

Efficacy of Antimicrobials on Bacteria Cultured in a Spaceflight Analogue

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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. Likewise, pathogens have been detected during preflight monitoring of spaceflight food, resulting in the disqualification of that production lot from flight. 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 and has previously been reported from Shuttle crew refuse, to the opportunistic pathogen *Staphylococcus aureus*, isolated numerous times from ISS habitable compartments and the crew. 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. 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. As antibiotics are the primary countermeasure after infection, determining if their efficacy during spaceflight missions is comparable to terrestrial application is of critical importance.

The NASA Rotating Wall Vessel (RWV) culture system has been successfully used as a spaceflight culture analogue to identify potential alterations in several key microbial characteristics, such as virulence and gene regulation, in response to spaceflight culture. We hypothesized that bacteria cultured in the low fluid shear RWV environment would demonstrate changes in efficacy of antibiotics compared to higher fluid shear controls. This study investigated the response of three medically significant microorganisms grown in the RWV to antibiotics that could be used on spaceflight missions.

Our findings suggest potential alterations in antibiotic efficacy during spaceflight and indicate that future studies on the antibiotic response require additional basic research using the RWV and/or true spaceflight. However, while this analogue has reinforced these potential alterations, the results suggest the best approach for applied forward work is evaluating an *in vivo* system during spaceflight, including human and rodent studies. The complex nature of the analysis for many antibiotics and organism suggests the best approach to determine *in vivo* responses during pharmaceutical treatment is evaluating an *in vivo* system during spaceflight.



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2014 NASA HRP Investigator's Workshop



Background

- Infectious disease does occur during spaceflight missions
- Current preflight risk mitigation efforts limit but do not eliminate the potential for pathogenic microorganisms, such as:
 - Obligate pathogens (*e.g.*, *Salmonella enterica* serovar Typhimurium)
 - Opportunistic pathogens (*e.g.*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*)
- Antibiotics are the primary post-infection countermeasure



Timbury, et al, 2004



Background

- Examples of Spaceflight Experiments

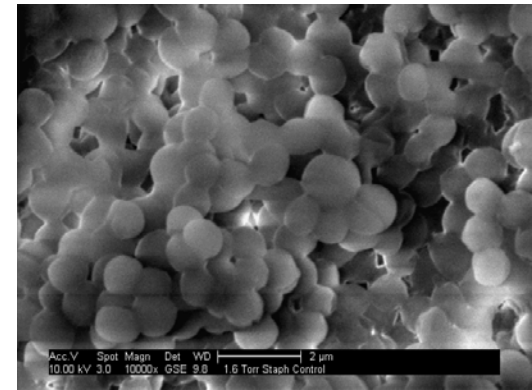


- A Cytos 2 experiment aboard Salyut 7 demonstrated increased resistance of *S. aureus* to oxacillin, chloramphenicol, and erythromycin and *Escherichia coli* to colistin and kanamycin (Tixador 1985).
- Juegensmeyer, *et. al.* observed both increased sensitivity and resistance by cultures of *S. aureus*, *P. aeruginosa*, *Bacillus subtilis*, and *E. coli* that had been re-grown after having been on the MIR space station for 4 months (Juergensmeyer 1999).
- Spaceflight differentially regulates expression of genes encoding antibiotic efflux pumps in *S. Typhimurium*, *P. aeruginosa*, and *Candida albicans* (Wilson, 2007; Crabbé 2011; Crabbé 2013).

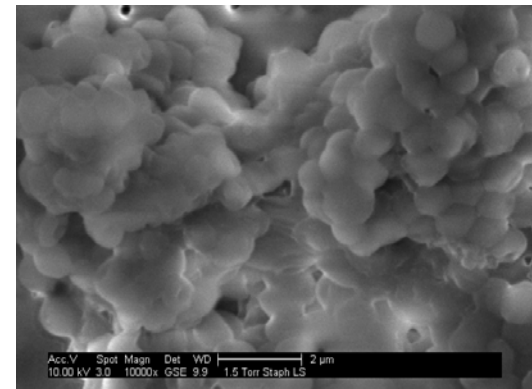


Challenges for Translation to Operations

- Microbial response to spaceflight may be altered by factors including:
 - Medium chemical composition (Wilson, 2008)
 - Growth substrate composition [agar v. liquid culture] (Kacena, 1999)
 - Microbial strain variations
 - Antibiotic mechanisms
 - Phase of growth
 - Single versus multispecies
- The design of any experiment may dramatically alter the question being answered
 - Are we investigating the mass transfer of the antibiotic to the organism?
 - Are we investigating the microbial responses to spaceflight culture that creates an alteration in the antibiotic resistance?
 - Are we measuring microbial kill or measuring minimum inhibitory concentration (MIC)?



