

## Evaluating *Staphylococcus aureus* infection dynamics in a rat joint PJI model

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**Disclosures:** Amita Sekar (N), Peyton Tierney (N), Madeline McCanne (N), Parker Jones (N) Yingfang Fan (N) Devika Dutta Kannambadi (N), Keita Fujino (N) Orhun Muratoglu (Exactech, Stryker, Corin, Mako, Iconacy, Renovis, ConforMIS, Meril Healthcare, Kyocera, Arthrex, Ceramtec, Alchemist, Cambridge Polymer Group, Orthopedic Technology Group), Ebru Oral (1-Corin, Iconacy, Renovis, Arthrex, ConforMIS, Meril Healthcare, Exactech; 3B – WL Gore & Assoc; 7/8 – JBMR; 9 – SFB, ISTA)

**INTRODUCTION:** Periprosthetic joint infections are a growing problem linked predominantly to *Staphylococcus aureus*<sup>[1]</sup>. Infection severity is increased by biofilm formation that aids the bacteria in evading host immune responses and resisting antibiotic treatments<sup>[2]</sup>. Clinically, biofilms have been strongly correlated to poor eradication and PJI recurrence, further emphasizing the need to determine in-vivo biofilm properties<sup>[3]</sup>. Multiple in vivo animal models have been developed to simulate PJI and study treatments. However, the nature of biofilm growth, microbial physiology, and antibiotic susceptibility profiles in PJI are not well understood. Previously, we developed a rat model of implant-associated *S. aureus* joint infection<sup>[4]</sup>. In this present study, we aim to understand infection dynamics in our joint model using a gentamicin-susceptible ‘low-risk’ strain and a gentamicin-resistant ‘high-risk’ strain of *S. aureus*.

**METHODS:** Control strain *Staphylococcus* ATCC 12600 (Methicillin-susceptible SA) and clinical strain L1101 (Methicillin-resistant SA) were used to establish a ‘low-risk and ‘high-risk’ PJI, respectively. Male Sprague Dawley rats (n=18) were assigned to ‘low-risk (n=3 per day) and ‘high-risk’ (n=3 per day) infection groups, and 10<sup>8</sup> CFU of bacteria were inoculated into the tibia before being implanted with stainless steel screws. The screws and peri-implant tissue (femoral and tibial) were harvested at postoperative day (POD) 1,3 and 7 to determine bacterial viability, gentamicin susceptibility, and bacterial gene expression. The screws and tissue samples were sonicated (40 mins) and homogenized respectively in PBS, and bacterial viability was determined using the plate count method. The gentamicin susceptibility of screw-adherent and tissue-colonized bacteria was evaluated by exposing the samples to a range of gentamicin concentrations (0,10,50, 100,300,500 µg/mL) and observing viability using the plate count method. Samples from no infection control (NIC) rats served as controls. Subsequently, RNA was extracted from the peri-implant tissues, and the relative gene expression profiles of bacterial genes associated with stress response (*vraR*), biofilm production (*icaA*, *icaD*), and adhesion (*ebpS*) were determined using the  $\Delta\Delta C_q$  method.

**RESULTS SECTION:** In the ‘low risk’(MSSA) infection model, the screw-adherent bacterial viability was consistent (~10<sup>7</sup> CFU/mL) for POD 1,3 and 7. In contrast, in the ‘high-risk’ (MRSA) infection model, high bacterial viability (~10<sup>7</sup> CFU/mL) was observed only at POD 1, which was reduced and stabilized (~10<sup>6</sup> CFU/mL) by POD 7. The viable bacteria count in femoral and tibial tissue was consistently high (10<sup>6</sup>-10<sup>7</sup> CFU/mg) for the entire period of the study (Fig 1). The viability of screw-adherent MSSA was slightly affected at a significantly low concentration of gentamicin (10µg/mL, ~1-2.5 log reduction) when compared to MRSA, for which viability was not affected even at the highest concentration of gentamicin tested (>500µg/mL) across all time points tested. On the other hand, the viability of tissue-colonized MSSA was significantly affected (>3log reduction) at a lower gentamicin concentration (50µg/mL) when compared to tissue-colonized MRSA from POD 1 and 3, which showed high viability across the gentamicin concentration range tested until POD 7 where the viability was reduced (~3log reduction) in the presence 300µg/mL gentamicin. Gene expression analysis for ‘low-risk’ MSSA showed a steadily increasing expression for all genes (+1-3log fold change) over seven days. For the ‘high-risk’ MRSA, the relative gene expression was significantly altered at POD7 (*ebpS* and *icaD*) and slightly altered (*vraR* and *icaA*, ~≤0.5log fold change) at POD 1,3 and 7 (Fig 2).

