Fiscal Year:	FY 2024	**Task Last Updated:**	FY 09/04/2023
PI Name:	Carr, Christopher Sc.D.		
Project Title:	Enterococci Evolution in Space: Environmental Adaptations, Antibiotic Resistance, and Clinical Implications		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	(1) Cell & Molecular Biology (2) Microbiology		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	(1) Translational (Countermeasure) Potential		
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Zip Code:	30332	**Congressional District:**	5
Comments:			
Project Type:	Flight	**Solicitation / Funding Source:**	2016-17 Space Biology (ROSBio) NNH16ZTT001N-FG. App G: Flight and Ground Space Biology Research
Start Date:	11/04/2020	**End Date:**	11/03/2024
No. of Post Docs:	0	**No. of PhD Degrees:**	0
No. of PhD Candidates:	1	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA ARC
Contact Monitor:	Griko, Yuri	**Contact Phone:**	650-604-0519
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Flight Program:			
Flight Assignment:	NOTE: End date changed to 11/03/2024 per NSSC information (Ed., 9/15/23)		
Key Personnel Changes/Previous PI:	No Co-PI/Co-I changes this year. PhD Student McKaig is transitioning to a NASA FINESST award effective 9/1/22.		
COI Name (Institution):	Burton, Aaron Ph.D. (NASA Johnson Space Center) Gilmore, Michael Ph.D. (Massachusetts Eye And Ear Infirmary) Wallace, Sarah Ph.D. (NASA Johnson Space Center)		
Grant/Contract No.:	80NSSC21K0234		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	Enterococci are gram-positive bacteria that originated when our ancient animal ancestors emerged from the oceans to live on land, and brought their gut flora with them. Enterococcus faecalis (EF) and Enterococcus faecium are common human commensals and can harbor multidrug resistance. Both have been previously isolated on the International Space Station (ISS). Likely as a consequence of their evolutionary origins, enterococci show remarkable stress resistance within, but also outside, their human hosts. Their antibiotic resistance, coupled with tolerance to desiccation, starvation, and disinfection, make some EF strains potent pathogens in the built environment (e.g., hospitals), and a potential risk to crew health during space missions. The proposed study includes flight components to: 1) Characterize the frequency and genomic identity of antibiotic resistant organisms, including enterococci, on the ISS; 2) Assess the evolutionary selective pressure of the space environment (microgravity, space radiation) using EF as a model system; 3) Characterize the "natural" evolutionary history of EF on Earth and in space to reveal mechanisms of microbial adaption including natural selection. The CS-05A: Genomic Enumeration of Antibiotic Resistance in Space (GEARS) payload is designed to fulfill specific aim 1, the characterization of the frequency and genomic identity of antibiotic resistant organisms on the ISS. The Co-Principal Investigators propose to carry out longitudinal sampling of ISS surfaces in a repeated measures design. The CS-05B: Enterococcus Growth Advantage on ISS via Tn-seq (EnteroGAIT) payload is designed to fulfill specific aim 2: to assess the evolutionary selective pressure of the space environment. The flight experiment will utilize on-board long-duration microbial growth to measure the selective pressure of the space environment on a defined microbial population: Enterococcus faecalis mutants are created by transposon insertional mutagenesis; selection is measured by sequencing (Tn-Seq) and occurs on timescales far shorter than natural or experimental evolution. The third study, Adaptation & Evolution of Resilient Enterococcus in Space (AERES) will generate complete whole genomes of Enterococcus isolates from the ISS and ground, combined with follow up characterization, to seek evidence of persistence and/or evolution in the space environment.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Antibiotic resistance is a growing threat to human health on Earth, resulting in infections in 2.8 million people, and causing 35,000 deaths annually (CDC data). Overuse or improper use of antibiotics is also contributing to this growing threat. Bacteria are evolving in response to the usage of antibiotics: for example, some strains of Staphylococcus aureus have acquired resistance to vancomycin from Enterococcus. Staphyloccoci and enterococci are the first and second leading causes of hospital-acquired infections, respectively. By studying the distribution of antibiotic resistant microbes on the International Space Station (ISS), a built environment similar in some ways to hospitals, we can also gain insight into how antibiotic resistant organisms survive, adapt, and evolve in response to their environment. Thus, this study will result in data that could also be relevant to human health on Earth.
Task Progress:	The Genomic Enumeration of Antibiotic Resistance in Space (GEARS) Science Verification Testing (SVT) has been completed, including selection of antibiotic (gentimicin), dose, nominal growth period, and growth on contact slides with antibiotics from International Space Station (ISS)-derived isolates from subculture and from surface swabs, followed by sequencing on MinION. Full scope testing also involved bioinformatics analysis, including genome assembly and genome-based antibiotic susceptibility assessment. Enterococcus Growth Advantage on ISS via Tn-seq * (EnteroGAIT) Study: SVT work was carried out using E. faecalis OG1RF (BSL-1 strain). First, filter tests were carried out, resulting in selection of the media exchange filter of the two-chamber BioCell, under development by BioServe. The BioCells use an insert to distribute hydrostatic or hydrodynamic loads across the filter, and also to permit flexing of the outer window during loading/unloading of media or sampling of cells. The biocompatibility of the insert material was assessed using growth curves and coupons of material; this verified that either material can be used in contact with E. faecalis cells. Initial growth tests verified growth of E. faecalis in the hardware, in support of assessing validity of the nominal 8 hour post-media-addition sampling timepoint. Additional testing is planned to confirm this timepoint and to carry out longer-duration testing with the goal of retiring risk related to biofouling during long-duration culturing. Implementation Partner BioServe has already completed some long-duration culturing (e.g., nominal 8 hour growth period followed by 14 days of no new media, followed by new media and another growth and stasis period). As of early September 2023, it is anticipated that the current BioCell hardware is representative of flight and will soon be subjected to additional SVT and Experiment Verification Testing (EVT). [See future work, below.] Adaptation & Evolution of Resilient Enterococcus in Space (AERES) Study: Oxford Nanopore Technologies (ONT) has recently developed a new kit (Kit 14) with higher accuracy. We tested this kit as part of GEARS SVT work. Based on these results, it seems likely we can simplify our AERES genome assembly work to perform nanopore only genome assemblies, instead of hybrid genome assemblies using Illumina and nanopore data. Based on the current nominal requested flight schedule, a no cost extension request is underway.
Bibliography Type:	Description: (Last Updated: 09/15/2023)
Abstracts for Journals and Proceedings	McKaig J, Mena C, Stahl-Rommel S, Nguyen HN, Sharp GM, Ryan K, Bryan N, Gilmore M, Moeller R, Grohmann E, Burton A, Wallace S, Carr CE. "Enabling Genomic Enumeration of Antibiotic Resistance in Space (GEARS)." American Society for Gravitational and Space Research (ASGSR) Conference, November 14-18, 2023, Washington DC. 39th Annual Meeting of the American Society for Gravitational and Space Research, Washington, DC, November 13-18, 2023. , Nov-2023