Spaceflight Microbiology

C. Mark Ott, PhD
Microbiology Laboratory
NASA Johnson Space Center, Houston, TX

Nature Conference on the Microbiology of Human Spaceflight
Microbiological Areas of Concern

• Astronaut Health
• Vehicle integrity
• Life support and other systems failure
  – Biofilm formation/biofouling
  – Biocorrosion and biodegradation
  – Trash and human waste containment
  – Risk of condensation
  – Astronaut hygiene areas
• Spaceflight foods
  – Impact of “pick and eat” foods on the environment
  – Impact of the environment on “pick and eat” foods
  – Rinsing food with potable water is impractical
• Planetary protection
  – How do we track what we are leaving versus what we are finding in our search for life on other planets?
Astronaut Health

• Preventative measures
  – Preflight medical exams and medical consult throughout a mission
  – Preflight crew quarantine
  – Stringent microbiological monitoring of spacecraft and its cargo

• Generally healthy crew
  – Infectious disease and allergic symptom rate on ISS has been estimated at 3.4 events/flight year (Crucian et al. 2016)
  – Survey of Space Shuttle missions (STS-1 through STS-89) indicated infectious disease accounted for 1.4% of all medical events (not including skin and subcutaneous tissue) (Risin 2009)

• Incidence is usually based on symptomology
  – Rash
  – Dry hacking cough
  – Diarrhea
Astronaut Health

• Examples of diseases during spaceflight missions
  – Upper respiratory infections
  – Urinary tract infections
  – Ear infections
  – Various fungal infections
  – Herpes Zoster
  – Rashes & skin disorders
  – Allergic reactions
  – Gastroenteritis

• Notable factors
  – Clear routes of infection have not been eliminated
  – No current environmental viral monitoring
  – Risks reflect the vehicle design and operations of specific missions
Protecting the Environment

• Prevention - Preflight
  – Extensive microbial monitoring and preflight disinfection
  – Preflight monitoring targets environmental areas that are probable routes of infection, including vehicle air and surfaces, spacecraft food, potable water, cargo, and a biosafety review of experimental payloads.
  – Flight requirements
    • **Vehicle air** - 100 CFU/m³ fungi; 1000 CFU/m³ bacteria
    • **Vehicle surfaces** - 100 CFU/100 cm² fungi; 10,000 CFU/100 cm² bacteria
    • **Potable water** – 50 CFU/ml heterotrophic plate count; no detectable coliforms in 100 ml; no detectable fungi in 100 ml; treatment technique to prevent transmission of parasitic protozoa.
Protecting the Environment

• Prevention – Vehicle design
  – Potable water recovery system
    • Recovers humidity condensate and urine
    • Final water recycling steps of the NASA water system include catalytic oxidizer (267 °F for 10 minutes); iodination; iodine removal; 0.22 micron filtration
  – Vehicle air
    • Air flow through HEPA filters
  – Vehicle surfaces
    • Use of materials that are not conducive to microbial growth
Constraints of Spaceflight

• When considering spaceflight hardware (e.g., monitoring equipment) and cargo (e.g., disinfection wipes, clothing, food), the benefit of the item is balanced against the need for:
  – Safe operation
  – Minimal power
  – Minimal mass
  – Minimal volume
  – Minimal crew time

No phase separation
Microbiological Monitoring on the ISS

**Surfaces**

**Air**

**Water**

Quantified in-flight and returned to JSC for identification
Environmental Health

• Microbial monitoring by JSC and others indicate ISS environmental flora is similar to a home (Pierson, et al. 2011; Lang, et al. 2017; Blaustein, et al. 2019).
• The bacteria in this environment reflect human-associated microorganisms.
• Media-based monitoring occasionally identifies opportunistic bacterial pathogens, such as *Staphylococcus aureus* and *Bacillus cereus*. No methicillin resistant *S. aureus* has been identified. *Enterobacter* and *Enterococcus* species are occasionally identified throughout the ISS.
The most prevalent fungal genera are *Aspergillus* and *Penicillium*. *A. flavus*, *A. niger*, and *A. fumigatus* have been identified from spaceflight samples. *Stachybotrys chartarum* has been isolated preflight.

