Fiscal Year:	FY 2014	**Task Last Updated:**	FY 10/23/2013
PI Name:	Ott, C. Mark Ph.D.		
Project Title:	Efficacy of Antimicrobials on Bacteria Cultured in a Spaceflight Analog		
Division Name:	Human Research		
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Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Space Human Factors Engineering		
Joint Agency Name:		**TechPort:**	No
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Human Research Program Risks:	(1) **Microhost**:Risk of Adverse Health Effects Due to Host-Microorganism Interactions		
Space Biology Element:	None		
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Space Biology Special Category:	None		
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Key Personnel Changes/Previous PI:			
COI Name (Institution):	Wotring, Virginia (Universities Space Research Association, Columbia) Nickerson, Cheryl (Arizona State University)		
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As humans travel into outer space, microorganisms will travel with them. While the current NASA standards limit the presence of infectious agents, they are not completely eliminated, and spaceflight missions maintain antibiotics as the primary countermeasure after infection.
One factor that could impact the efficacy of antibiotics is the change in microbial resistance. Previous experiments have confirmed that the spaceflight environment alters a variety of microbial characteristics (1-3). Most notably, alterations in microbial virulence in Salmonella Typhimurium (4, 5) and virulence characteristics in S. Typhimurium and Pseudomonas aeruginosa (4-6) have been demonstrated in response to spaceflight, thus influencing our perception of infectious disease risk during missions. Several spaceflight experiments have shown alterations in antibiotic resistance. During the Cytos 2 experiment aboard Salyut 7, the minimum inhibitory concentration (MIC) of oxacillin, chloramphenicol, and erythromycin for Staphylococcus aureus and of colistin and kanamycin for Escherichia coli were

Task Description:	compared to those of ground controls (7). These results indicated an increased resistance of both S. aureus and E. coli to all antibiotics used in this experiment (7). In 1999, Juegensmeyer, et. al. observed both increased sensitivity and resistance of S. aureus, P. aeruginosa, Bacillus subtilis, and E. coli that had been re-grown after having been on the MIR space station for 4 months (8). Ground-based spaceflight analog systems provide a practical approach to understanding this change. The rotating-wall vessel (RWV) culture apparatus was developed to produce a low-shear, low-turbulence environment for suspension culture that models aspects of spaceflight (9-11). This analog does not completely reproduce all of the effects of microgravity, but has been shown to be predictive of trends that will be seen during spaceflight (1, 12).\ Hypothesis: Bacteria cultured in the low fluid shear RWV environment will demonstrate changes in efficacy of antibiotics commonly used during spaceflight missions compared to higher shear controls. Aims: The MIC of antibiotics currently manifested during spaceflight missions will be evaluated on three medically significant model organisms (Salmonella Typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus) that have either been isolated from spaceflight vehicles or have a clear route of infection. Deliverables: Improve the Quantification of Health Risk by determining the degree to which microbial resistance is altered in a spaceflight analog. Gap Mapping: Risk of Adverse Health Effects Due to Alterations in Host-Microorganism Interactions. IRP GapAEH10: What changes are occurring to the efficiency of current countermeasures against microbial associated risks during human exploration of space that could affect crew health? Risk of Therapeutic Failure Due to Ineffectiveness of Medication ; IRP Gap PH15: Are the antimicrobials carried onboard effective against microbes that exhibit spaceflight-related changes? References 1. C. A. Nickerson, C. M. Ott, J. W. Wilson, R. Ramamurthy, D. L. Pierson, Microbiol Mol Biol Rev 68, 345 (Jun, 2004). 