Adaptation of vancomycin-intermediate *Staphylococcus aureus* to intracellular compartment leading to bacterial reservoir responsible for chronic infection

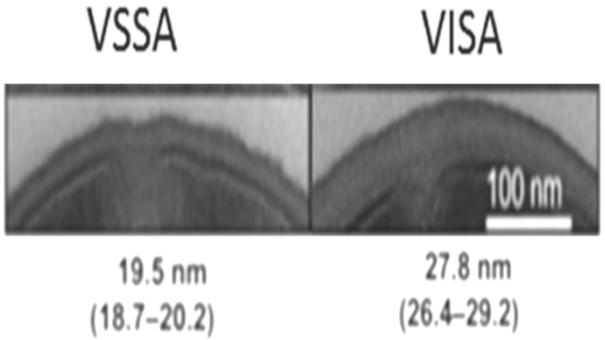
Sophie Trouillet-Assant, Virginie Tafani, David Cameron,
Benjamin Howden, Anton Peleg, **Frédéric LAURENT**

Department of Bacteriology – North Hospital, Hospices Civils de Lyon, France
French National Reference Centre for Staphylococci, Lyon, France
International Centre for Infectiology Research - INSERM U1111, Lyon, France
Monash University, Melbourne, VIC Australia

S. aureus : the VISA strain issue

- ✓ MSSA ➡ overuse of antibiotics ➡ Emergence of MRSA
- ✓ Vancomycine ➡ antibiotic of choice for treating MRSA
- ✓ in 1997 ➡ emergence of vancomycin-intermediary *S. aureus* **VISA**

✓ Features of VISA vs. VSSA isolates



- Phenotypically: **thickening of the wall**
- Genetically: **cumulative point mutations** in diverse regulatory loci including regulatory system
- Clinically: treatment failures, persitent bacteriemia and prolonged hospitalization

BUT - not acute clinical instability
- no higher mortality

➡ Chronic infection

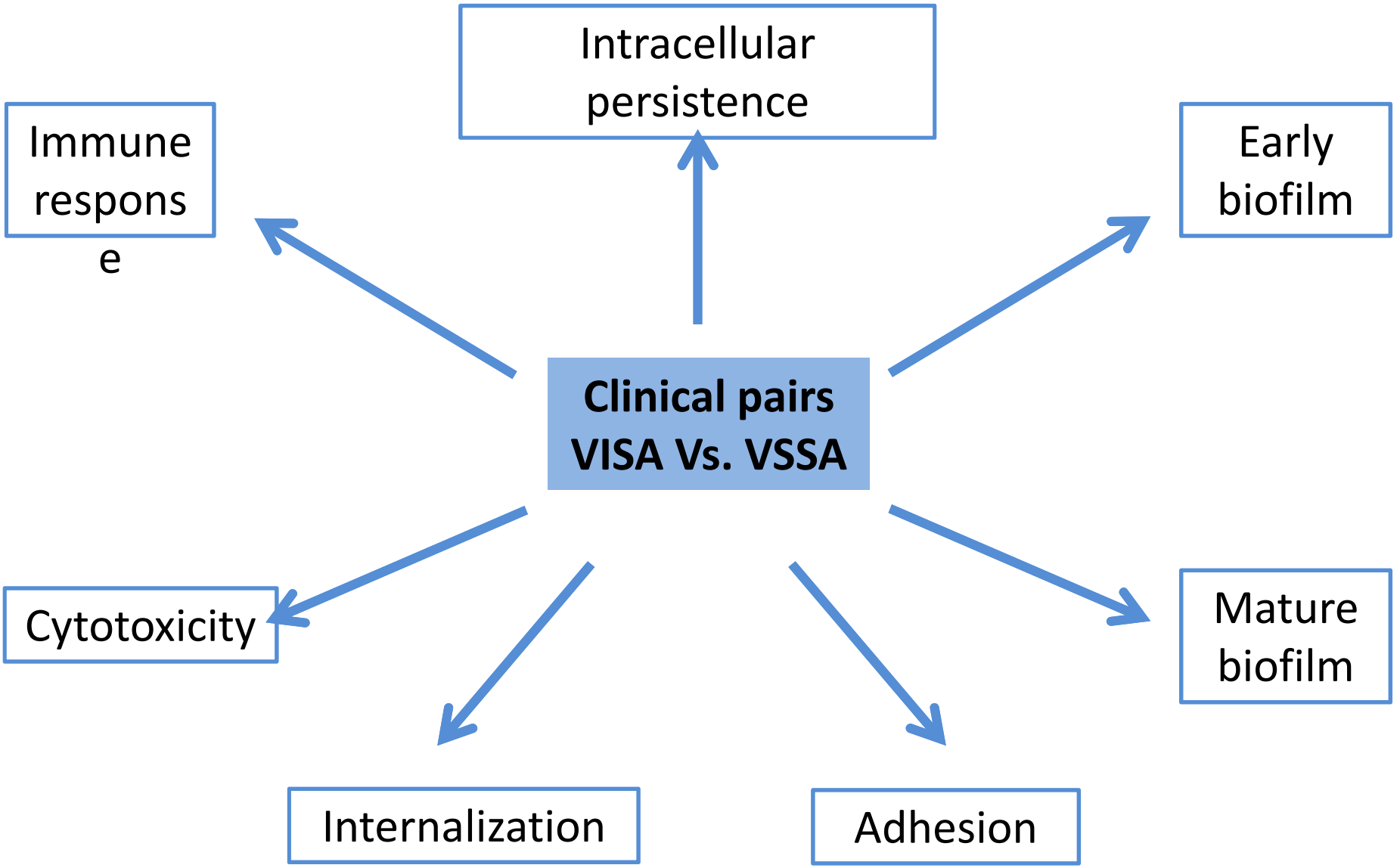
From VSSA to VISA : switch of resistance … and virulence ?

