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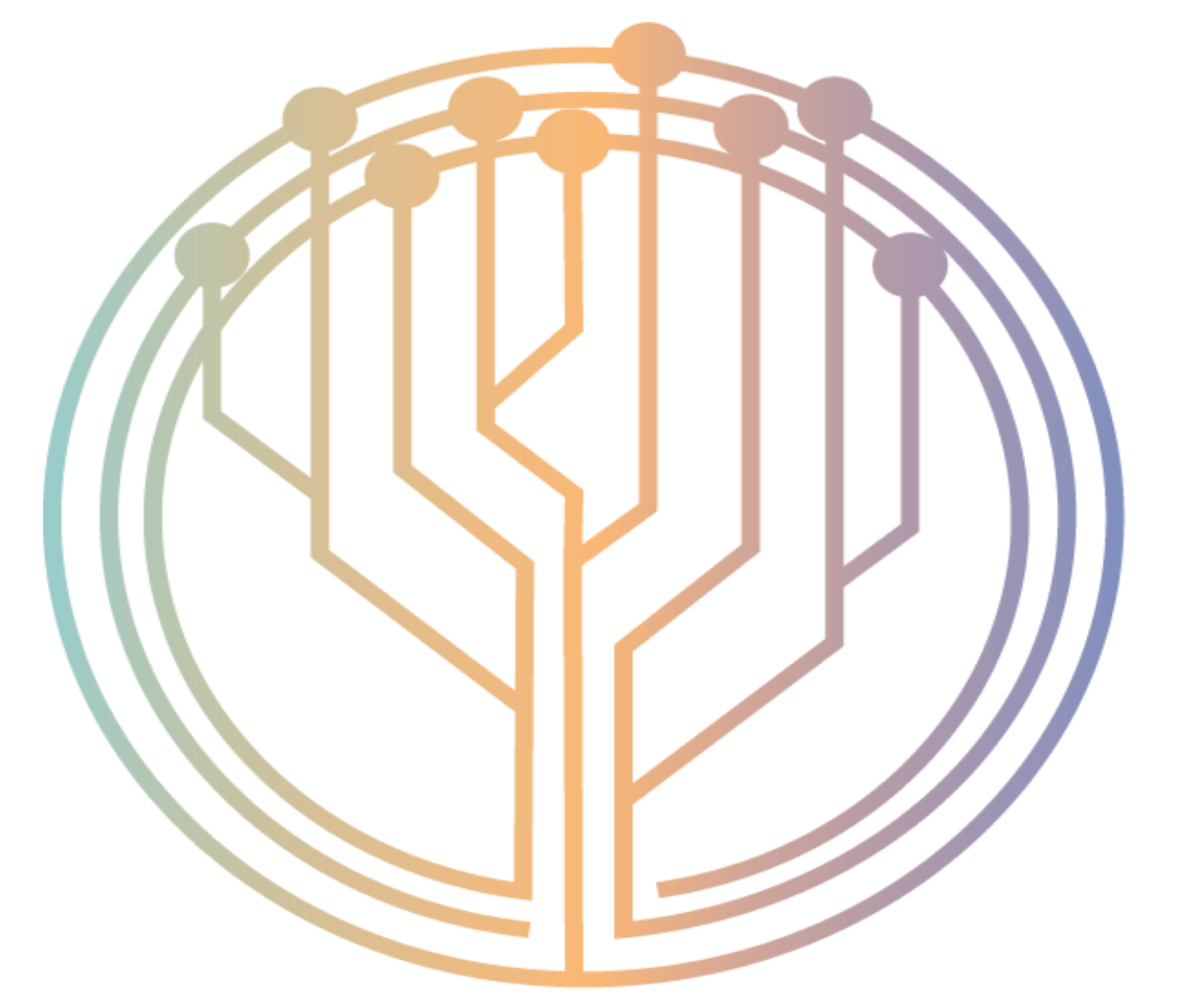
Assessing the ability of a microgravity environment to promote the transfer of antibiotic resistance genes between bacteria

Tristan R. Grams^{1,2}, Camilla Urbaniak², Kasthuri Venkateswaran², Andrea M. Henle¹