Control Culture



Spaceflight Analogue Culture



Study Approach

- Employ the Rotating Wall Vessel (RWV) bioreactor as a spaceflight analogue to culture *S. enterica* serovar Typhimurium, *P. aeruginosa*, and *S. aureus* (methicillin resistant/MRSA)
- Evaluate these organisms outside of the RWV with various concentrations of antibiotics that could be used during a spaceflight mission
 - Lethal Concentration, 50% kill [LC_{50}]
- Challenges:
 - Biofilm formation in stationary phase of *P. aeruginosa* and *S. aureus*
 - Media choice





Study Approach

Organism	Antibiotic	Mechanism
<i>S. Typhimurium</i>	Ceftriaxone	Cephalosporin β -lactam that interferes with cell wall synthesis
	Azithromycin	Azalide that interferes with protein synthesis
	Levofloxacin	Fluoroquinolone that inhibits bacterial DNA gyrase and topoisomerase IV
<i>P. aeruginosa</i>	Ceftriaxone	Cephalosporin β -lactam that interferes with cell wall synthesis
	Azithromycin	Azalide that interferes with protein synthesis
	Levofloxacin	Fluoroquinolone that inhibits bacterial DNA gyrase and topoisomerase IV
<i>S. aureus</i>	Amoxicillin	β -lactam that interferes with cell wall synthesis
	Clindamycin	Lincosamide that interfere with protein synthesis
	Levofloxacin	Fluoroquinolone that inhibits bacterial DNA gyrase and topoisomerase IV



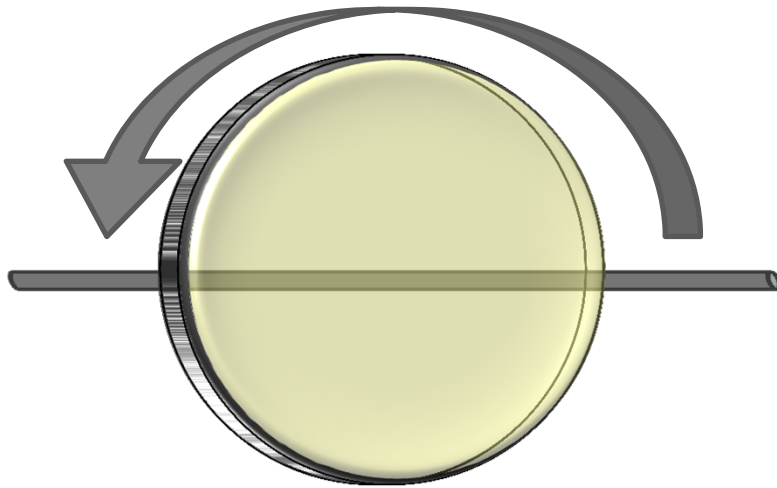
Rotating Wall Vessel Bioreactor

- Rotating Wall Vessel (RWV)
 - Solid body rotation in the reactor simulates several secondary effects of culture during spaceflight
 - Correlation shown with virulence response, media composition impact, and molecular genetic impact of the Hfq global regulator (Wilson 2007; Wilson 2008)
 - Enables relatively high throughput
 - Capability to follow up spaceflight findings without the delays associated with true spaceflight experiments

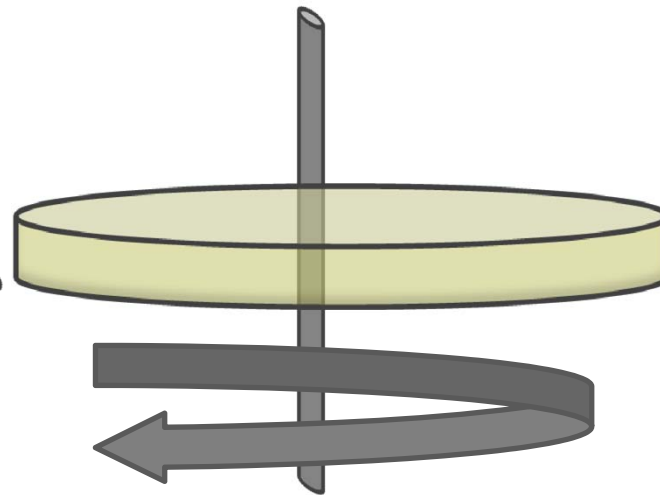




Operating positions of the bioreactors



Low-Fluid-Shear Orientation
(Low-Shear Modeled
Microgravity: LSMMG)