Hypothesis: the various mutations that appeared in VISA may also impact upon pathogenicity

What are the virulence mechanisms impacted by the switch from VSSA to VISA?

3 pairs of VSSA-VISA clinical isolates collected from three patients with persistent bacteremia treated with vancomycin

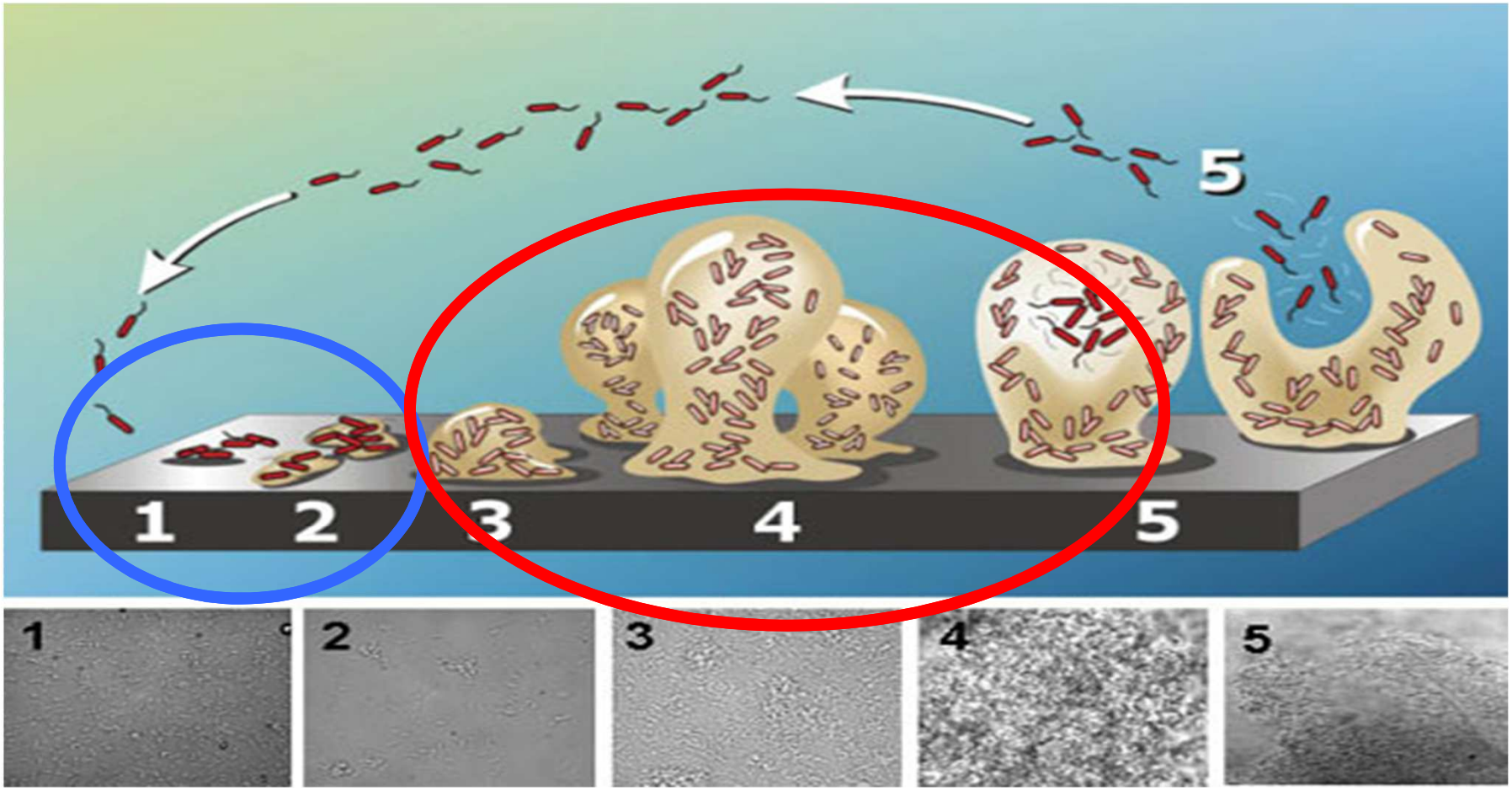
Which virulence mechanisms were impacted by the switch from VSSA to VISA ?