¹Department of Biology, Carthage College, Kenosha, WI

²Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA

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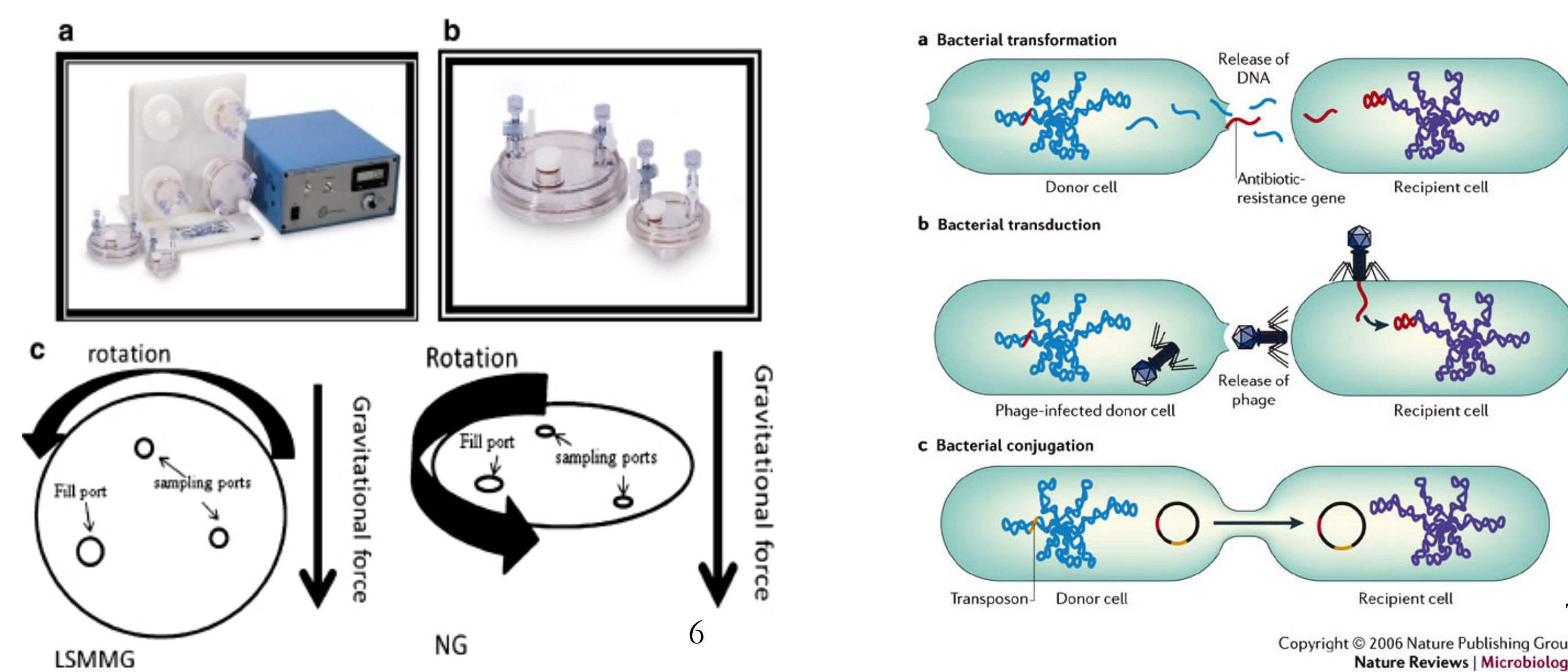
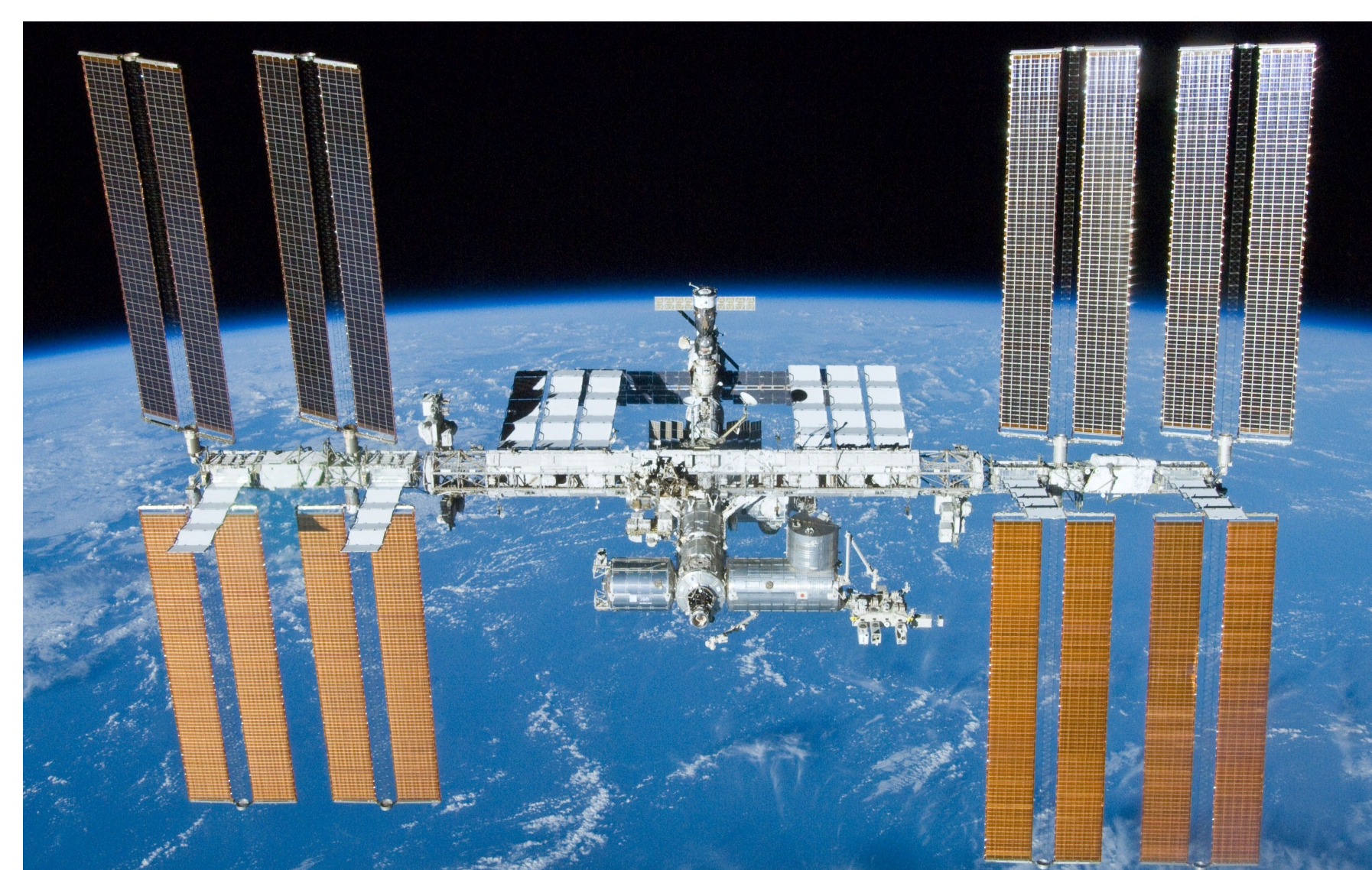
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Abstract

