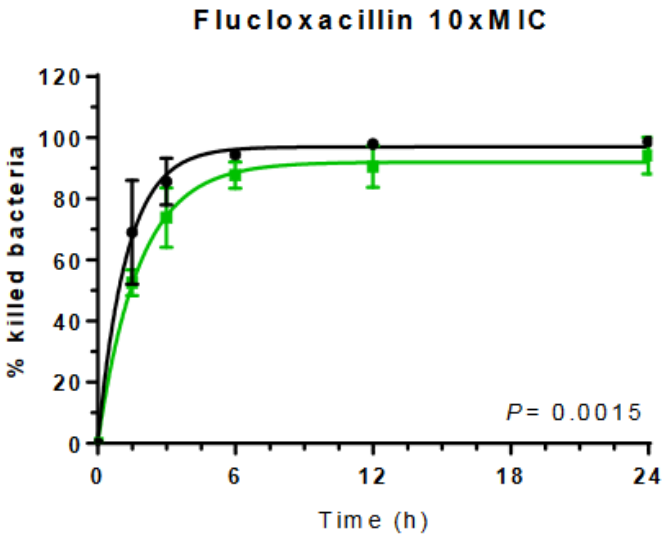
Nature communications 2018

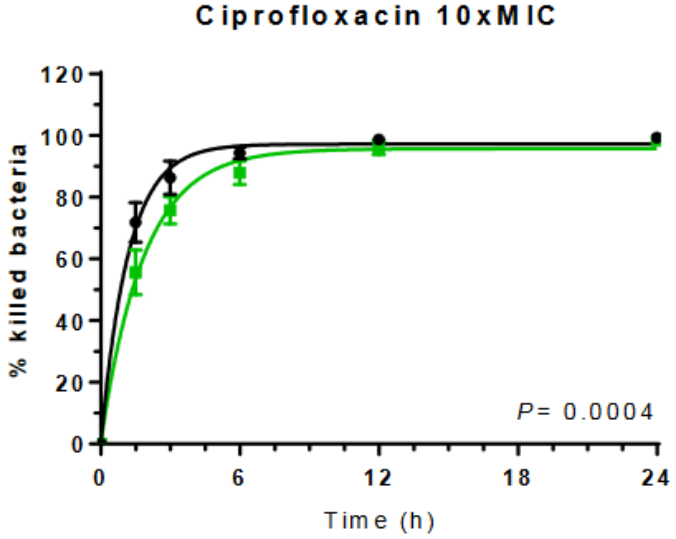
Non dividing bacteria withstand antibiotics

