Characterization of an *in vitro* polymicrobial peri-prosthetic infection (PJI) model

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INTRODUCTION: PJI is a devastating complication associated with total joint replacement with over 25% of cases being reported as polymicrobial infections [1]. Gram-positive and gram-negative bacteria co-exist in biofilms and use complex interactions to regulate each other's physiology, virulence, and drug resistance [2]. These factors increase the severity of PJI leading to poor clinical outcomes [3]. *Staphylococcus aureus*, which is the predominant causative organism, is known to be accompanied by members of Enterobacteriaceae including *Escherichia coli* in a polymicrobial PJI [4,5]. However, not much is known about their infection dynamics which could aid in developing therapeutic approaches that are effective against polymicrobial PJI. In this present study, we aim to characterize an in-vitro poly-microbial infection and develop a robust model for testing PJI therapeutic strategies.

METHODS: 10^5 CFU/mL each (1:1 ratio) of *S. aureus (*ATCC 12600) and *E. coli* (ATCC 25922) were grown on 316L stainless steel (SS) plates (10mm × 3mm ×1mm) immersed in 1mL of tryptic soy broth for a period of 6, 24 and 48 hours. The biofilm dynamics of each bacterial species were determined by rinsing the plate-adhered biofilms with sterile 1 × PBS (3 times) and sonicating in 1mL PBS to dislodge the biofilm. The adherent bacteria viability for *S. aureus* and *E. coli* was determined using selective and differential Mannitol salt agar and MacConkey agar respectively via the spread plate method. Subsequently, the biofilms were also subjected to fluorescent gram staining to visualize, differentiate, and quantify the bacterial populations using fluorescence microscopy. To understand the susceptibility profiles of both strains for broad-spectrum antibiotic gentamicin, minimum inhibitory concentration (MIC) tests were performed according to CLSI protocol (M07-A10). The effect of co-existence on the susceptibility of *S. aureus* and *E. coli*, in biofilm status, to gentamicin was determined by exposing dual-species biofilms to a range of gentamicin (broad-spectrum) concentrations for a period of 24 hours. Following drug exposure, the bacterial viability was assessed using the colony count method, and minimum biofilm eradication concentration (MBEC) for gentamicin was determined for both *S. aureus* and *E. coli* using differential agar. Mono-species biofilms grown on SS plates served as controls for all experiments.

RESULTS SECTION: In a 6-hour dual-species biofilm, the SA viability was not altered in the presence of EC and was comparable to the viability observed from a mono-species SA biofilm. However, in a 24-hour dual-species biofilm, the SA viability was significantly reduced (~3 log) in the presence of EC when compared to the mono-species SA biofilm viability. The EC showed overall poor adherent bacteria viability at 6 hours and comparable viable adherent bacteria count at 24 hours regardless of the presence of SA (Fig 1). The fluorescent gram staining revealed distinct cocci and rod-shaped morphology for SA and EC respectively. The gram stain-based quantification of the images also supported the bacterial viability data. The MIC for gentamicin was 0.5±2 (SA) and 0.25±2 µg/mL (EC). The MBEC gentamicin for SA, in the presence of EC, was significantly reduced in both 6-hour and 24-hour biofilms when compared to MBEC for SA alone (Fig 2). On the other hand, the MBEC for EC, in the presence of SA, was reduced only slightly in 24-hour biofilms with no viable bacteria observed for 6-hour biofilms due to poor initial adherence.

DISCUSSION: We have determined the feasibility of an implant material-based in-vitro polymicrobial PJI infection model by co-culturing gram-positive SA and gram-negative EC on stainless steel plates. The study showed that the co-existence of two species in a biofilm setting significantly altered the biofilm growth dynamics and morphology for SA. The data also indicated significant regulation of gentamicin susceptibility profiles for both SA and EC in a polymicrobial infection, which provides valuable information on the effect of co-existence in modifying bacterial characteristics. These preliminary findings reveal more information about the infection dynamics in a polymicrobial infection and aid in the development of a robust in-vitro polymicrobial PJI model for testing effective therapeutic strategies.

SIGNIFICANCE/CLINICAL RELEVANCE: Polymicrobial PJI is a complex complication that warrants novel treatment strategies for effective eradication. Understanding polymicrobial infections using robust models would enable us to devise effective eradication strategies thereby reducing the morbidity and mortality associated with PJI.

REFERENCES: *1. Razii N et al, Bone Jt Open. 2021; 2. Ruhal and Kataria, Microbiol Res. 2021; 3. Lenguerrand E et al, Lancet Infect Dis. 2018; 4. Lora-Tamayo J et al, Clin Infect Dis. 2013; 5. Ull C et al, Orthop Rev (Pavia). 2020*

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1.

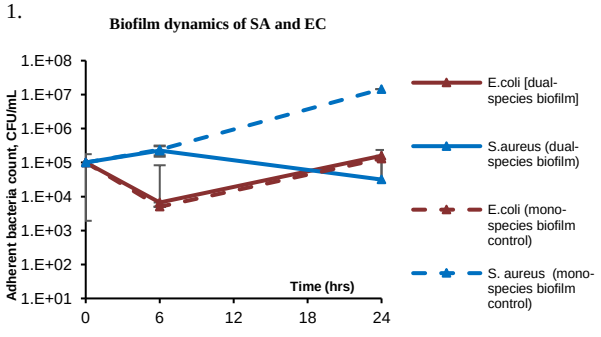

Fig 1. Adherent viable bacteria count of SA and EC in 6hr and 24hr dual species-biofilms grown on SS plates. Adherent bacteria from 6hr and 24hr mono-species biofilms served as controls.

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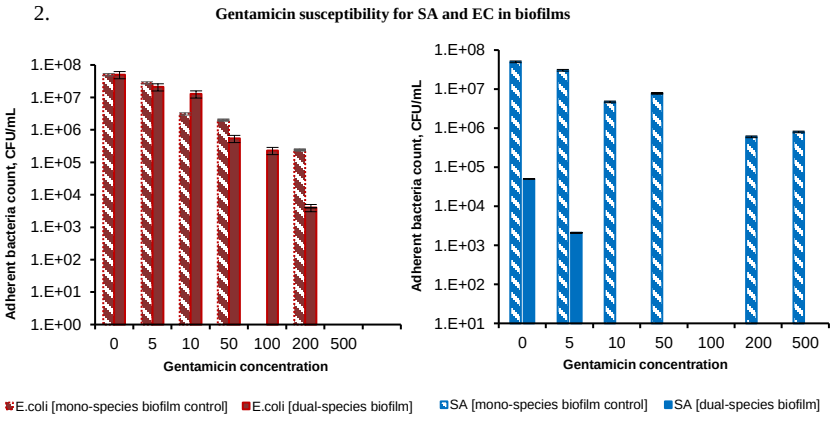

Fig 2. Adherent viable bacteria count for SA and EC in a 24-hour dual-species biofilm after exposure to indicated gentamicin concentrations. Adherent bacteria mono-species biofilms exposed to the same gentamicin concentration range served as controls. MBEC was determined as >3 log reduction in viability.