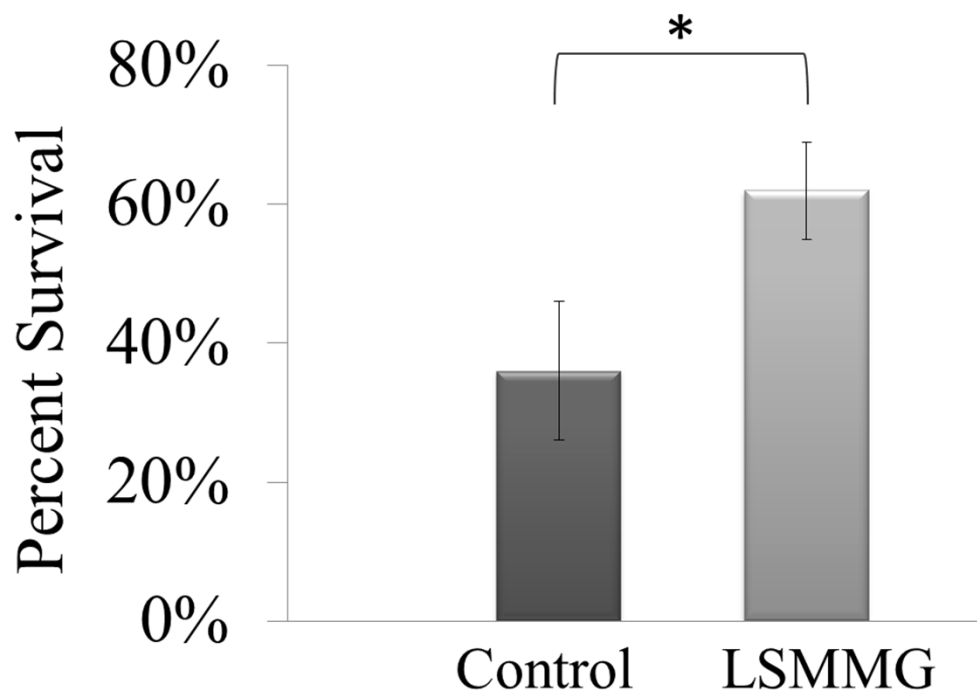


Control Orientation



RWV studies with *Staphylococcus aureus*

- *S. aureus* cultured to stationary phase in the RWV (20 hours) and challenged with 25 µg/ml ciprofloxacin
- Increased resistance likely due to increased mucoidy of the *S. aureus* cultures



Levels of antibiotic resistance for control and LSMMG cultured *S. aureus* N315 (*, $P < 0.05$)
Castro, et. al. 2011



Overview of Findings

- Generally, the organisms did not display significant differences when compared to appropriate controls; however, a few interesting exceptions were noted.
- *S. Typhimurium* exhibited alterations in antibiotic sensitivity to azithromycin, which interferes with protein synthesis, but only at one concentration when longer exposure times were adopted.
- Interestingly, *P. aeruginosa* displayed reproducible changes in resistance to azithromycin depending on the level of fluid shear, which was modulated through the incorporation of beads added to the RWV; suggesting a more complex shear associated mechanism with this antibiotic.
- Methicillin resistant *S. aureus* (MRSA) strain N315 also demonstrated an enhanced resistance under microgravity-analogue culture conditions to levofloxacin, a DNA gyrase inhibitor.



Conclusion

- Collectively, these findings reinforce previous spaceflight investigations suggesting potential alterations in antibiotic efficacy during spaceflight.
- As a high number of variables could impact the microbial response to antibiotics, future work using the RWV would be considered basic/mechanistic.
 - Should be leveraged into Gap, AEH 14: *We need to determine how physical stimuli specific to the spaceflight environment, such as microgravity, induce unique changes in the dose-response profiles of expected medically significant microorganisms.*
- The results also suggest the best approach for applied forward work in determining the impact on pharmaceutical use during spaceflight would be better served using a less reductionistic model, such an *in vivo* system (human or rodent) during spaceflight.