2. K. J. Dickson, ASGSB Bulletin 4, 151 (1991). 3. J. A. Rosenzweig et al., Appl Microbiol Biotechnol, (Oct 22, 2009). 4. J. W. Wilson et al., Proc Natl Acad Sci U S A, (Sep 27, 2007). 5. J. W. Wilson et al., PLoS One 3, e3923 (2008). 6. A. Crabbe et al., Appl Environ Microbiol 77, 1221 (Feb, 2011). 7. R. Tixador et al., Aviat Space Environ Med 56, 748 (Aug, 1985). 8. M. A. Juergensmeyer, E. A. Juergensmeyer, J. A. Guikema, Microgravity Sci Technol 12, 41 (1999). 9. T. G. Hammond, J. M. Hammond, Am J Physiol Renal Physiol 281, F12 (2001). 10. C. A. Nickerson et al., J Microbiol Methods 54, 1 (Jul, 2003). 11. R. P. Schwartz, D. A. Wolf, T. T. Trinh. (1991). 12. D. M. Klaus, H. N. Howard, Trends Biotechnol 24, 131 (Mar, 2006).
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Infectious disease remains a major cause of morbidity and mortality worldwide. As the incidence of antibiotic resistance increases, the need for a better understanding of the mechanisms behind antibiotic resistance and novel approaches for new antibiotics remains a high priority for the general public in the 21st century. Likewise, the primary countermeasure against infectious disease during a spaceflight mission remains those antibiotics carried during a mission. The efficacy of those antibiotics may directly impact the success of a spaceflight mission. By studying the antibiotic sensitivity of microorganisms in a spaceflight analog system, NASA will gain a better understanding of the use of antibiotic countermeasures for the crew. Novel insight may also be gained toward our understanding of microbial resistance against antibiotics on Earth.
	As humans travel in space, they will interact with microbial flora from themselves, other crewmembers, their food, and the environment. While evaluations of microbial ecology aboard the Mir and ISS suggest a predominance of common environmental flora, the presence of (and potential for) infectious agents has been well documented (1-3). Likewise, pathogens have been detected during preflight monitoring of spaceflight food, resulting in the disqualification of that production lot from flight (3). These environmental and food organisms range from the obligate pathogen, Salmonella enterica serovar Typhimurium (S. Typhimurium), which has been responsible for disqualification and removal of food destined for ISS (3) and has previously been reported from Shuttle crew refuse (4), to the opportunistic pathogen Staphylococcus aureus, isolated numerous times from ISS habitable compartments (1) and the crew (5). Infectious disease events have affected spaceflight missions, including an upper respiratory infection that delayed the launch of STS-36 and an incapacitating Pseudomonas aeruginosa urinary tract infection of a crewmember during Apollo 13 (6, 7). These observations indicate that the crew has the potential to be exposed to obligate and opportunistic pathogens. This risk of exposure is expected to increase with longer mission durations and increased use of regenerative life support systems. Efficacy of antibiotics on microorganisms cultured during spaceflight. The consequence of infectious disease during the human exploration of space is greatly dependent on the efficacy of the countermeasures used to minimize the impact of the infection. As antimicrobials are the primary post-infection countermeasure against infectious disease aboard spacecraft, understanding the pharmacodynamics of antibiotics during a mission is of critical importance to mitigate the health, safety, and performance risk for the crew. Previous spaceflight experiments have indicated alterations in bacterial antibiotic resistance, though the specific mechanisms behind these changes have not been elucidated. During the Cytos