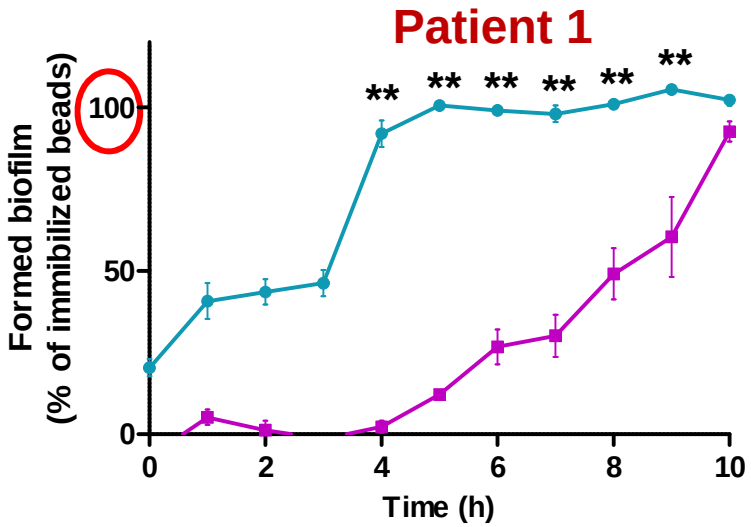
From VSSA to VISA: which impact on biofilm formation?

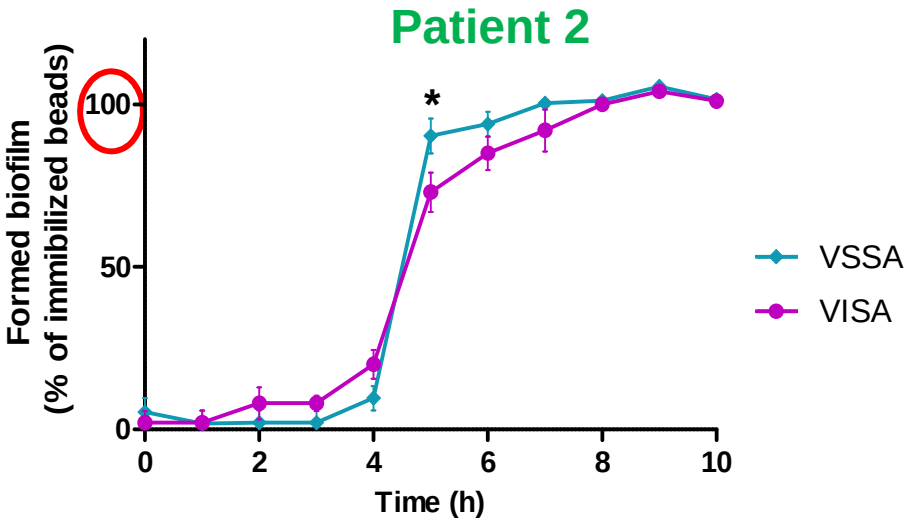
Adhesion to materials
Early biofilm

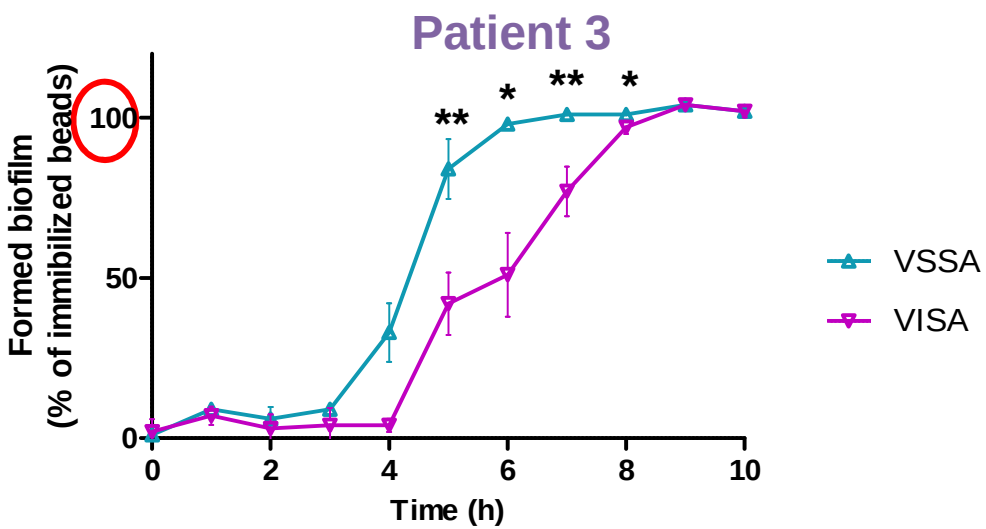
Mature
biofilm



From VSSA to VISA: which impact on biofilm formation?