Tolerance by growth

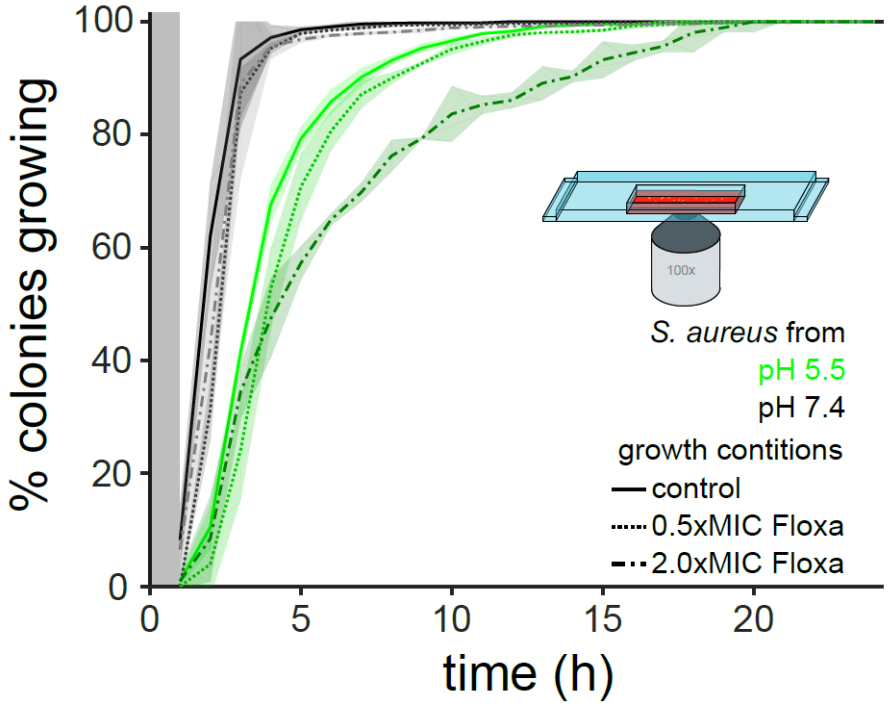


S. aureus from
pH 5.5
pH 7.4





single cells' first divisions
from liquid culture



Nature communications 2018

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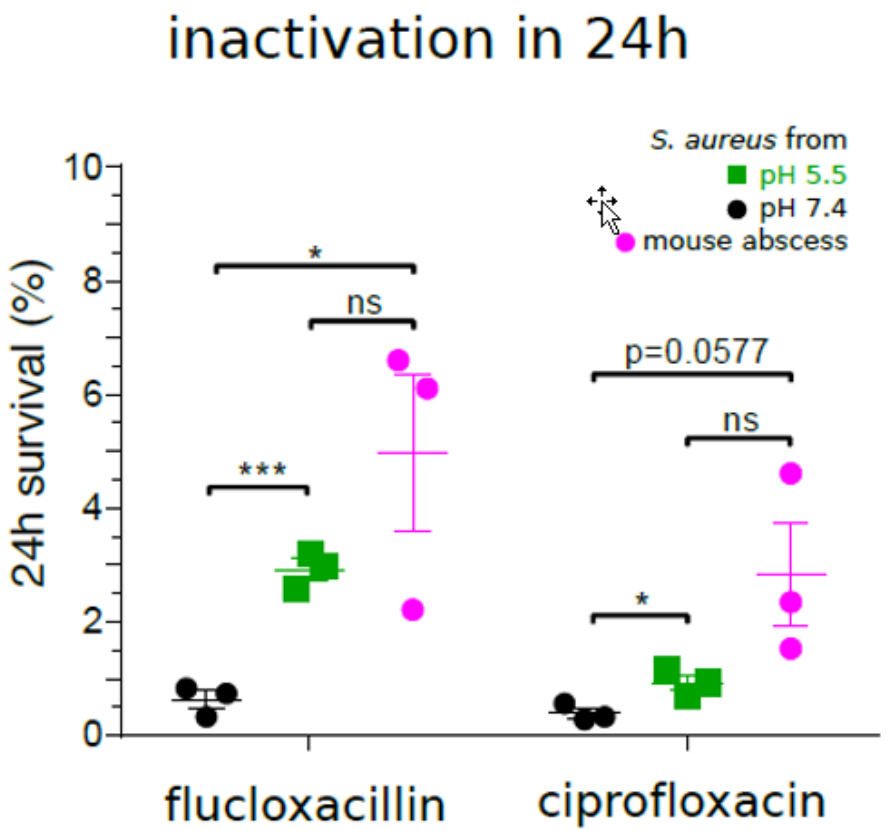
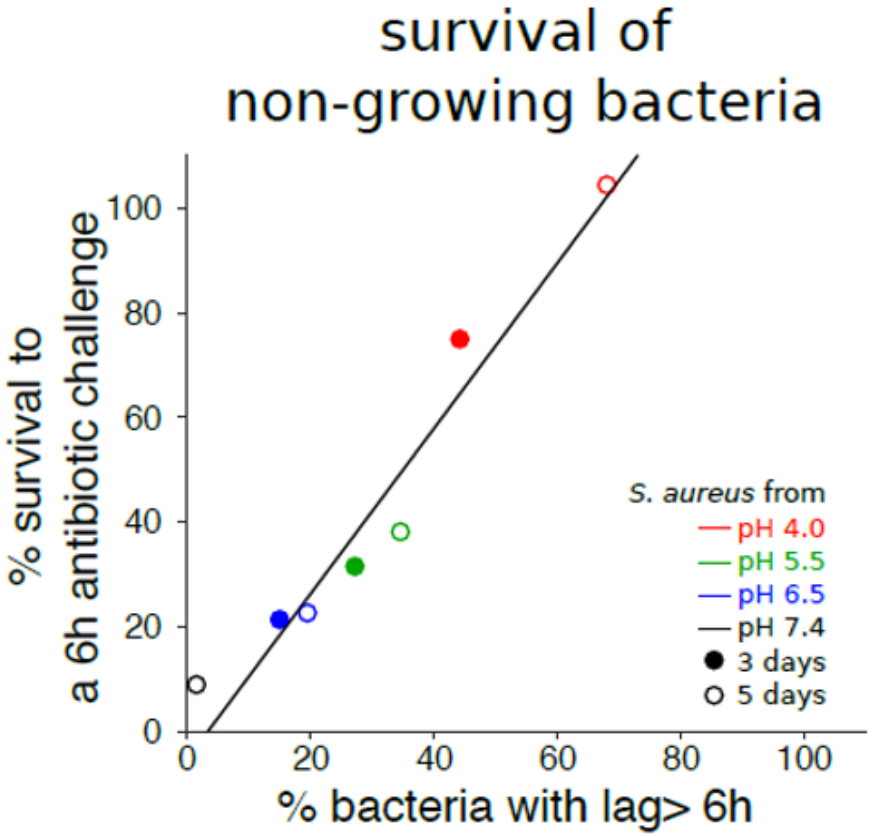
The proportion of bacteria in lag phase correlates with the proportion of bacteria surving antibiotics





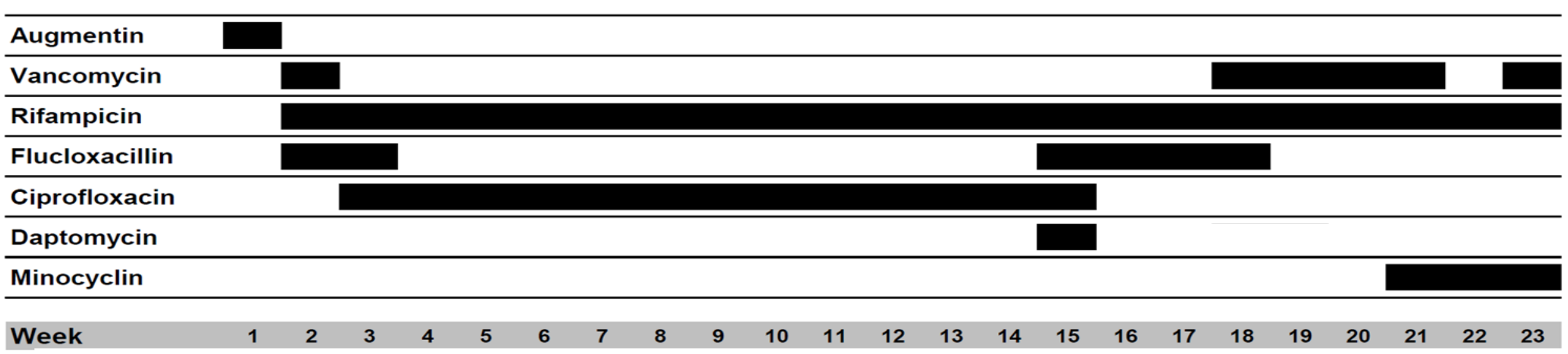
ARTICLE

DOI: 10.1038/s41467-018-06527-0 OPEN

Prolonged bacterial lag time results in small colony variants that represent a sub-population of persisters

Clément Vulin[1,2], Nadja Leimer[3,4], Markus Huemer[3], Martin Ackermann[1,2] & Annelies S. Zinkernagel[3]

