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[O122] ADAPTATION OF VANCOMYCIN-INTERMEDIATE STAPHYLOCOCCUS AUREUS TO INTRACELLULAR COMPARTMENT LEADING TO BACTERIAL RESERVOIR RESPONSIBLE FOR CHRONIC INFECTION

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

Method: Methods: Three pairs of VSSA/VISA clinical isolates 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 (mature biofilm) and the Biofilm test based on measurement of superparamagnetic microbeads mobility trapped by biofilm (early biofilm), ii) cytotoxicity and immune response by quantifying lactate dehydrogenase (LDH) and Interleukin(IL)-6 release and iii) intracellular bacterial persistence using in vitro "lysostaphin protection" infection model of human osteoblasts.

Results: Comparing between individual pairs, the 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). In addition, using the Biofilm test*, VISA isolates required more time to immobilize magnetic beads than VSSA, reflecting delayed early biofilm-forming ability. For instance, the number of beads immobilized by VISA isolates composing pair 1, 2 and 3 was 8.29-, 1.23- and 1.91-fold lower than VSSA parental isolates respectively ($p<0.05$ for all).
The two lysostaphin-susceptible pairs tested in the in vitro infection model revealed that VISA strains harbored a lower capacity to adhere to and invade osteoblasts, compared to VSSA. Regardless of the time post-infection (up to 14 days post-infection), the percentage of intracellular bacteria recovered after host cells lysis 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). These results were consistent even after adjusting for the number of intracellular bacteria between the VSSA and VISA pairs.

Conclusions: Our results suggest that once internalized, VISA were well-adapted to the intracellular compartment, which led to the formation of an intracytoplasmic bacterial reservoir that could explain the chronicity and the persistence observed during infection caused by VISA.
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