2 experiment aboard Salyut 7 in 1982, the minimum inhibitory concentration of oxacillin, chloramphenicol, and erythromycin for Staphylococcus aureus and of colistin and kanamycin for Escherichia coli were compared to those of ground controls (8). These early results indicated an increased resistance of both S. aureus and E. coli to all antibiotics used in this experiment. In 1999, Juegensmeyer, et. al. observed both increased sensitivity and resistance by cultures of S. aureus, Pseudomonas aeruginosa, Bacillus subtilis, and E. coli that had been re-grown after having been on the MIR space station for 4 months (9). Keener insight into the impact of the spaceflight environment on the efficacy of antibiotics has been limited due to the paucity of opportunities for spaceflight experiments, suggesting a need for ground-based analogues that provide (a) preliminary data indicative of spaceflight-induced cellular responses prior to a true spaceflight experiment, and (b) a technique to follow up spaceflight findings without the delays associated with true spaceflight experiments.

The RWV as a ground based spaceflight analogue. Early studies indicated phenotypic changes were occurring to microorganisms cultured in the spaceflight environment (10-12); however, a full understanding of the mechanisms behind those changes and associated clinical implications for spaceflight were limited by the relatively few spaceflight opportunities. One solution that was pioneered by Fang, et. al. in 1997 is the culture of microorganisms in the NASA-designed RWV bioreactor (13-15), which creates a growth environment in which planktonic cells experience low fluid shear culture similar to that which they would encounter during spaceflight (16-18). This spaceflight analogue is a thin, cylindrical vessel that, when completely filled with culture medium and rotated on an axis parallel to the ground, results in solid body rotation (16). Microorganisms in this environment are maintained in suspension and experience a physiologically relevant, low level of fluid shear over their cell surface, previously described as low-shear-modeled microgravity (LSMMG) (16, 18). Mathematical modeling has demonstrated that the fluid shear level within the RWV is less than 1 dyne/cm2. Control cultures are identical except the RWV orientation is adjusted such that the axis of rotation is parallel to the gravity vector, which is predicted to result in a higher fluid shear level (16). Using S. Typhimurium as a model organism, the results from the RWV have been found to be exceptionally accurate as an indicator of trends determined from true spaceflight culture. Specifically, using a murine model of infection, the 50% lethal dose (LD50) of S. Typhimurium grown in the RWV was decreased 5.2 fold compared to controls (19). In comparison, cultures grown in similar media during spaceflight displayed an LD50 decrease of 2.7 fold and 6.9 fold aboard Space Shuttle missions STS-115 and STS-123, respectively (20, 21). Gene expression data also demonstrated similar responses between S. Typhimurium cultured in LSMMG and true spaceflight, including a role for the global regulatory protein Hfq in mediating these responses (20). Collectively, these results strongly suggested that the RWV would provide excellent insight into microbial responses during exposure to antibiotics in the spaceflight environment.

Selection of model organisms and antibiotics. The model organisms selected for this proposal represented Gram negative and positive microbes that have been isolated from spaceflight vehicles and have a clearly defined route for crew infection. S. typhimurium is currently one of the organisms selected by NASA in the preflight microbial monitoring of spaceflight food and has been recovered from Shuttle crew refuse. P. aeruginosa has been found in ISS environmental samples (22) and was responsible for a debilitating infection aboard Apollo 13 (7). S. aureus has been isolated from both ISS environmental samples (1) and Space Shuttle crewmembers (5). All of these model organisms have also demonstrated alterations in molecular genetic and phenotypic characteristics in response to LSMMG culture (19, 23, 24).

Alterations in biofilm characteristics were observed in both the RWV culture of P. aeruginosa (23) and S. aureus (24), respectively, again suggesting the potential for enhanced antibiotic resistance. Indeed, as a part of characterizing the increased biofilm production of S. aureus N315 in response to LSMMG, Castro, et. al. evaluated ciprofloxacin resistance compared to control cultures (24). RWV cultures from both LSMMG and control orientations were grown to stationary phase (20 hours) and challenged with 25 µg/ml ciprofloxacin, corresponding to 50 times the minimum inhibitory concentration. To ensure that fluid shear force was no longer a variable, the vessels were allowed to sit statically at room temperature. Resulting cell concentrations were determined after 24 hours and compared to identically grown, untreated LSMMG and control cultures, defined as 100% survival. Averaging the findings of three independent biological samples, S. aureus cultured in LSMMG was found to be 1.72-fold more resistant than bacteria cultured in the control orientation.

Task Progress:

The antibiotics selected for this study are likely therapeutic agents in the event of infection during spaceflight. To better understand the alterations that may occur in antibiotic function during spaceflight, we selected three antibiotics for each organism that either affect cell wall synthesis, interfere with protein synthesis, or inhibit bacterial DNA gyrase and topoisomerase IV. Specifically, the Gram negative S. typhimurium was challenged with ceftriaxone, a third generation cephalosporin ß-lactam that interferes with cell wall synthesis, azithromycin, an azalide that interferes with protein synthesis, and levofloxacin, a fluoroquinolone that inhibits bacterial DNA gyrase and topoisomerase IV(25). Gram negative P. aeruginosa was challenged with ceftriaxone and azithromycin. The Gram positive S. aureus was challenged with amoxicillin, a broad spectrum ß-lactam, clindamycin, and lincosamide that interfere with protein synthesis, and levofloxacin.

Hypothesis and Significance. We hypothesized that bacteria cultured in the low fluid shear RWV environment would demonstrate changes in efficacy of antibiotics commonly used during spaceflight missions compared to higher fluid shear controls. As previous studies have demonstrated the potential to affect the primary post-infection countermeasure against infectious disease during spaceflight missions, this study was proposed to investigate the use of the RWV as a ground based analogue to investigate potential spaceflight induced changes in antibiotic efficacy during a spaceflight mission. This proposal was designed to provide information to the NASA Human Research Program to enable a better understanding, and corresponding decrease in uncertainty regarding both the Risk of Adverse Health Effects Due to Alterations in Host- Microorganism Interactions and Risk of Therapeutic Failure Due to Ineffectiveness of Medication.

Publications are in preparation for journal submission.

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