Staphylococcus epidermidis
pacemaker associated endocarditis



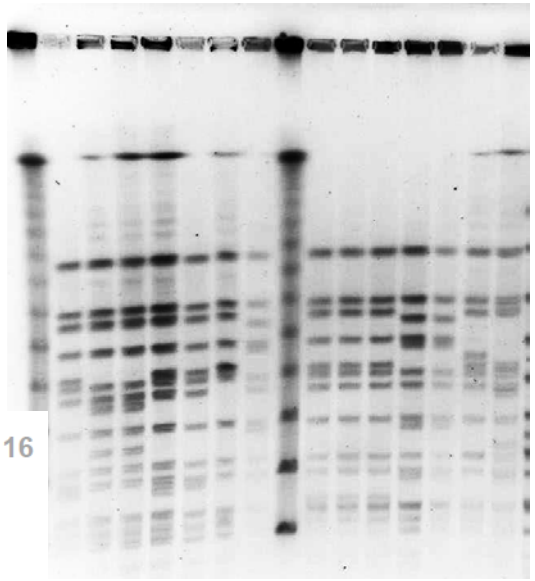
Pacemaker pocket infection in 40 year old male, debridement of the pacemaker pocket, vacuum assisted closure therapy (VAC), antibiotics

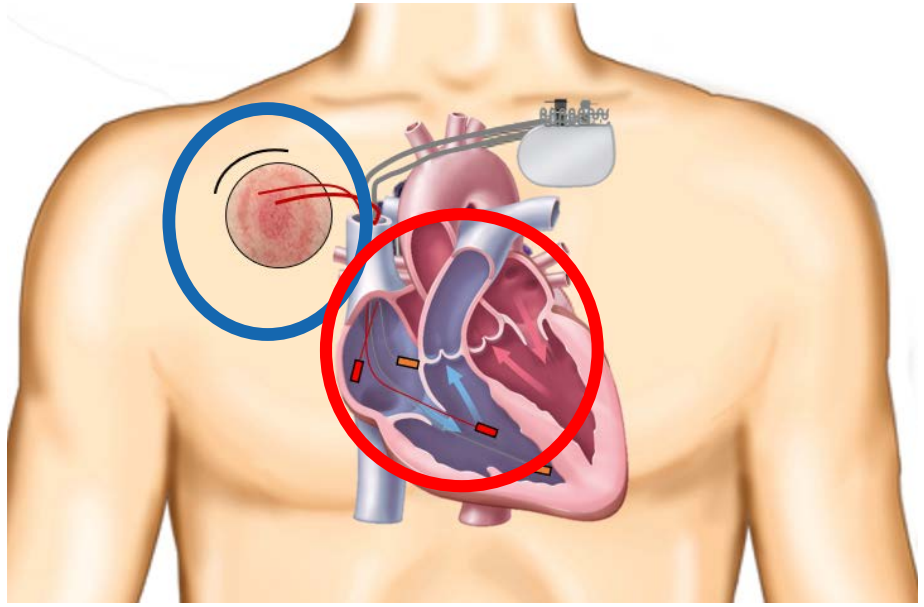
Bacteremia, vegetation in echocardiography

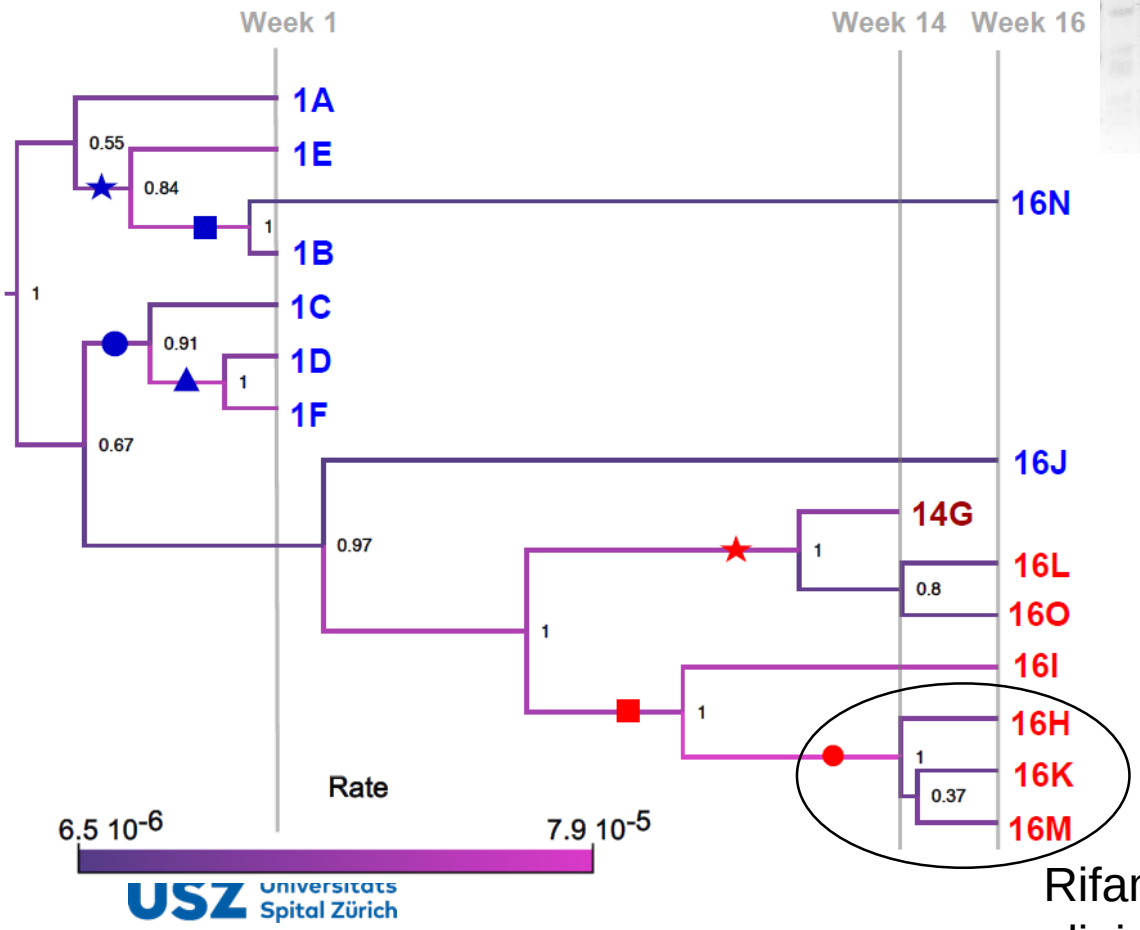
Explantation of pacemaker

The 'Endocarditis Team'