Potable water reflects common non-pathogenic bacterial species. The 0.22 micron filter upstream of the water ports have limited increases in diversity.

The ISS is only semi-closed and “new” bacterial and fungal isolates are regularly identified.
Environmental Anomalies

• Mir Space Station
  – Power failures and problems with temperature control eventually caused large amounts of free-floating condensate
  – Samples of condensate revealed the presence of high diversity, including *E. coli*, *Serratia marcescens*, *Legionella* species, and protozoa (Ott *et al.*, 2005)

• ISS anomalies
  – Surface contamination is often associated with uncontrolled water
  – Air contamination and unpleasant odors do occur; however, the sources are often unclear. Luckily, these events have been transient and/or handled by the HEPA filtration system
ISS Environmental Anomalies
• DNA sequencing in space was first performed with the Biomolecular Sequencer in 2016 using Oxford Nanopore Minion (Castro-Wallace et al. 2017).
  – No decrease in sequencing performance
  – Demonstrated flow cell reuse and shelf-life stability to at least 6 months in space
• Microbial cells from a media plate used for surface sampling were processed and sequenced on ISS.
  – The Minion accurately identified the three isolates that were selected.
Advances in Environmental Monitoring

• Spaceflight experiments on ISS are currently being performed focusing on:
  – Swab-to-sequencer (non-culture based identification)
  – Cellular evolution
  – Direct RNA sequencing
• Earth-based investigations comparing sequencing and media-based results are being performed to provide insight into how to translate sequencing results for crew health purposes.
Multiple experiments over the past 50 years indicate unique microbial responses when cultured during spaceflight. The environmental stimulus (stimuli) initiating the response mechanisms are unclear.
Ground Based Spaceflight Analogues

- Rotating Wall Vessel (RWV)
  - Solid body rotation in the reactor simulates several aspects of culture in microgravity
  - Enables relatively high throughput
  - Provides predictive trends for spaceflight experiments
  - Capability to follow up spaceflight findings without the delays associated with true spaceflight experiments
Microbial Responses to Spaceflight

• PI: Cheryl A. Nickerson, Arizona State University
  • Seminal studies evaluating the response of the enteric pathogen, *Salmonella enterica* serovar Typhimurium
  • First studies to effectively study **microbial virulence and global molecular genetic responses of microorganisms** when cultured in the spaceflight and spaceflight analogue environment.

• PI: Duane Pierson, NASA (Emeritus)
  • A series of experiments investigating the **reactivation of Epstein Barr Virus (EBV), Cytomegalovirus, and Varicella Zoster Virus (VZV)** in crewmembers during a mission
  • Increased concentrations of EBV and VZV in astronaut saliva during a mission. **VZV subclinically reactivates in healthy individuals after acute stress.**
Biofilm Development during Spaceflight

- **PI:** Cynthia Collins, Rensselaer Polytechnic Institute
  - Identified a unique biofilm architecture produced by *Pseudomonas aeruginosa* in response to spaceflight culture, described by the authors as “a column-and-canopy structure”.

- **PI: Bob McLean, Texas State University**
  - Investigation into polymicrobial biofilm development during spaceflight by *P. aeruginosa* and *E. coli*.
  - The study focuses on biofilm architecture, disinfection, and corrosion potential during spaceflight.
Astronaut Microbiome

• **PI: Hernan Lorenzi, J. Craig Venter Institute**
  • Baseline evaluation of the microbiome of from two skin sites, nostrils, and fecal samples from 9 astronauts during spaceflight missions (preflight, inflight, post-flight). Blood and saliva were also collected for immunological assays.
  • The composition of the intestinal microbiota became more similar across astronauts in space, mostly due to a drop in the abundance of a few bacterial taxa.
  • Alterations in the skin microbiome that might contribute to the high frequency of skin rashes/hypersensitivity episodes experienced by astronauts in space were also observed.