Anti-microbial resistance (AMR) is a major concern worldwide, which prompted the World Health Organization (WHO) this year to publish the first ever list of antibiotic resistant "priority pathogens" that pose the greatest threat to human health. While AMR is a serious problem on Earth, it is an even bigger issue in space, as astronauts become immune-compromised and therefore more prone to infection. This, coupled with the fact that bacteria become more virulent and antibiotic resistant when grown in space, make the study of AMR under microgravity a high priority. It is not yet known what causes bacteria grown in space to become resistant or more susceptible to antibiotics, but we believe it could be due to increased horizontal gene transfer (HGT) of AMR genes and/or increased mutations in AMR genes, leading to a gain of function when these bacteria are exposed to microgravity. A microbial monitoring study of the International Space Station (ISS), isolated various strains of *Acinetobacter pittii*, which were resistant to 6 antibiotics, one of which was oxacillin. Oxacillin is used to treat penicillin-resistant *Staphylococcus aureus*; however, oxacillin resistant *S. aureus* are becoming more prevalent. To carry out our HGT hypothesis, *S. aureus* isolated from the ISS, and negative for *bla*_{OXA}, will be incubated with *A. pittii*, which carries OXA75 and OXA421, under simulated microgravity using the High Aspect Ratio Vessel (HARV). Transfer of these genes to *S. aureus* will be assessed by PCR using primers against the *A. pittii* OXA75 and OXA421 genes. Functionality of these transferred genes will be assessed by growing on plates supplemented with various doses of oxacillin. In parallel, *Staphylococcus epidermidis*, positive for *mecA* will be co-cultured with our ISS *S. aureus* strains to assess *mecA* transfer, following the same procedure as outlined above. To assess our second hypothesis mutations in the *A. pittii ampC* gene will be compared from cultures grown under simulated microgravity and at 1g and functionality of the gene will be assessed by using nitrocefin, a substrate for *ampC*. Determining the cause of increased AMR will help protect astronauts on future long-term space missions.