Phylogenetic tree of the clinical *S. epidermidis* isolates

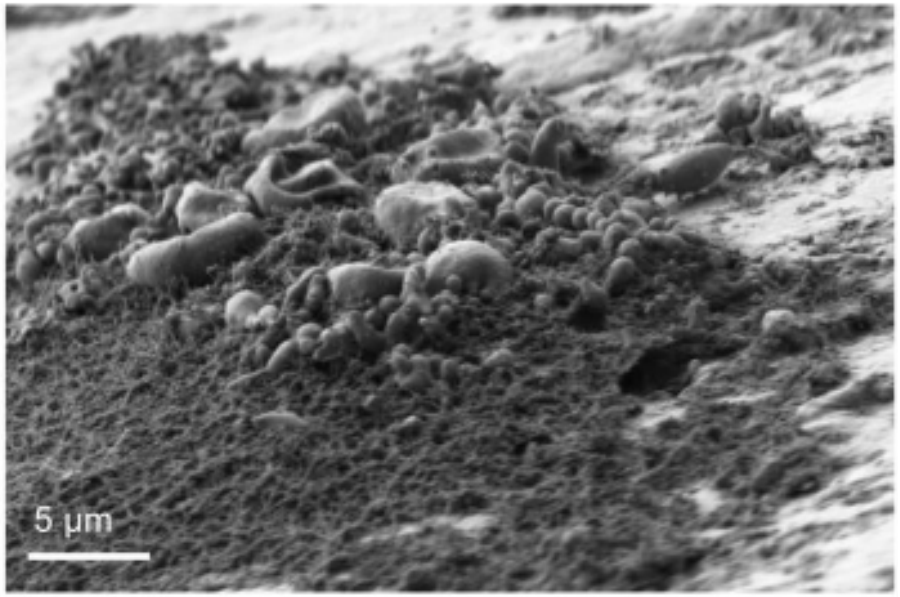




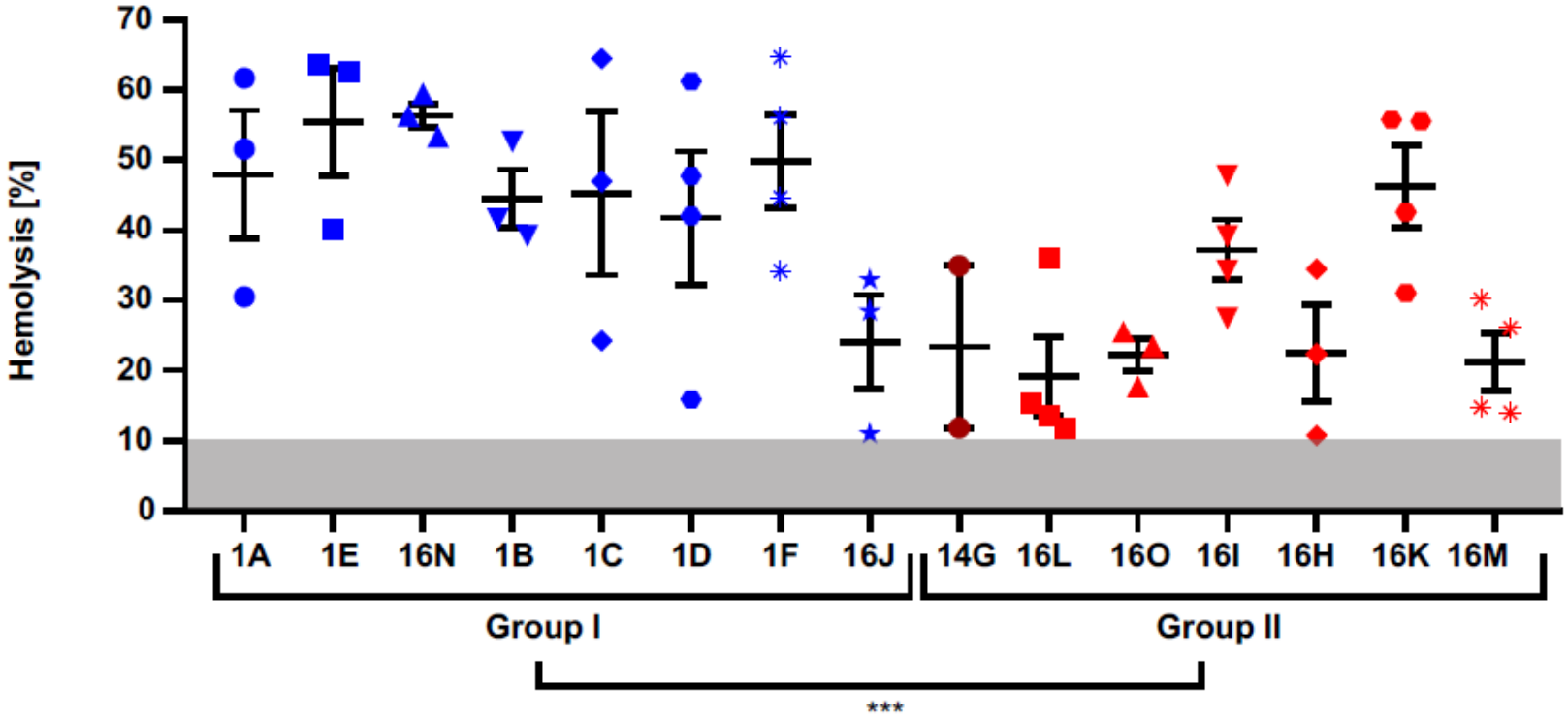


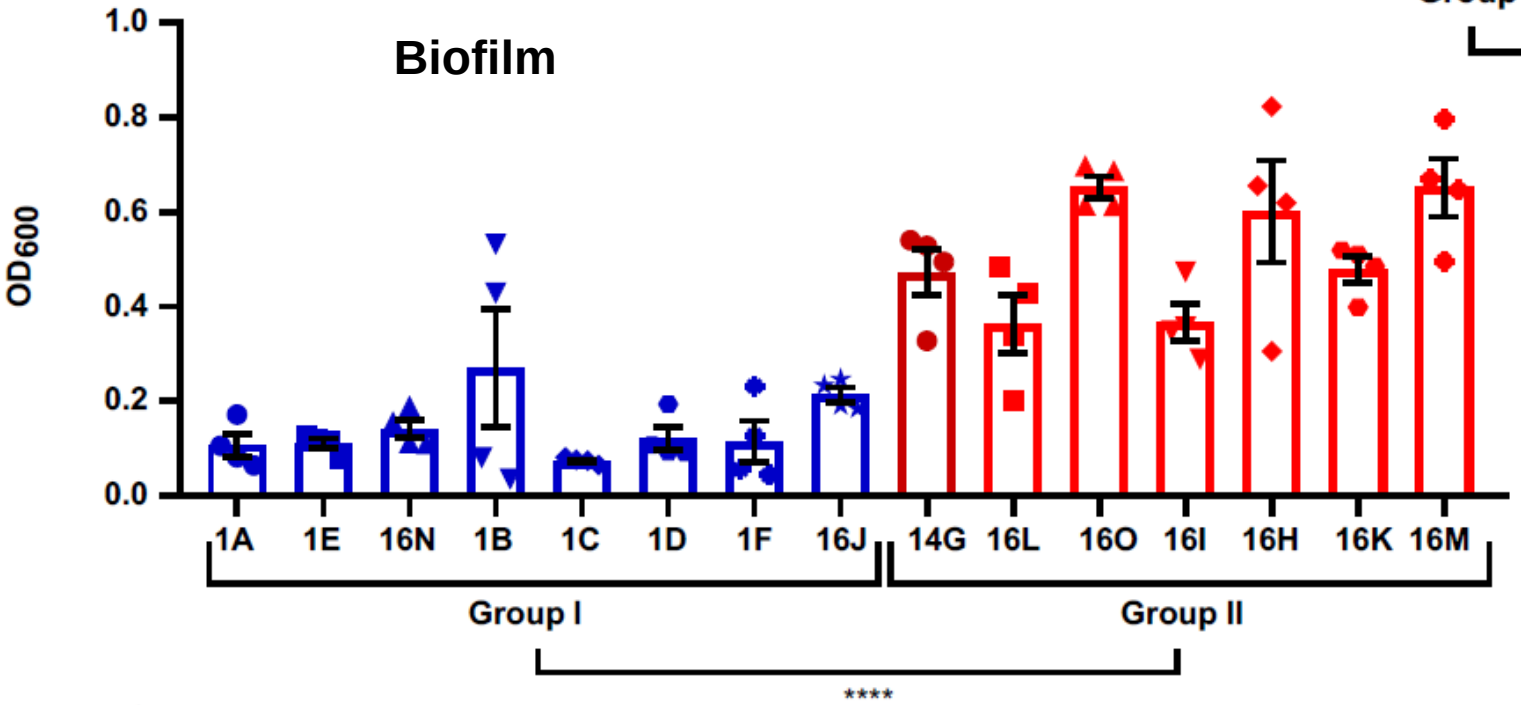
Rifampicin resistent clinical isolates

Bacterial isolate	Isolation week	Laboratory internal CI number	Isolation origin	Pacemaker area	Sequence Type
1A	1	835	Wound extraction of PM pocket	Old	378
1B	1	836	Wound extraction of PM pocket	Old	378
1C	1	837	Tissue	Old	378
1D	1	838	Tissue	Old	378
1E	1	839	Electrode	Old	378
1F	1	841	Electrode	Old	378
14G	14	753	Blood	-	378
16H	16	842	n.d	New	378
16I	16	848	Aggregate	New	378
16J	16	849	Aggregate	New	378
16K	16	788	Ventricle electrode	New	378
16L	16	792	Atrial electrode	New	378
16L	16	792	Atrial electrode	New	378
16M	16	797	Ventricle electrode	New	378



