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O1136 Sb-1 bacteriophage for treatment of methicillin-resistant *Staphylococcus aureus* biofilm: administration in a *Galleria mellonella* model of implant-associated infection

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Background: Implant-associated infections (IAIs) are difficult to treat due to the involvement of drug-tolerant biofilm-associated microorganisms. Previously, we showed that Sb-1 bacteriophage eradicated biofilm-embedded methicillin-resistant *Staphylococcus aureus* (MRSA) *in vitro* when tested in combination with antibiotics. Here, we investigated the efficacy of Sb-1, alone and in combination with daptomycin, to reduce MRSA biofilm in a *Galleria mellonella* model of IAI.

Materials/methods: The stability of Sb-1 in *G. mellonella* larvae was investigated by injecting 10^8 PFU and evaluating the presence of phages in haemolymph at different time points by plaque assay. For infection experiments, sterile stainless-steel K-wires (4 mm, 0.6 mm Ø) were implanted into the last proleg. After 2 days, larvae were infected with MRSA ATCC 43300 ($1X10^5$ CFU) and incubated at 37 °C for further 2 days. Implanted larvae were treated 3X/day with 10µL of Sb-1 (10^7 PFU), Daptomycin (4mg/kg), PBS(24h)/Daptomycin(24h) and Sb-1(24h)/Daptomycin(24h). An untreated control was also added. Either 2 days post-infection or post-treatment, larvae were dissected for K-wire explanting and the material was sonicated and plated for colony counting. Biofilm on K-wires was detected by scanning electron microscopy (SEM).

Results: Sb-1 treatment resulted in a reduction of PFU number after 12 h administration in haemolymph of *G. mellonella* larvae (Figure 1A). Two days post-infection of K-wire implanted larvae, ≈$2x10^7$ CFU/ml MRSA were found on the material. K-wires from larvae treated with Sb-1 or Daptomycin showed a *S. aureus* CFU/ml reduction of 4log compared to the CFU/ml values of the untreated control (Figure 1B). The staggered administration Sb-1/Daptomycin determined higher CFU reduction (≈ 5.5 log) (Figure 1B). SEM analysis confirmed CFU counting data.

Conclusions: As Sb-1 is stable in haemolymph for 8h, a 3X/day administration resulted in an effective treatment. Sequential combination of Sb-1 and Daptomycin strongly reduced biofilm formed on K-wire implanted in larvae. *G. mellonella* represents a useful *in vivo* model to study the biofilm formation on implanted materials and alternative treatment options.

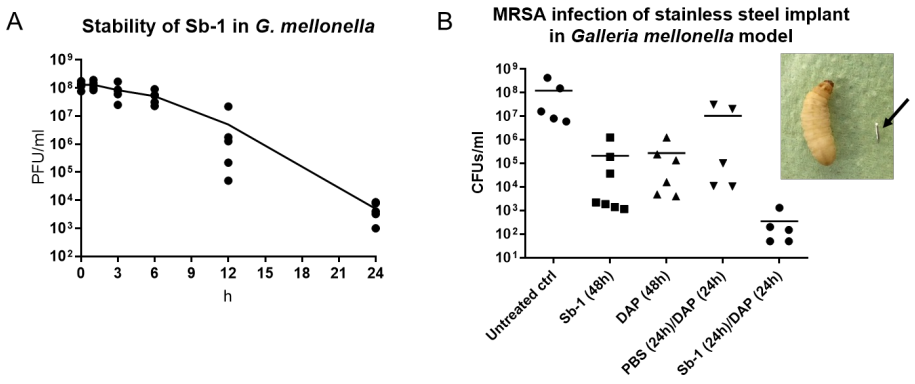

Figure1. A) Stability of Sb-1 in haemolymph of *G. mellonella*. B) CFU/ml number of *S. aureus* from K-wires-implanted larvae treated with different combination of phage and daptomycin (DAP). Black arrow indicates a K-wire.