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Intracytoplasmic compartment of host cells: a bacterial reservoir for Vancomycin-Intermediate *Staphylococcus aureus* isolates

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Introduction

Vancomycin-intermediate *Staphylococcus aureus* **(VISA)** was associated with **persistent infection** and **treatment failure**. To date, two staphylococcal virulence mechanisms have been associated with chronicization and therapeutic failure, leading to host immune system evasion and antibiotic activity protection: i) **bacterial internalization** in non-professional phagocytic cells and ii) **biofilm formation**. The present study aimed **to compare paired clinical isolates** composed by VISA and their Vancomycin-Susceptible (VSSA) progenitors toward these **bacterial adaptive mechanisms**.

Materials And Methods

Three pairs of VSSA/VISA clinical isolates (A5937/A5940, A9635/A9639, A8090/A8094) have been isolated from persistent bloodstream infections during prolonged antibiotic therapy. Clinical pairs were compared for different features : **i) biofilm formation ability** using the crystal violet staining method and the Biofilm RingTest™, **ii) cytotoxicity and immune response** by quantifying lactate dehydrogenase (LDH) (Siemens) and interleukin (IL)-6 release (Elisa) and **iii) intracellular bacterial persistence** (plate counting) using *in vitro* "lysostaphin protection" infection model of human osteoblasts MG-63 (only performed on the two lysostaphin-susceptible isolates).

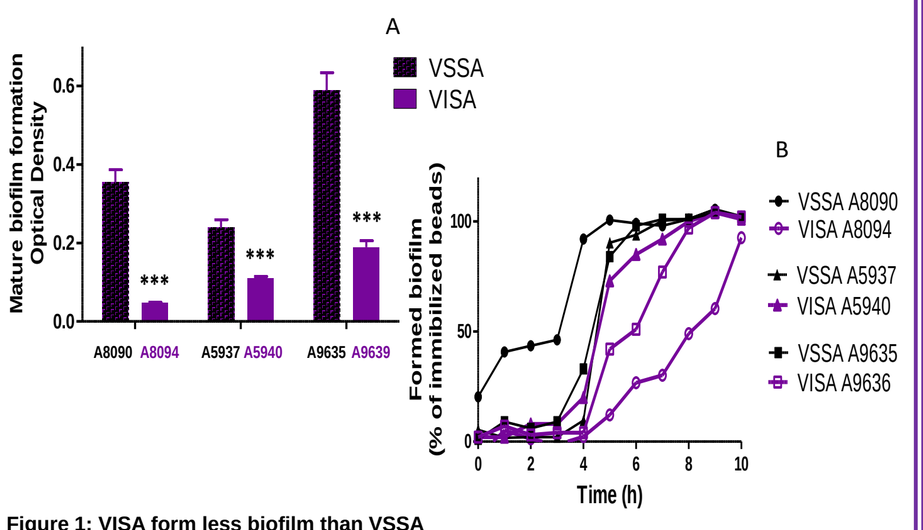

Figure 1: VISA form less biofilm than VSSA

(A) Mature biofilm formation was quantified using the tissue culture plate method with crystal violet. Bars represent the mean +/-SEM of three experiments realized in quadruplicate. All of the P-values were calculated using the Mann-Whitney test. (B) Kinetics of early biofilm formation was assayed by the Biofilm Ring Test method for VISA and VSSA strains.

Results

The two isolates (VSSA vs VISA) within each clinical pair were compared:

For biofilm formation ability:

- **Crystal violet staining method** after 24h or 48h of incubation revealed that VISA isolates **formed significantly less mature biofilms** than VSSA ($p<0.001$ for all pairs).
- Using the **Biofilm RingTest™**, VISA isolates required more time to immobilize magnetic beads than VSSA, reflecting **longer biofilm-forming ability**.

For intracellular behavior:

- VISA strains harbored a **lower capacity to adhere and to invade osteoblasts**, compared to VSSA.
- The **percentage of intracellular bacteria** recovered after 14 days post-infection was always **significantly greater in VISA**- than VSSA-infected wells ($p<0.01$ for all) reflecting a **higher intracellular persistence ability**.
- The **IL-6** and **LDH** released from the osteoblasts infected with VISA strains were **significantly lower** than those from the cells infected with VSSA strains within each pair ($p<0.01$ for all).
- **To avoid a bias related to a lower internalization capacity of VISA strains in cells**, we added an experimental condition to obtain the same number of intracellular bacteria after the invasion step for VSSA and VISA strains.