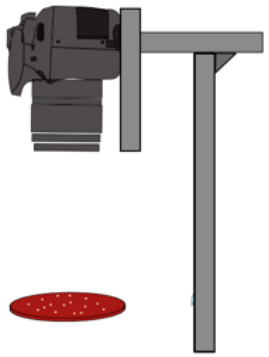
Scanning Electron Microscopy





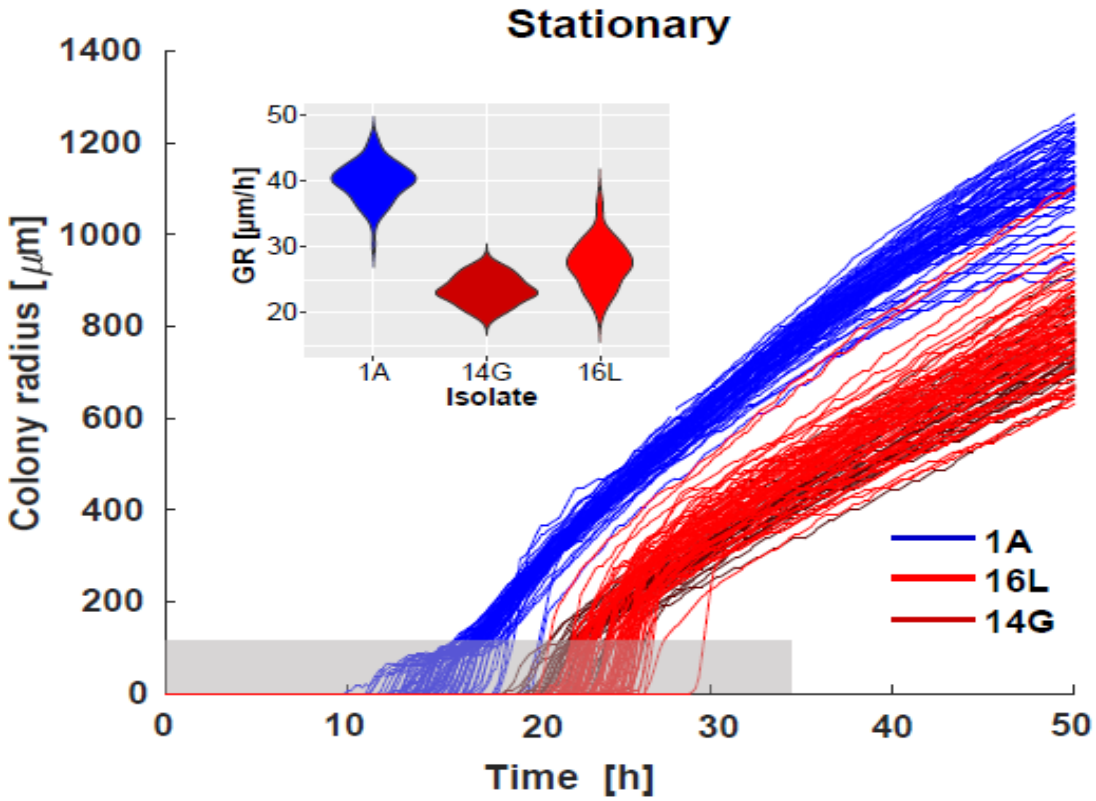
Nature communications 2019

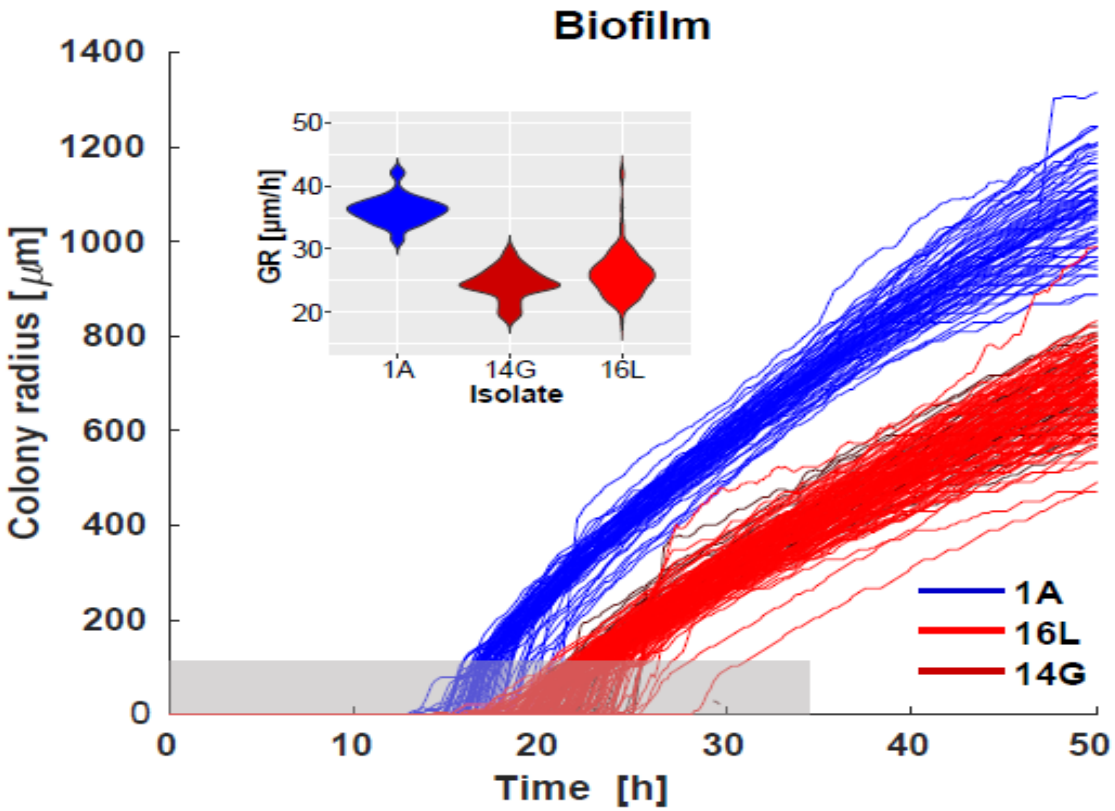
Colony time lapse



Vulin et al. 2018, Nat. Commun.

Late isolates show reduced growth rate and increased lag time





Appearance of colonies on blood agar plates

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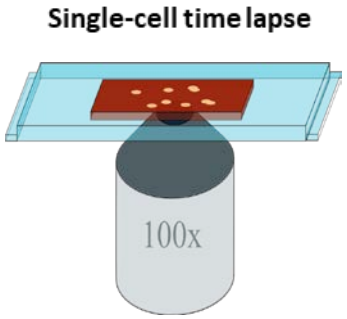
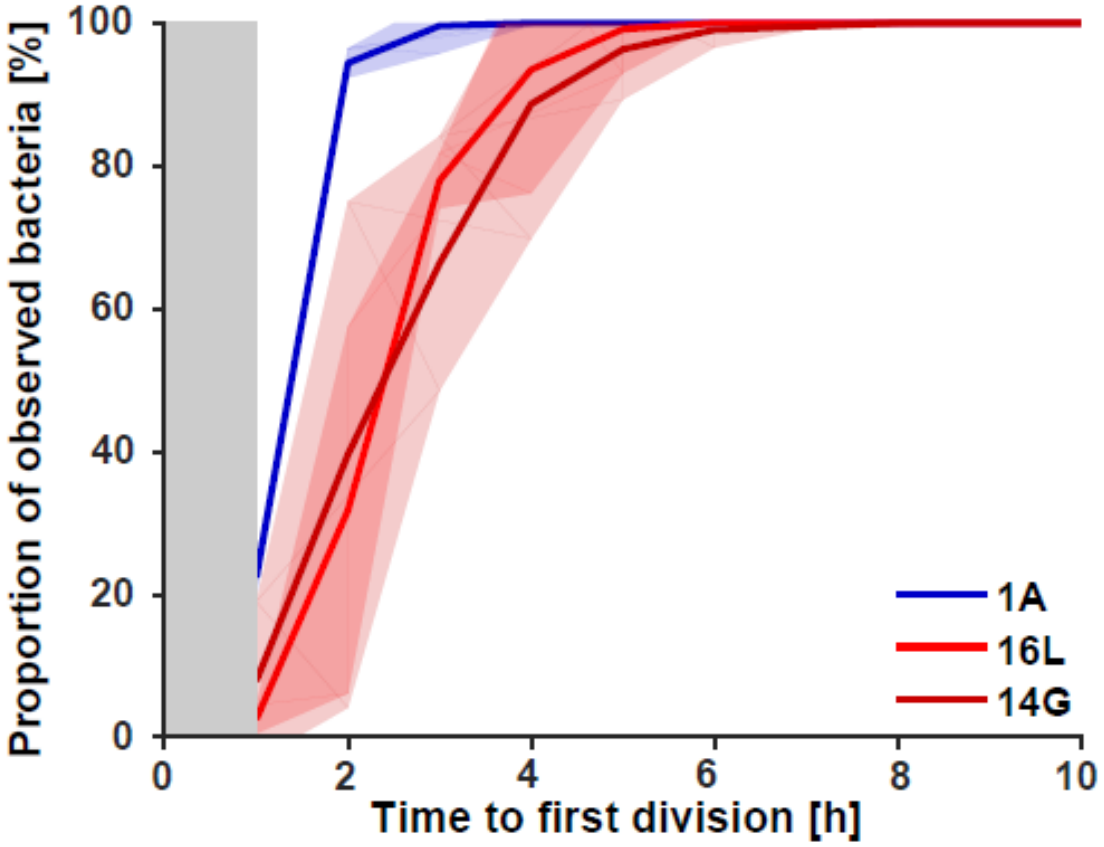