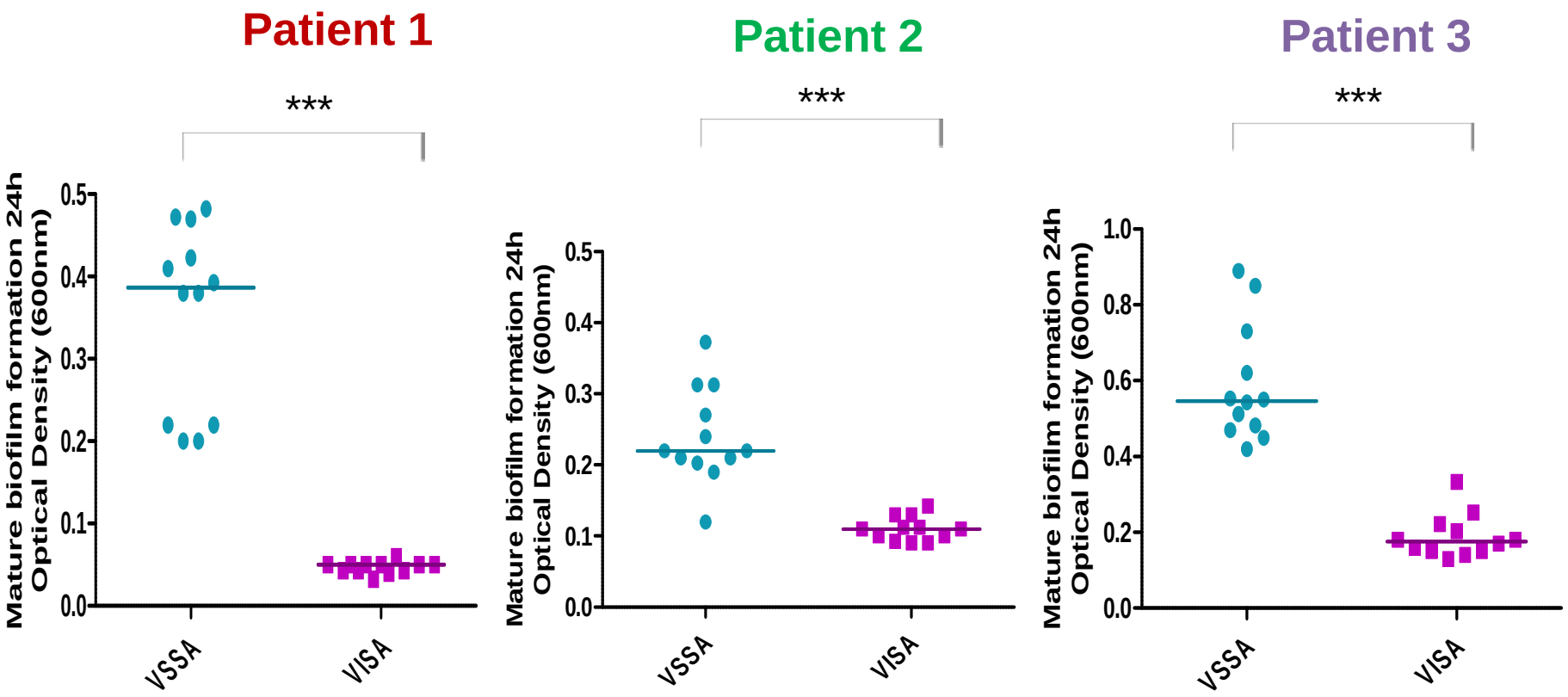
Early biofilm
First hours

3 experiments performed in duplicate
Mann-Whitney test bilateral(a=0.05)
(*p<0.05, **p<0.01, ***p<0.001)

Initiation of biofilm is **slower in VISA** than in VSSA
Variability from one patient to another

From VSSA to VISA: which impact on biofilm formation?