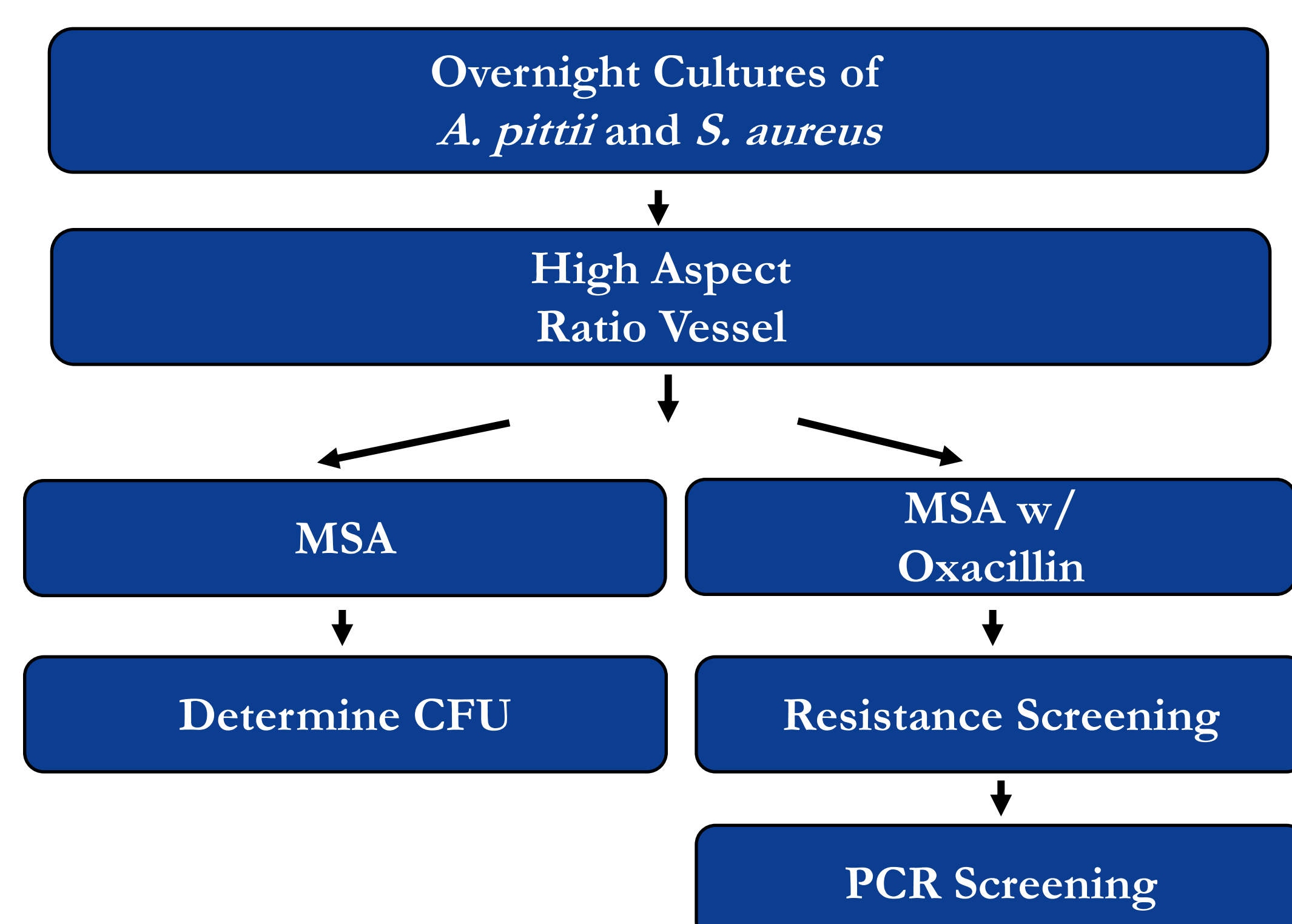
Introduction

- On Earth, anti-microbial resistance (AMR) is a serious issue, which prompted the WHO to issue a report in Feb 2017 outlining pathogens that are the greatest risk to human health¹
- While AMR is a serious issue on Earth, it may be more problematic in space as studies have shown that pathogens acquire more resistance genes when grown on the ISS compared to Earth²
- *Acinetobacter pittii* is a critical priority pathogen which contains genes encoding oxacillin resistance³
- *Staphylococcus aureus* is a common human commensal which can become harmful if resistance genes are acquired⁴
- The goal of this research project is to determine whether increased horizontal gene transfer (Aim 1) and/or increased mutations of AMR genes, leading to a gain of function (Aim 2), is responsible for increased AMR under microgravity conditions