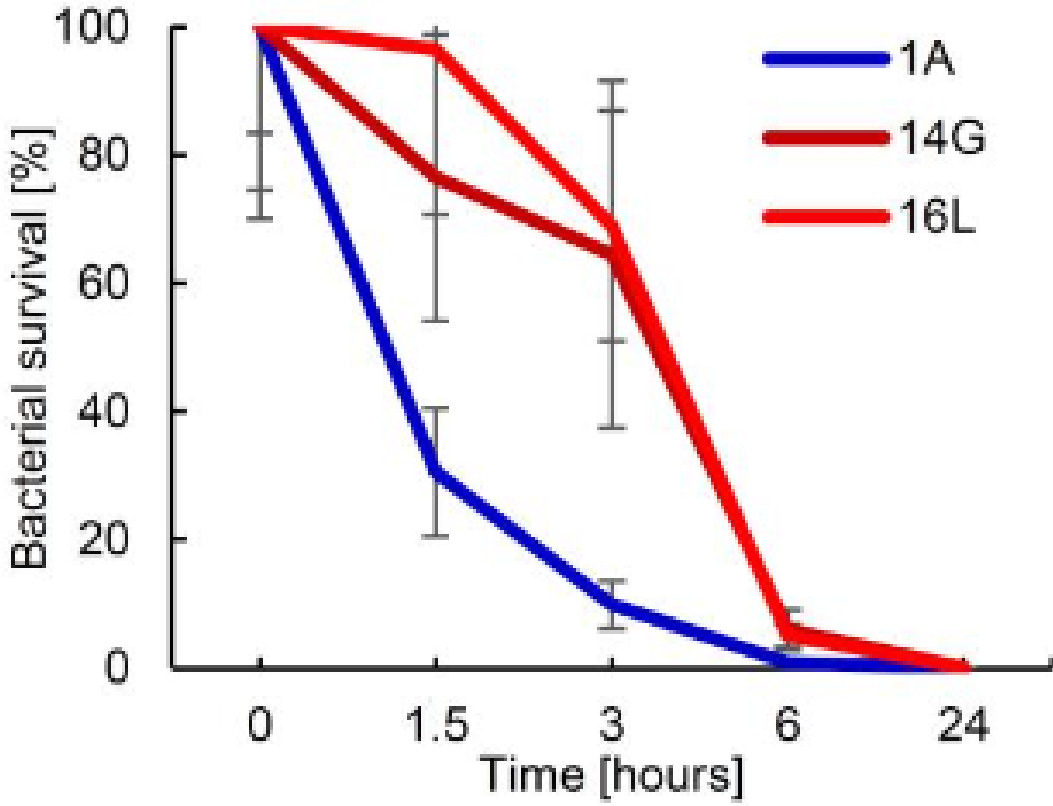
Vulin et al. 2018, Nat. Commun.

