**Abstract:**

Immunization with planktonic and biofilm *Staphylococcus aureus* antigens prevents peritoneal abscesses and osteomyelitis.

**Author Block:**

J. M. Harro¹, Y. Achermann², M. E. Shirtliff¹; ¹University of Maryland, Baltimore Dental School, Baltimore, MD, ²University Hospital of Zurich, University of Zurich, Zurich, SWITZERLAND.

**Background:** *Staphylococcus aureus* causes a wide range of acute and chronic diseases, which result in 340,000 hospitalizations and 50,000 deaths annually in the United States. New therapeutics are essential due to the increased incidence of antibiotic-resistant S. aureus isolates as well as the hampered resolution of infections by the host immune system or antimicrobial agents due to biofilm growth. We previously observed that in a rabbit osteomyelitis challenge model, an anti-staphylococcal, biofilm-specific quadrivalent vaccine coupled with adjunctive vancomycin treatment eliminated *S. aureus* burden in 87.5% of rabbits compared to 55.6% or 22.2% of the antibiotic-treated or vaccinated rabbits, respectively. We proposed that incorporation of a planktonic-specific antigen, a lipoprotein homologous to SACOL0119, could supplant antibiotic therapy needed to target the planktonic population within a chronic infection.

**Methods:** Vaccine efficacy of the pentavalent vaccine was evaluated in the murine peritoneal abscess model and the rabbit osteomyelitis model. BALB/c mice were immunized with 12.5 μg/biofilm antigen and 25 μg/planktonic antigen at 35 and 21 days prior to intraperitoneal injection of 3.5 × 10⁸ CFUs or 1 × 10⁹ CFUs of *S. aureus*. Animals were monitored for mortality for 21 days and then *S. aureus* counts in peritoneal abscesses were enumerated from surviving mice. New Zealand white rabbits were immunized with 75 μg/antigen at 20 and 10 days prior to intramedullary injection of 3.0 × 10⁶ CFUs of *S. aureus*. At 20 days post-infection, tibias were assessed for the severity of bone disruption and *S. aureus* counts in the tibias were enumerated. Protective efficacy of sera IgG elicited in response to the antigens was also evaluated in the murine model during challenge with 5-8 × 10⁸ CFUs of *S. aureus*. Results: BALB/c mice had a significant reduction in mortality of immunized versus non-immunized mice (16.7% vs 91.6%, p<0.001) or (37.5% vs 87.5%, p<0.05) following challenge with 3.5 × 10⁸ CFUs or 1 × 10⁹ CFUs of *S. aureus*, respectively. While bacterial persistence was observed after challenge with the higher dose, *S. aureus* was eliminated from 66.7% of the immunized mice versus 8.3% of the control animals after challenge with 3.5 x 10⁸ CFUs (p<0.05). Similarly, we found *S. aureus* was eliminated from 62.5% of the immunized rabbits versus none of the non-immunized animals (p<0.05). Passively immunized BALB/c mice also had a significant reduction in mortality (0% vs 63.6%, p<0.05) and increased clearance of *S. aureus* (33.3% vs 0%) compared to non-immunized cohorts. **Conclusions:** Data from our vaccine studies demonstrate that targeting both the planktonic and biofilm phenotypes of *S. aureus* biofilm generates an immune response capable of eradicating a *S. aureus* biofilm-infection. Furthermore, sera IgG provides protection from mortality and reduces bacterial burden in the absence of activated immune T cells.