

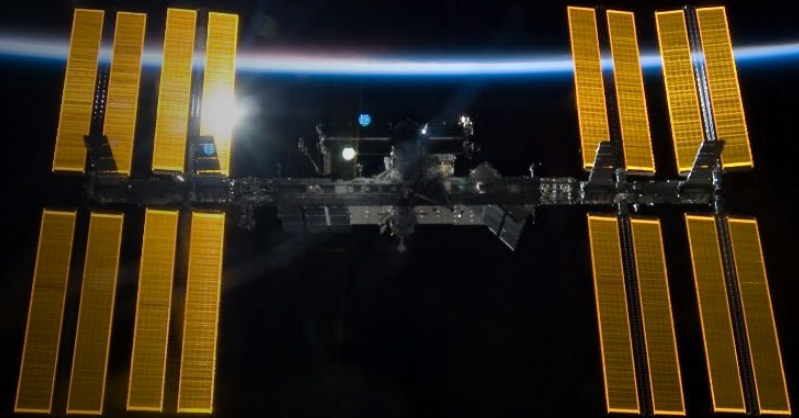


Redefining Microbiological Risk Mitigation during Spaceflight

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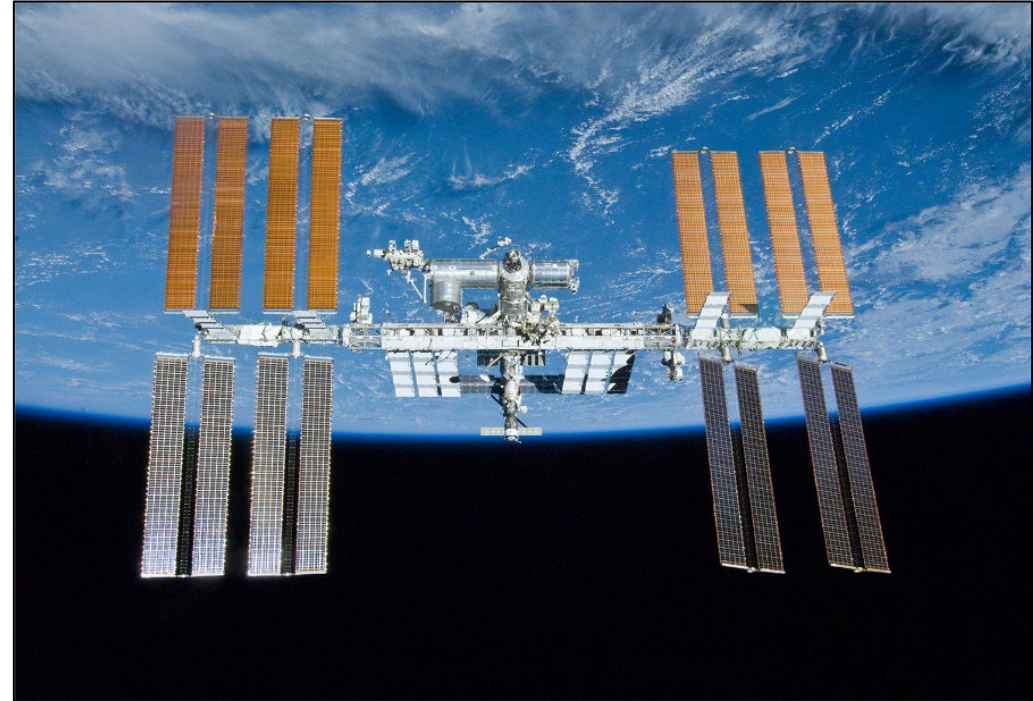
NASA Johnson Space Center, Houston, TX





Why Microbiology?

- Astronaut health
- Spaceflight foods
- Vehicle integrity
- Life support and other systems
 - Biofilm formation/biofouling
 - Biocorrosion and biodegradation
 - Hygiene areas
 - Trash and human waste containment
- Planetary protection
 - How do we protect the crew and extraterrestrial environments in our search for life on other planets?





Where we began...

- Historically, spaceflight missions have relied on stringent microbiological requirements for vehicle design and operations.
 - Microbiological monitoring targeted astronauts, spaceflight foods, vehicle air/surfaces, potable water, hardware, and cargo bags.
 - Microbial concentrations targeted “essential sterility” in the environment.
 - Preflight crew quarantine beginning in 1971.

How stringent?

- ✓ As recently as the late 1990s, NASA potable water requirements limited preflight microbial concentrations to 1 Colony Forming Unit (CFU) per 100 ml.





What have we learned?