Late isolates show increased lag time of single cells' first division

Ciprofloxacin MIC 40 x time kill curves



First division of a single bacterial cell



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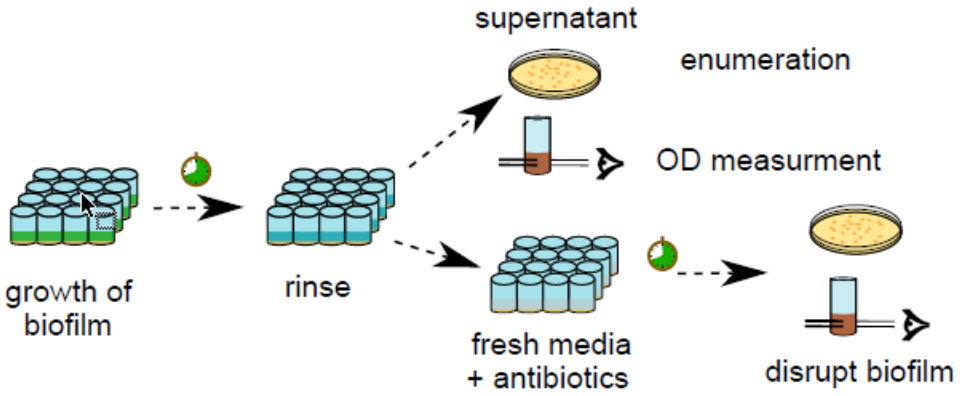
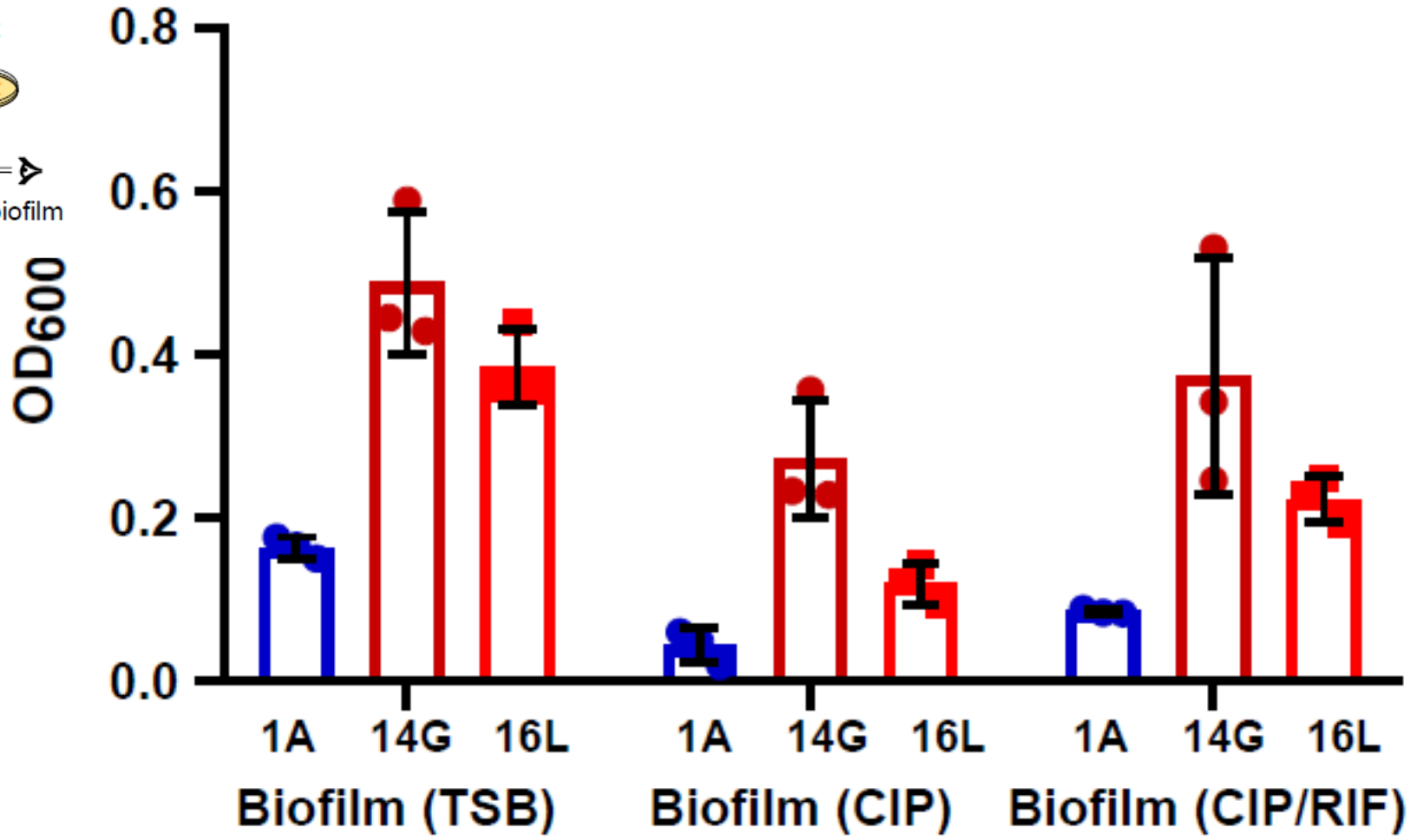
Late isolates show thicker biofilm after ciprofloxacin and rifampicin exposure



Biofilm thickness



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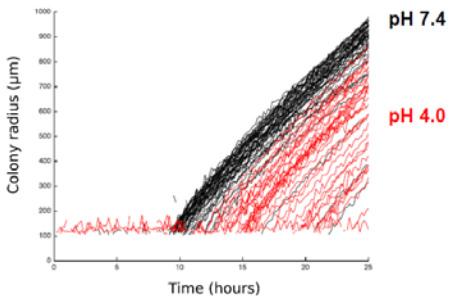
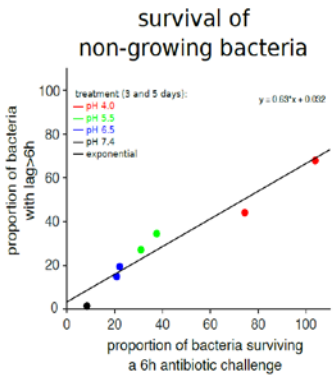
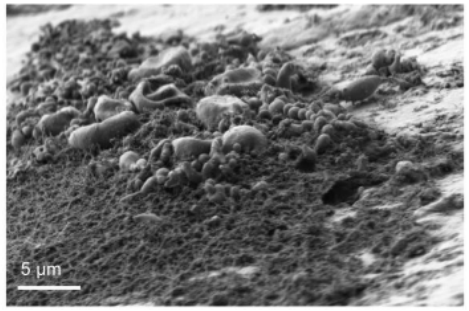
Lag phase in bacteria directly derived from patients - implications for therapy in IE







- **Staphylococcus ssp. endocarditis**
  - Surgery crucial
  - Long antibiotic treatments
  - Heterogenous population

The 'Endocarditis Team'

- **Prolonged bacterial lag time results in SCV that represnt a sub-population of persisters**
  - The proportion of bacteria in lag phase correlates with the proportion of bacteria surving antibiotics

- ***S.epidermidis*** in host evolution

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SCHWEIZERISCHER NATIONALFONDS
FONDO NAZIONALE SVIZZERO
SWISS NATIONAL SCIENCE FOUNDATION

Many thanks

inkernagel lab

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Claudia Zürcher
Katrin Schilcher
Rey Gaffner

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Kim Röthlin
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Roger Kouyos
Denise Kühner

→ collaborations with
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