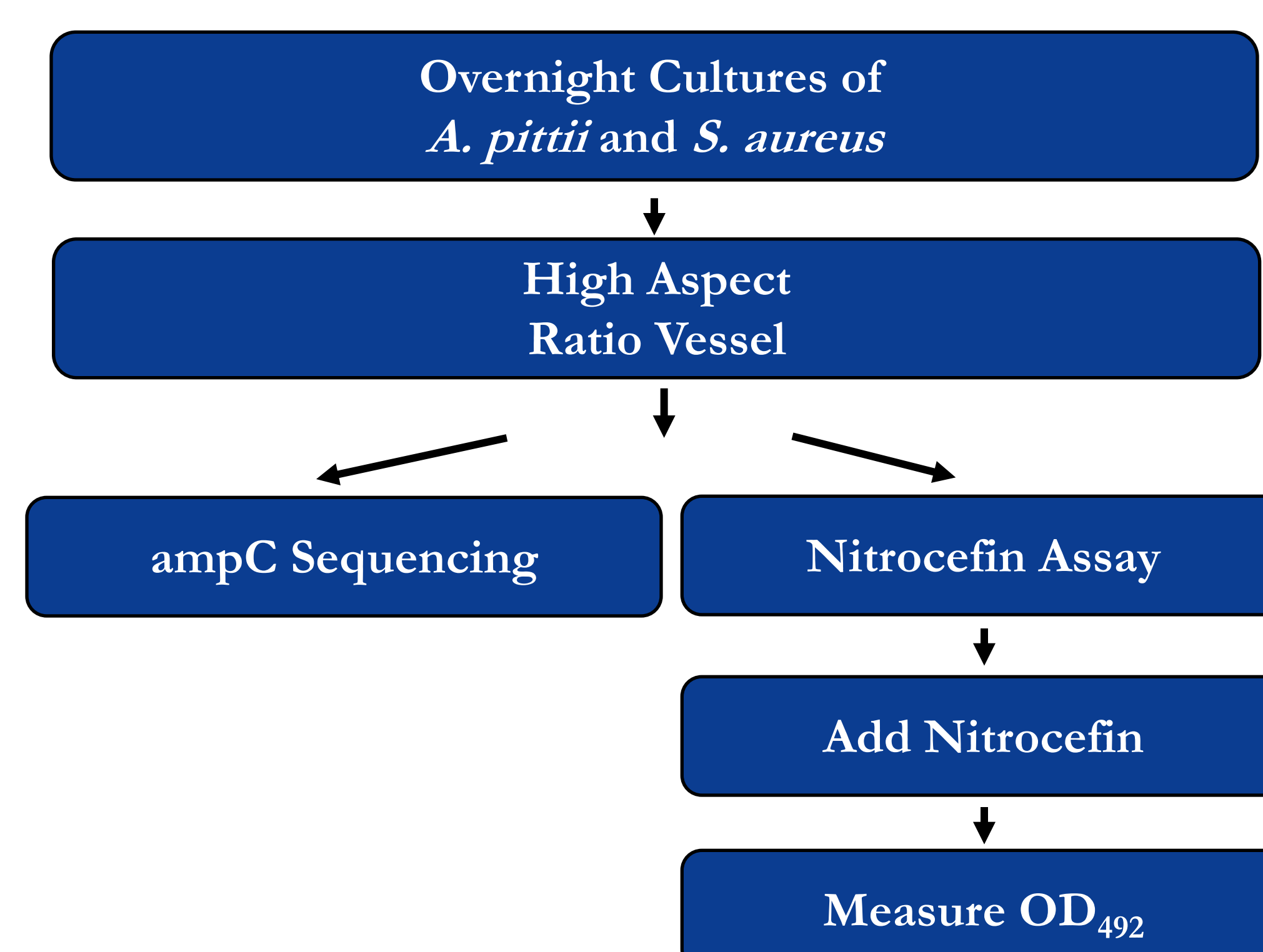


Methods

Aim One



Aim Two



Results

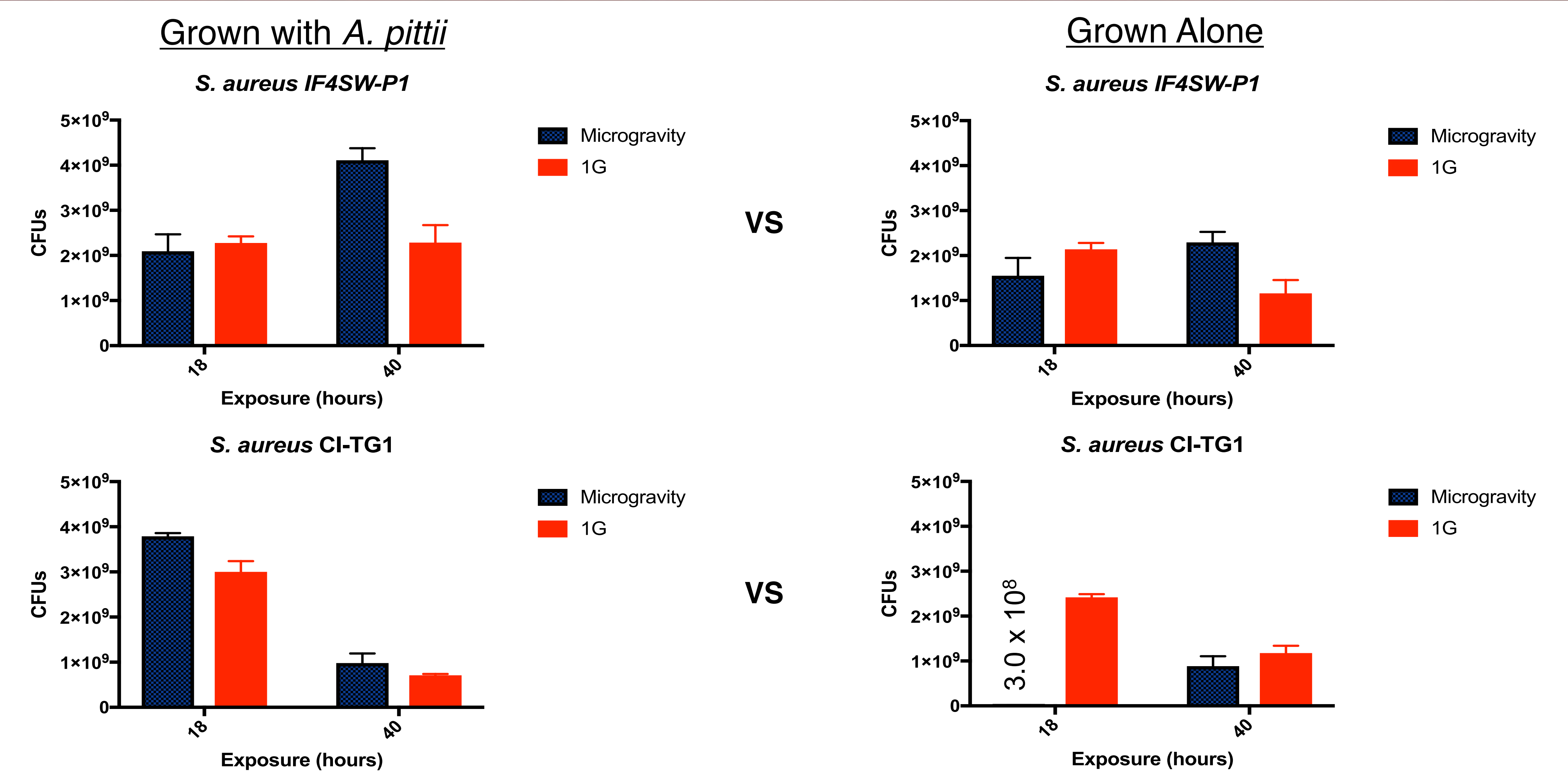


Figure 1. Growth of *Staphylococcus aureus* IF4SW-P1 and CI-TG1 with or without *Acinetobacter pittii*. Simulated microgravity (blue bars) HARV end-point was measured by serial dilution and CFU/ml plating at $t=18$ and 40 hours ($n=2$). To isolate *A. pittii* serial dilutions were plated on Leeds agar and *S. aureus* was isolated by plating on mannitol salt agar. *S. aureus* IF4SW-P1 is an International Space Station isolate, while *S. aureus* CI-TG1 is an earth-based clinical isolate. Error bars= standard error of the mean.