- For over 20 years, the International Space Station (ISS) has provided extensive microbiological operations and research data characterizing the complex interface between humans and their spaceflight habitat.
- This experience has provided many **findings and questions** on the actual risks and benefits from living and working in space.
- **We found the best mitigation technique is preflight prevention.**





Prevention - Spacecraft design

- Vehicle surfaces
 - Materials that do not promote microbial growth
- Vehicle air
 - Air flow through HEPA filters
- The water recovery system that recycles urine and cabin humidity relies on multiple microbial mitigation techniques, including:
 - Catalytic oxidizer (267°F for 10 minutes)
 - Iodination/iodine removal
 - 0.2-micron filtration





Prevention – Mission operations



Health Stabilization Program

<u>Mission</u>	<u>Illness (Crew)</u>
• Apollo 7	Upper respiratory infection (3)
• Apollo 8	Viral gastroenteritis (3)
• Apollo 9	Upper respiratory infection (3)
• Apollo 10	Upper respiratory infection (2)
• Apollo 11	
• Apollo 12	Skin infection (2)
• Apollo 13	Rubella (1)
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• Apollo 14	
• Apollo 15	
• Apollo 16	
• Apollo 17	Skin infection (1)
• Skylab-2	
• Skylab-3	Skin infection (2)
• Skylab-4	Skin infection (2)



Prevention – Food monitoring

- Spaceflight foods that are considered commercially sterile (e.g., thermostabilized, irradiated) do not require microbiological monitoring.
- Non-thermostabilized foods may be microbiologically monitored for total aerobic counts, Enterobacteriaceae, *Salmonella*, and/or Yeast and Molds.
- Preflight monitoring of ISS spaceflight food has indicated the presence of
 - *Salmonella enterica* serovar Typhimurium
 - *Staphylococcus aureus*
 - *Klebsiella pneumoniae*
 - *Enterobacter cloacae*
 - *Cronobacter sakazakii*
 - *Aspergillus flavus*
 - *Aspergillus fumigatus*.





Prevention – Environmental monitoring

- Extensive microbial monitoring and preflight disinfection
- Preflight monitoring targets environmental areas that are probable routes of infection, including vehicle air and surfaces, potable water, and cargo.
- Environmental microbiological requirements (Missions over 30 days)
 - 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.
- Spaceflight experimental payloads also receive a biosafety review to support the determination of appropriate levels of containment and control.