• **Sloan**
  • Investigation into polymicrobial biofilm development during spaceflight by *P. aeruginosa* and *E. coli*.
  • The study focuses on biofilm architecture, disinfection, and corrosion potential during spaceflight.
Astronaut Microbiome

- **PI:** Hernan Lorenzi, J. Craig Venter Institute
- **Baseline** study of changes in the microbiome of 9 astronauts during spaceflight missions
  - Samples were collected preflight, in-flight, and post-flight samples and included two skin sites, nostrils, fecal samples, potable water, as well as blood and saliva for immunological evaluations.
  - Tightly monitored metadata (e.g., temperature, humidity, crew diet, medications).
- The composition of the intestinal microbiota became more similar across astronauts in space, mostly due to a drop in the abundance of a few bacterial taxa.
- Alterations in the skin microbiome were observed that might contribute to the high frequency of skin rashes/hypersensitivity episodes experienced by astronauts in space.
• **Postdoctoral Fellowships** focusing on the **Microbiology of the Built Environment** using bacterial from the ISS archives
  
  – **Fellow: Michael Lee; PI-Advisor: Craig Everroad**, NASA Ames Research Center
    Microbial evolution and transmission aboard the ISS: inferring mutation rates, assessing pangenomes, and tracking microbiome transmission between astronauts and the space-based built environment. [NASA Space Biology Program]
  
  – **Fellow: Aubrie O’Rourke; PI-Advisor: William Nierman**, J. Craig Venter Institute
    Virulence and drug resistance of *Burkholderia* species isolated from ISS potable water systems. [NASA Space Biology Program]
  
  – **Fellow: Noelle Bryan; PI-Advisor: Maria Zuber**, Massachusetts Institute of Technology
    Genomic and functional analysis of biofilm morphotypes of International Space Station isolated *Staphylococcus epidermidis* and their pathogenicity in *Caenorhabditis elegans*. [NASA Space Biology Program]
  
  – **Fellow: Blake Stamps; PI-Advisor: John Spear**, Colorado School of Mines
    Biodeterioration and Biocorrosion in Spaceflight Ecosystems: Implications for Material/Microbiome Interactions on the International Space Station. [Sloan Foundation]
  
  – **Fellow: Jiseon Yang; PI-Advisor: Cheryl Nickerson**, Arizona State University
    Developing predictive model systems of polymicrobial biofilm formation and susceptibility to chemical disinfectant: A longitudinal study with implications for spaceflight systems integrity and health risks. [Sloan Foundation]
Considerations for Future Research

• Historically, spaceflight research has often been burdened by sensationalized or unsubstantiated conclusions.
  – Highly visible field
  – Limitations in experimental reproducibility
  – Misconceptions from these reports can last for years and actually be damaging to our progress

• Our current resources and ability to perform science on ISS enables solid hypothesis driven science.

• Recommendations and reviews of future space research should be sensitive to the need for the same levels of evidence and credibility that terrestrial studies require.
  – Proper controls
  – Conclusions that reflect the evidence
Microbiology Research aboard ISS

• NASA NSPIRES System
  – Solicitation information
  – https://nspires.nasaprs.com/external/

• NASA Space Biology
  – Microbiology is one of five science Elements in the Space Biology Science Plan

• NASA Human Research Program
  – Microbiology information is under the “Risk of Adverse Health Effects Due to Host-Microorganism Interactions” in the HRP Research Roadmap
  – https://humanresearchroadmap.nasa.gov/
BACKUP SLIDES
ISS Air and Surface Monitoring

Fungal Isolates

Nature Conference on the Microbiology of Human Spaceflight
ISS Air and Surface Monitoring
Bacterial Isolates

Nature Conference on the Microbiology of Human Spaceflight
U. S. Potable Water Dispenser

- Provides “hot” and “ambient” potable water
- Processing includes:
  - Catalytic oxidizer
  - Iodine disinfection
  - In-line filter (0.2 micron)
- Common isolates
  - *Ralstonia pickettii*
  - *Burkholderia multivorans*
  - *Sphingomonas sanguinis*
  - *Cupriavidas metallidurans*