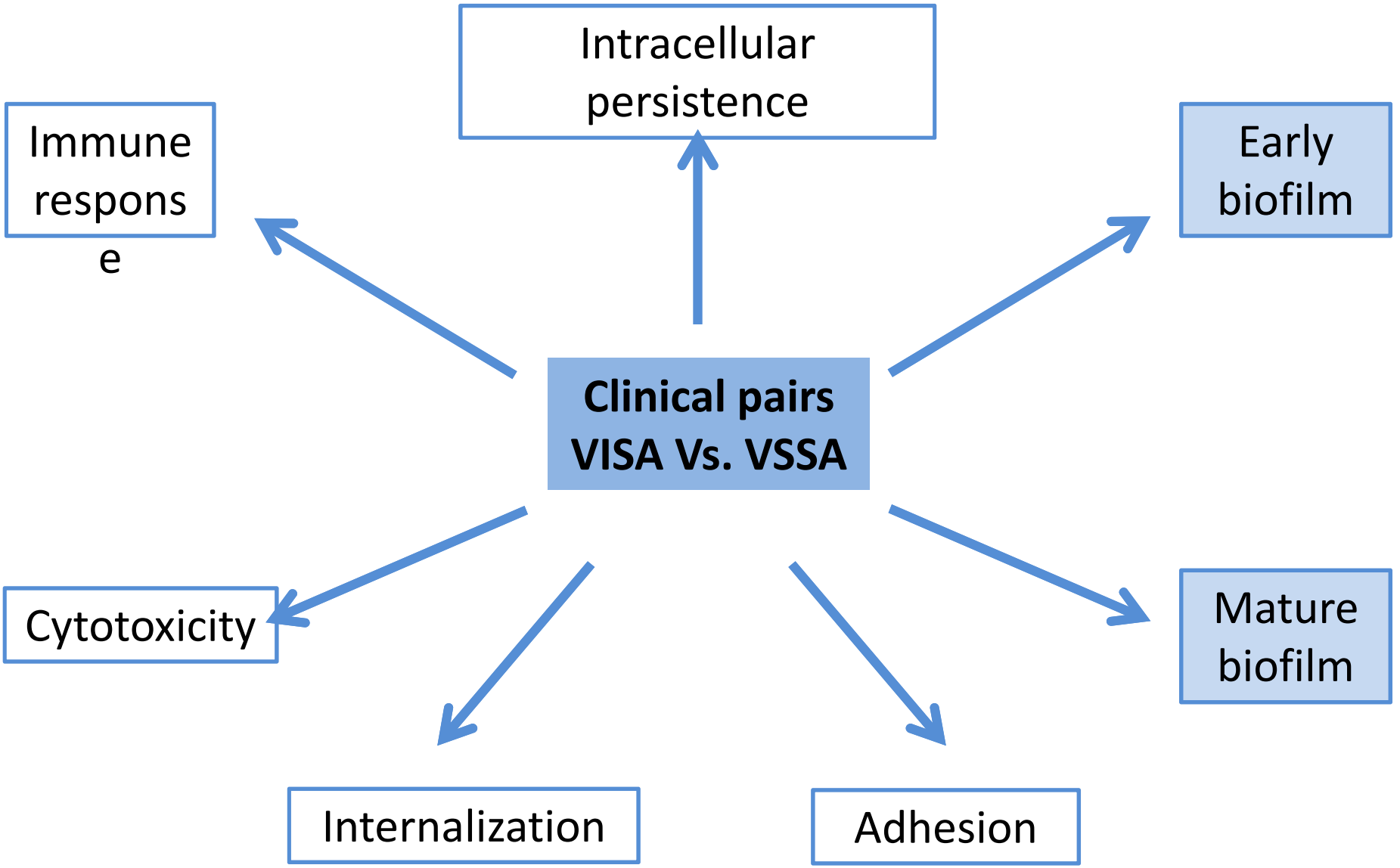
Mature biofilm
Afetr 48h



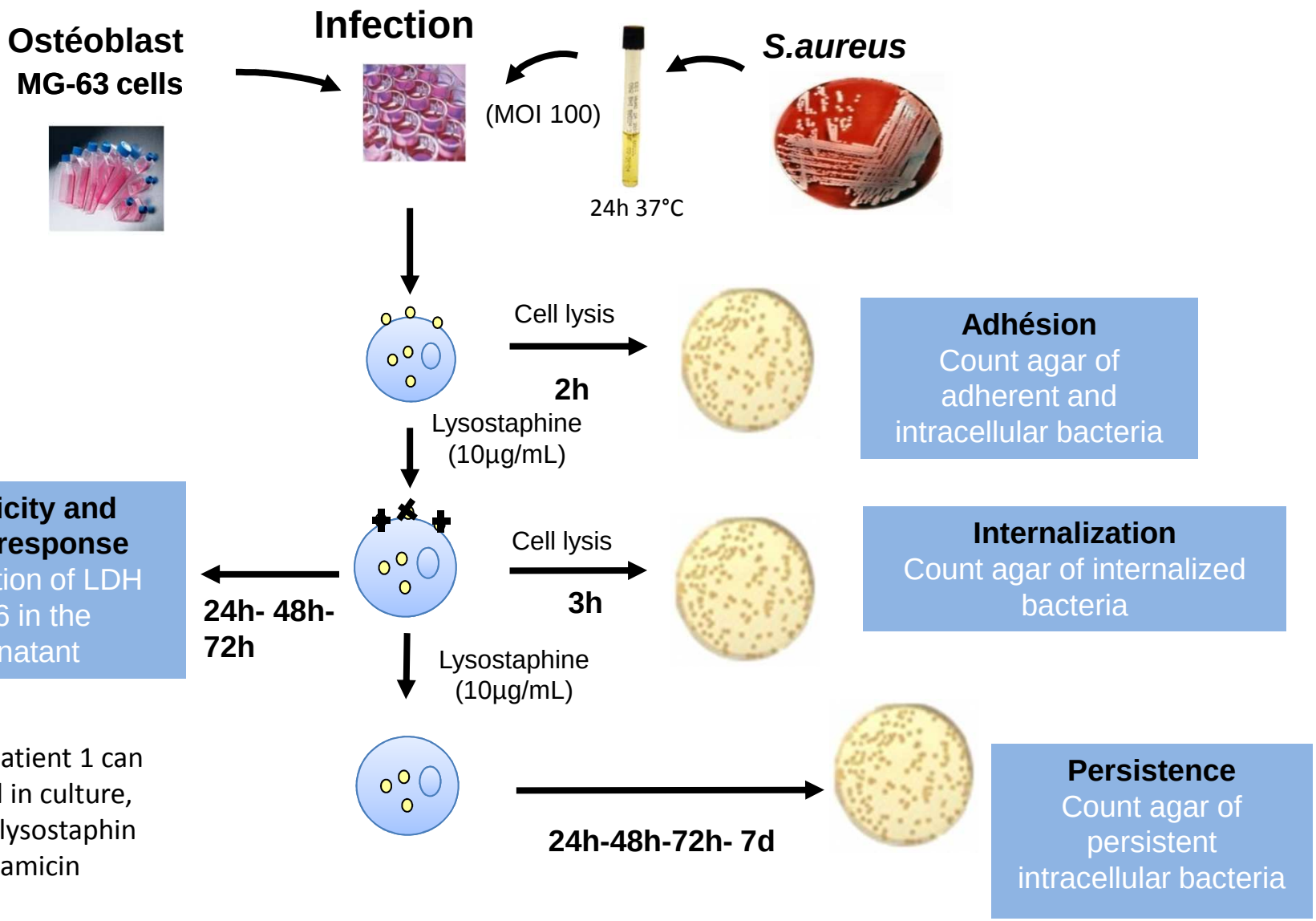
VISA forms **less mature biofilm** than VSSA