➢ We showed that VISA strains **persisted longer** in the **intracellular compartment**; induced a **lower cytotoxicity** and a **lower inflammatory response**, compared to VSSA counterpart.

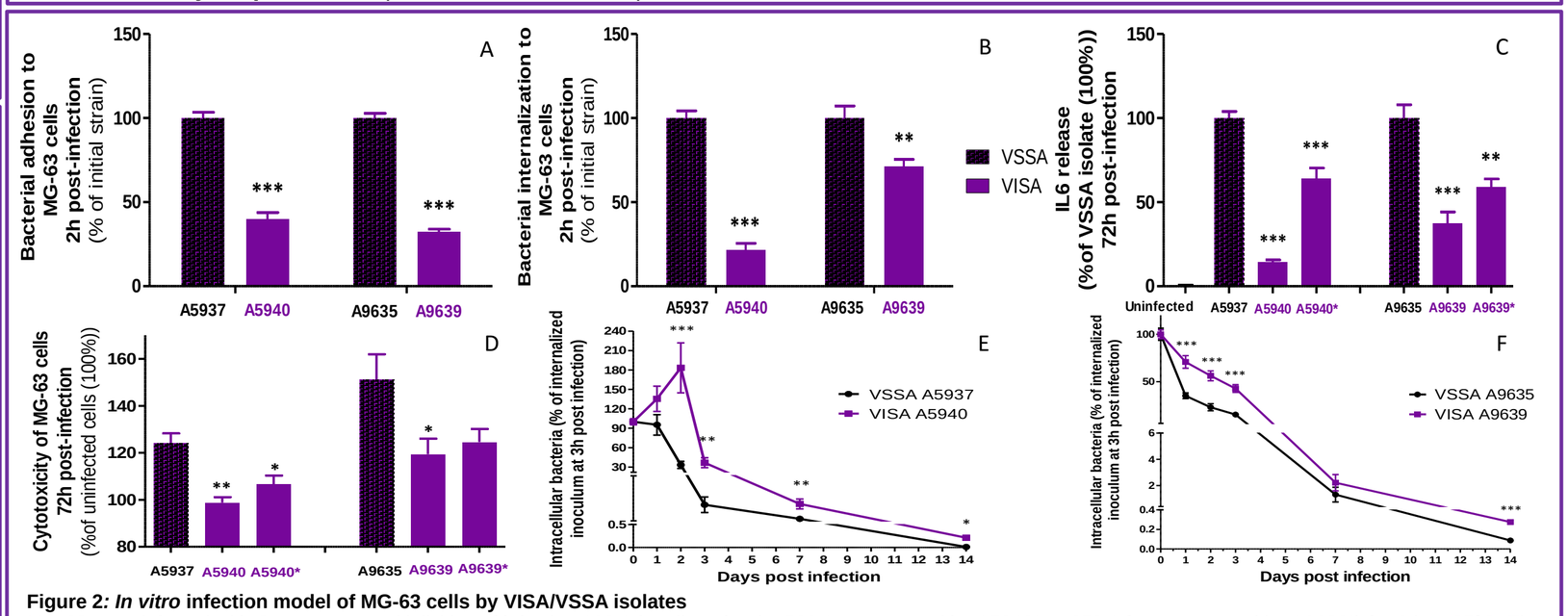

Figure 2*: In vitro* infection model of MG-63 cells by VISA/VSSA isolates

MG-63 were infected with S. aureus at multiplicities of infection 100:1 for all strains (or 500:1 represented by A5940* or 250:1 for represented by A9639*) for 2 h at 37°C. The adhesion levels to MG-63 were estimated at 2 h post-infection (A). The invasion capacities were assessed by quantifying the viable intracellular bacterial loads at 3 h post-infection after lysostaphin treatment (B). Quantification of IL-6 (C) and LDH, reflecting cytotoxicity (D) were performed on culture supernatant at 72h post infection. A kinetic of intracellular bacterial persistence capacities was performed (E-F). All of the results are expressed as percentages of the values obtained for the initial strains. The horizontal bars denote the means derived from three independent experiments conducted in triplicate. The statistical analyses were performed using the Mann-Whitney test.

Conclusions

Our results suggest that **VISA** are **better adapted to the intracellular compartment than VSSA.** This could contribute the enhanced **formation of an intracytoplasmic bacterial reservoir for VISA** and so could explain the chronicity and the persistence observed during infection caused by VISA.