**DISCUSSION:** We have established an in-vivo ‘low-risk and ‘high-risk *S. aureus* PJI model. The study revealed biofilm growth dynamics for MSSA and MRSA with comparable viable bacteria recovered from both the implant surface and surrounding tissue, indicating no preferential colonization. However, there was a distinct difference in the gentamicin susceptibility profiles of screw-adherent bacteria vs. tissue-colonized bacteria, which strongly suggested the crucial role of biofilm properties and the environment (implant vs. tissue) in determining treatment outcomes. The preliminary gene expression data also revealed marked differences in the MSSA and MRSA’s response to the host environment, emphasizing the importance of having infection risk and severity assessment as tools to aid treatment decisions.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Understanding the infection characteristics using robust preclinical models will aid in defining realistic ranges of antibacterial activity and in devising effective treatment strategies against PJI.

**REFERENCES:** 1. Liukkonen RJ et al, J Arthroplasty. 2023 2. Cangui-Panchi SP et al, Curr Res Immunol. 2023; 3. Svensson Malchau K et al, Orthop Translat. 2021; 4. Fan et al, J Orthop Res, 2020

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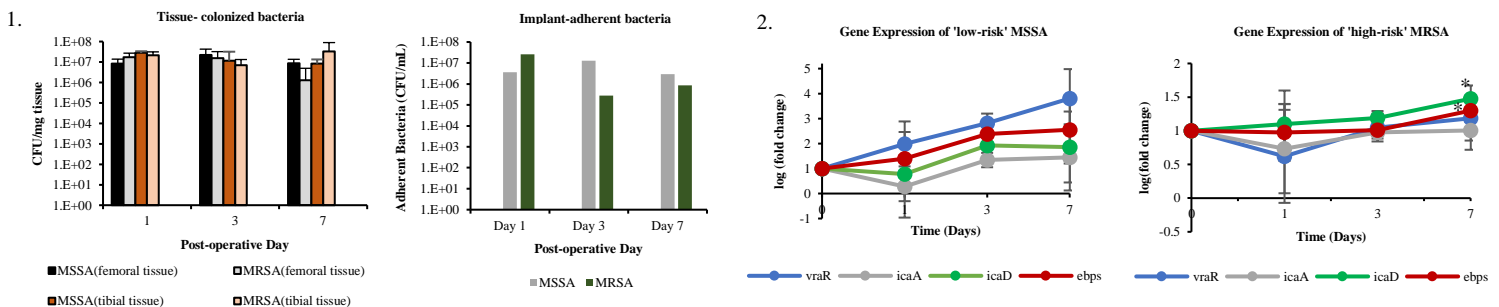


Fig 1. The viability of tissue-colonized and implant-adherent MSSA and MRSA for POD1, 3, and 7. Error bars represent standard deviation (n=3).

Fig 2. Relative gene expression of bacterial genes *vraR*, *icaA*, *icaD* and *ebpS* of tissue colonized MSSA and MRSA at POD1, 3 and 7. The  $\Delta\Delta C_q$  was calculated by normalizing the expression to housekeeping gene *16sRNA* and to planktonic *S. aureus* gene expression to determine the log fold change. The error bars represent standard deviation (n=3). Wilcoxon rank sum test was performed and \* indicates p-value ≤ 0.1.