Presence of Oxacillin Resistance Gene Transfer to *S. aureus*

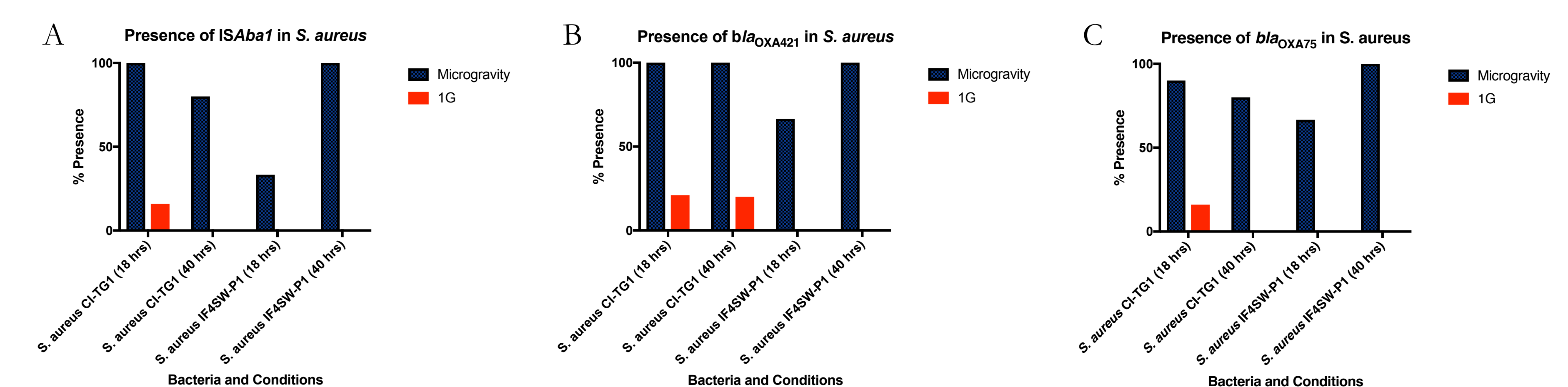


Figure 2. Percent presence of *bla*_{OXA421}, *bla*_{OXA75}, and *ISAb1* oxacillin resistance genes in *S. aureus* measured by PCR Screening. *S. aureus* CI-TG1 (Earth based clinical isolate) or *S. aureus* IF4SW-P1 (ISS isolate) was co-cultured with *A. pittii* in the High Aspect Ratio Vessel at 25rpm. Samples were removed from HARV after $t=18$ and 40 hours. Samples were then measured for phenotypic resistance of oxacillin by plating on mannitol salt agar containing 4 μ g/ml of oxacillin. CFUs positive for phenotypic resistance of oxacillin were screened for resistance genes via PCR for *bla*_{OXA421}, *bla*_{OXA75}, and *ISAb1*. Figure 2A displays the percent presence of the *ISAb1* gene in CFUs that grew on MSA with oxacillin. Figure 2B displays the percent presence of the *bla*_{OXA421} gene in CFUs that grew on MSA with oxacillin. Figure 2C shows the percent presence of the *bla*_{OXA75} gene in CFUs that grew on MSA with oxacillin. Sample size of $n>10$.

Conclusions and Further Direction

- Non-ISS *S. aureus* grew slower than ISS strains, but still acquired resistance genes from *A. pittii*
- Transfer of AMR genes from *A. pittii* to *S. aureus* was confirmed via PCR Screening
- AMR gene transfer occurred more frequently in microgravity compared to 1G
- No difference in frequency of gene transfer to ISS *S. aureus* vs clinical *S. aureus* isolate
- Repeat experiments using *S. aureus* ISS strains and establish ground strains that have been exposed of microgravity.
 - Proper ground control to have bacteria rotating in a HARV set up for 1G
- Obtain a ground isolate *A. pittii* to compare with our ISS strain
- Determine whether increased mutations of AMR gene leads to gain of function under microgravity conditions
- Plate colonies that grew on MSA + oxacillin to determine whether the acquired gene persists over a few replications

References & Acknowledgements

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