3 experiences in quadriplicata
Mann-Whitney test bilateral (a=0.05)
(*p<0.05, **p<0.01, ***p<0.001)

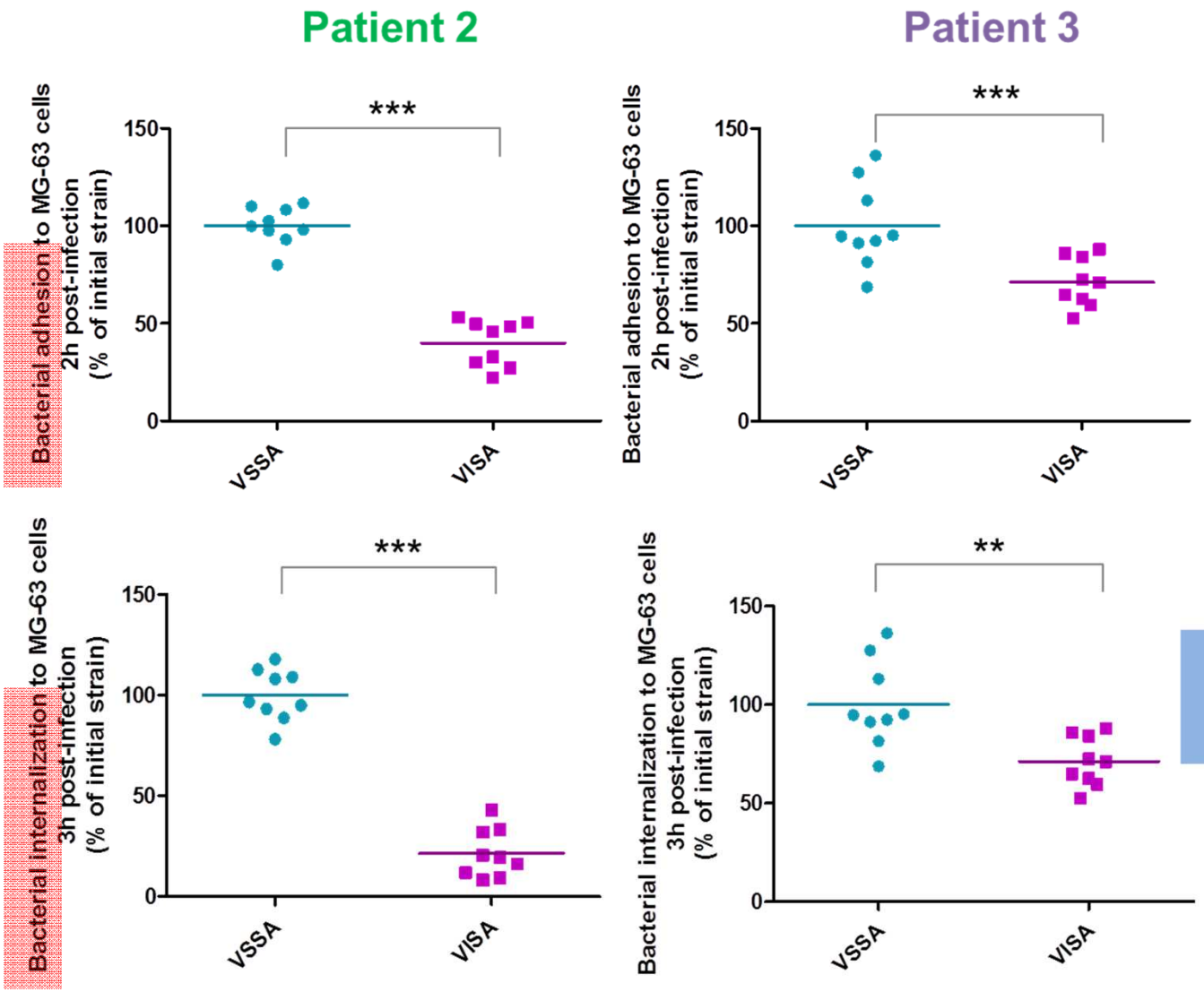
Which virulence mechanisms were impacted by the switch from VSSA to VISA ?



Infection in vitro on osteoblast MG-63 (on pairs 2 and 3)



From VSSA to VISA: which impact on adhesion/internalization?

