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) cytolysis 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).

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 intracellular 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.

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.