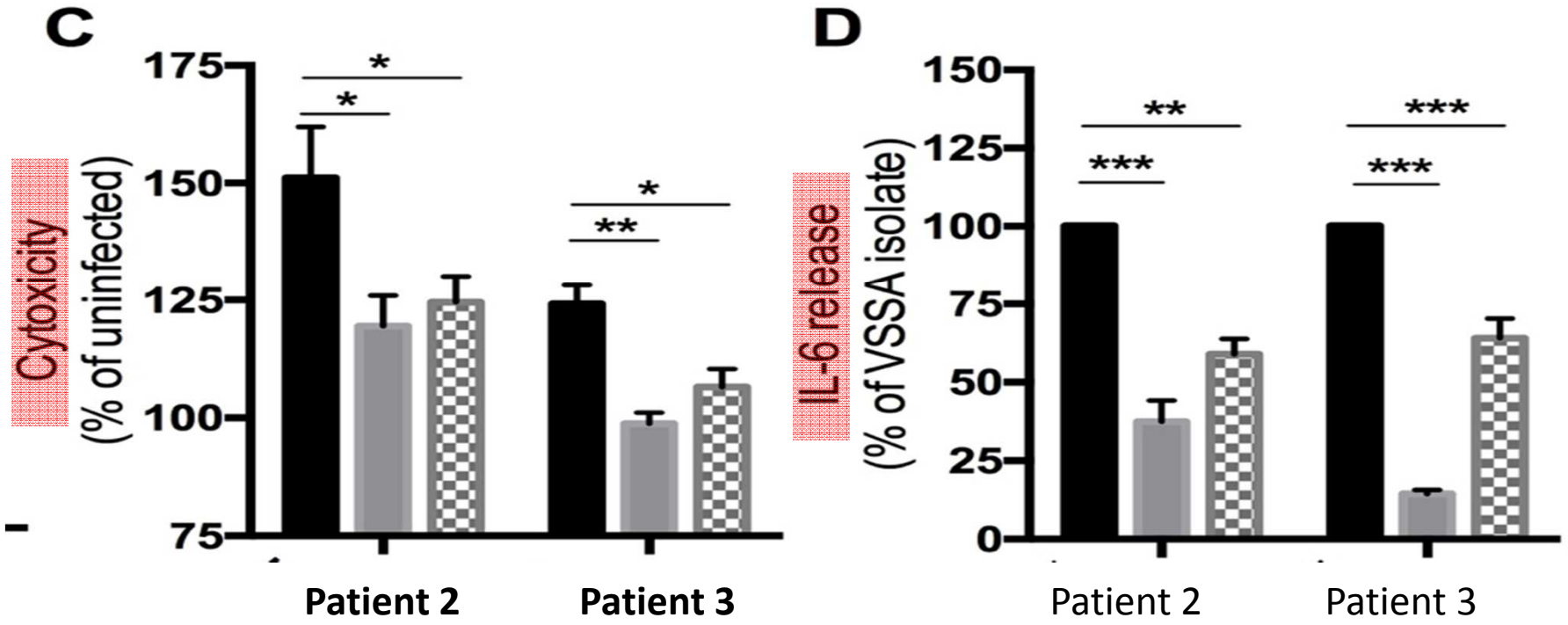


Significant Lower adhesion of VISA

Significant lower internalization of VISA

From VSSA to VISA: which impact on cytotoxicity/immune response?

VSSA VISA VISA with adjusted inoculum

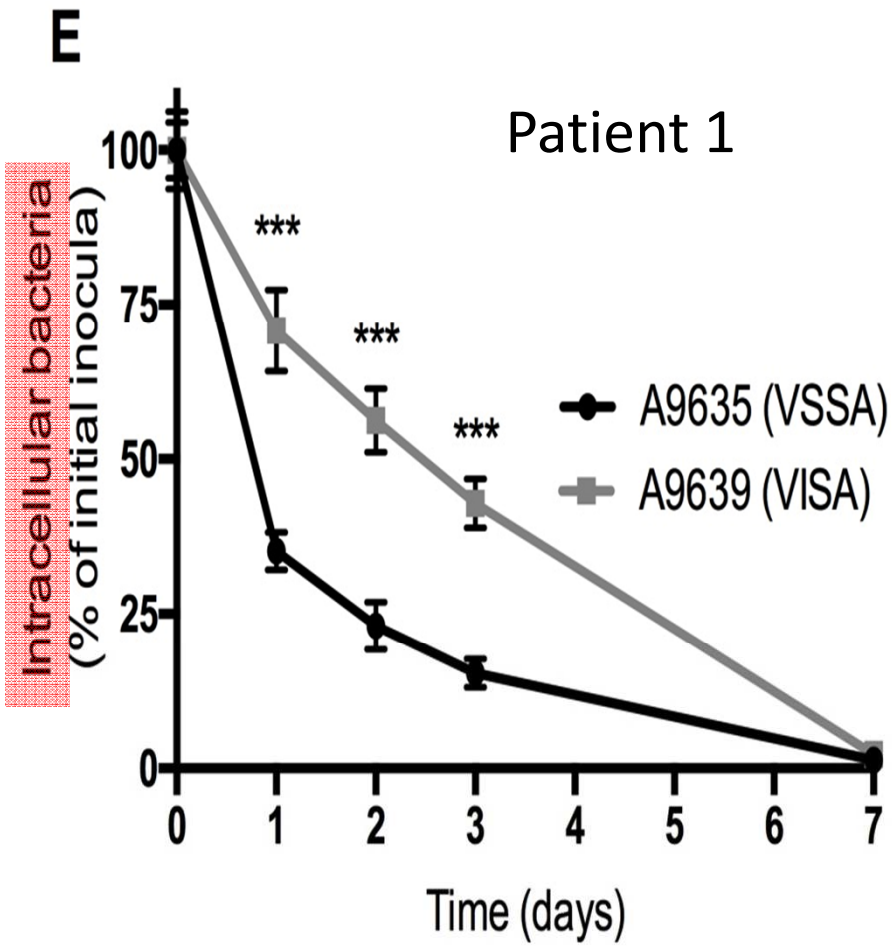


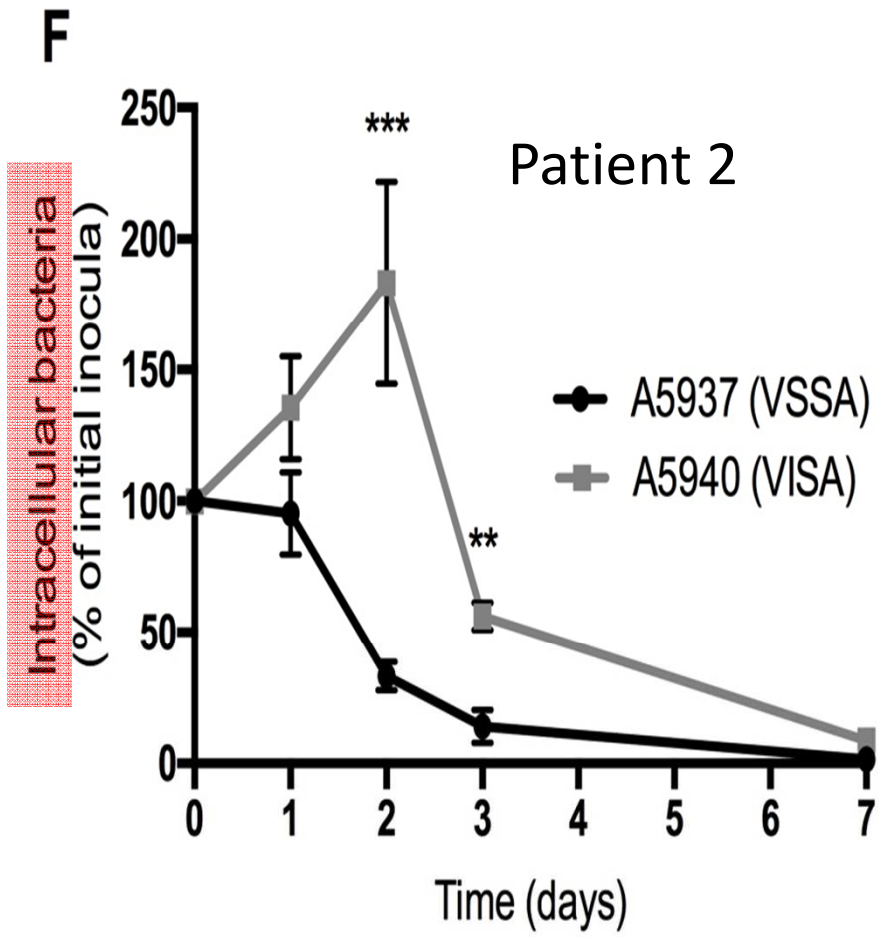
- Perforated columns = VISA using adjusted MOI to reach the same number of internalized CFU in cells to prevent biais due to decreased internalization

3 experiments performed in triplicate
Mann-Whitney test bilateral (a=0.05)
(*p<0.05, **p<0.01, ***p<0.001)

VISA are **less** cytotoxic than VSSA
VISA induce a **decreased** osteoblast inflammatory response

From VSSA to VISA: which impact on persitence?





3 experiments performed in triplicate
Mann-Whitney test bilateral (a=0.05)
(*p<0.05, **p<0.01, ***p<0.001)

VISA persist **longer** in the intracellular compartment

Which virulence mechanisms were impacted by the switch from VSSA to VISA ?

✓ VISA = confirms remarkable adaptability of *S. aureus*

- **reduced** antibiotic susceptibility
- **alteration** of the expression of pathogenic factors to circumvent the host-immune response

… to favour intracellular persistence over acute virulence.

✓ intriguing decrease of biofilm formation, adhesion and internalization biofilm is not the way of persistence for VISA

- adhesion and internalization likely impact by the modification of bacterial wall related to vancomycin resistance in VISA

✓ Take home ”clinical” message for VISA

- VISA = intracellular sanctuarization = no place for vancomycin: use antibiotic with very good intracellular penetration
- peristent *S. aureus* BJI = bacteria to be tested for VISA which need very specific methods = contact your ”favorite” microbiologist

Research for understanding BJI

Fundamental research, clinical trials, clinical studies, …

Management of patients suffering with BJI

Orthopaedic surgeons, ID clinicians, microbiologists, radiologists, histopathologists, …

Centre International de Recherche en Infectiologie

CRIOAc LYON

Hospices Civils de Lyon

Céline DUPIEUX
Jason TASSE
Jean-Philippe RASIGADE
Yousef MAALI
Sacha FLAMMIER
Virginie TAFANI
William MOUTON
Régis VILLET
…

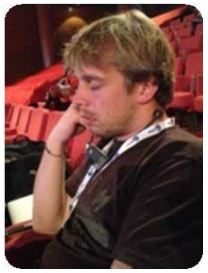
STAPHYLOCOQUES Centre National de Référence

Hospices Civils de Lyon

Sophie ASSANT-TROUILLET

Pr. Francois VANDENESCH
Pr. Jérome ETIENNE
Pr. Gérard LINA
Dr. Anne TRISTAN
Dr. Michèle BES
Dr. Olivier DAUWALDER
Dr. Oana DUMITRESCU
Dr. Hélène MEUGNIER

Pr. Tristan FERRY



Dr. Florent VALOUR

MONASH University

Pr Sébastien LUSTIG

Patricia SIMOES

Anton Peleg

David Cameron

Benjamin Howden