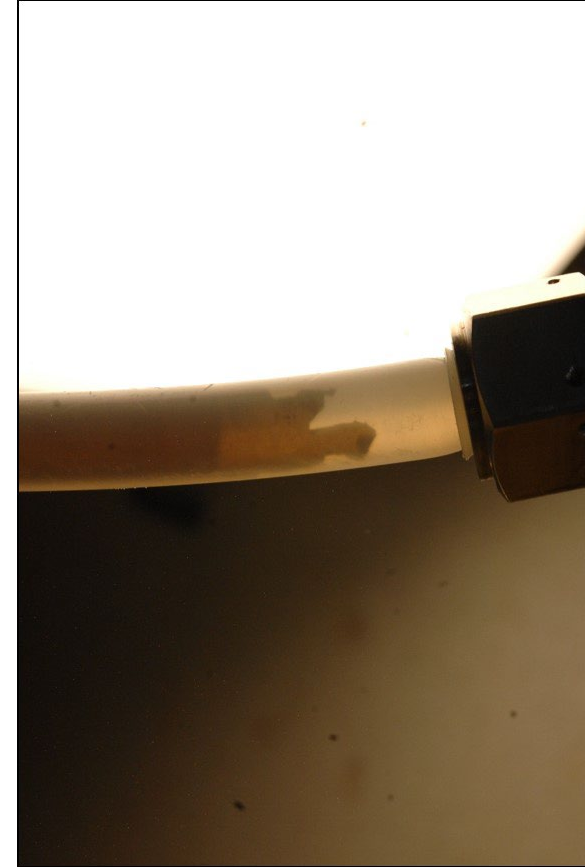
We found that contamination happens



Trash and waste control



Astronaut activities, such as eating and hygiene



Biofilm formation in life support system water lines



Microbiological monitoring on the ISS

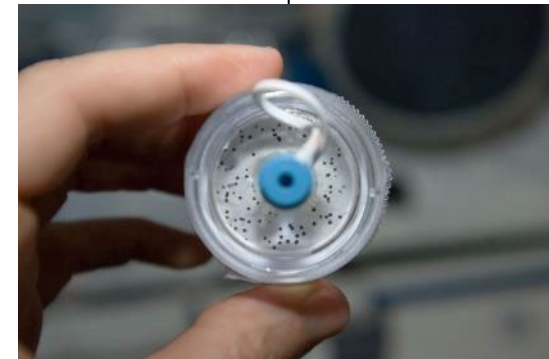
Surfaces



Air



Water



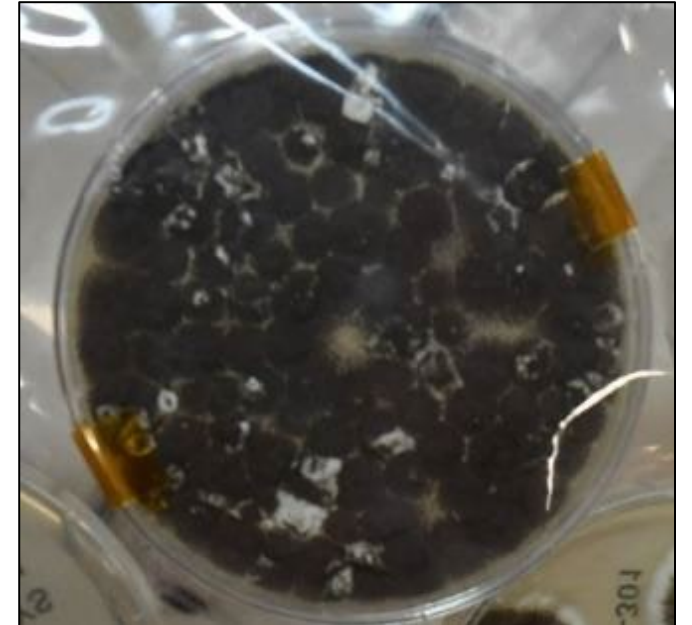
Quantified in-flight and returned to JSC for identification



Environmental health

Microbial monitoring by NASA and others indicate ISS environmental flora reflect human-associated microorganisms commonly found in terrestrial homes.

- 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.





Environmental health

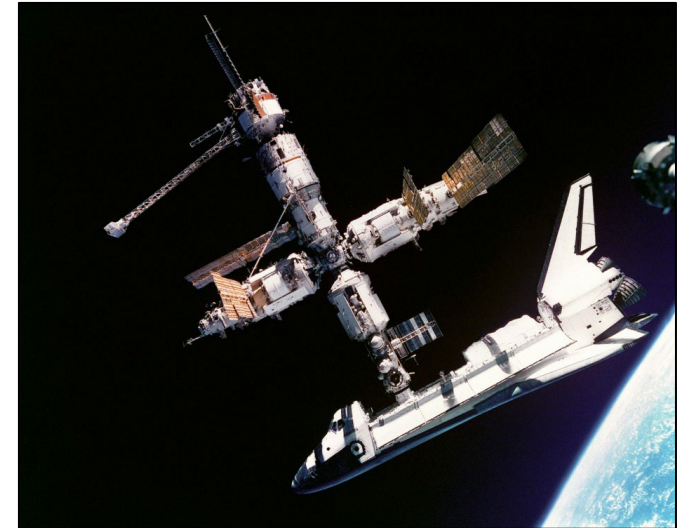
- The most prevalent fungal genera are *Aspergillus* and *Penicillium*.
- *A. flavus*, *A. niger*, and *A. fumigatus* have been identified from spaceflight samples. *Stachybotrys chartarum* has been isolated preflight.
- Potable water reflects a limited diversity of common environmental bacterial species, including
 - *Ralstonia pickettii*
 - *Burkholderia multivorans*
 - *Sphingomonas sanguinis*
 - *Cupriavidas metallidurans*





Environmental anomalies

- Mir Space Station
 - Samples of free-floating condensate caused by power failures and problems with temperature control revealed high microbial diversity, including *Escherichia coli*, *Serratia marcescens*, *Legionella* species, and protozoa.
- International Space Station
 - Surface contamination is often associated with uncontrolled water
 - Air contamination and unpleasant odors do occur; however, the sources are often unknown. These events have been transient and/or handled by the HEPA filtration system.
 - Biofilm formation in ISS water lines have caused clogged lines and pump failure.





We found that astronauts still get sick

- Diagnosis is often based on symptomology (e.g., headache, rash, dry hacking cough, diarrhea).
- Examples of infectious diseases during spaceflight missions include upper respiratory infections, urinary tract infections, ear infections, herpes zoster, rashes and skin disorders, and gastroenteritis.
- A survey of Space Shuttle missions indicated infectious disease accounted for 1.4% of all medical events (not including skin and subcutaneous tissue).



Why do exceptionally health astronauts in a pristine environment still contract infectious diseases?

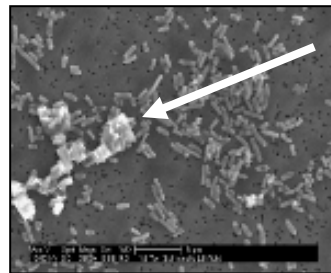


We found that microorganisms respond to the spaceflight environment in unexpected ways

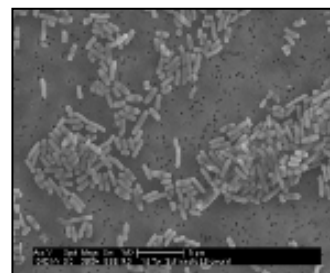
- In 2006, the MICROBE Experiment (PI: Cheryl Nickerson, Arizona State University) identified **alterations in microbial virulence** in spaceflight grown cultures of *Salmonella enterica* serovar Typhimurium.
 - Proteomic profiling identified **73 differentially regulated proteins**, and microarray analysis identified **167 differentially regulated, globally distributed genes** compared to ground controls
 - Common molecular regulatory protein (Hfq) associated with 32% of the differentially regulated genes
 - In-flight grown *S. Typhimurium* showed the presence of an extracellular material not seen in ground control.



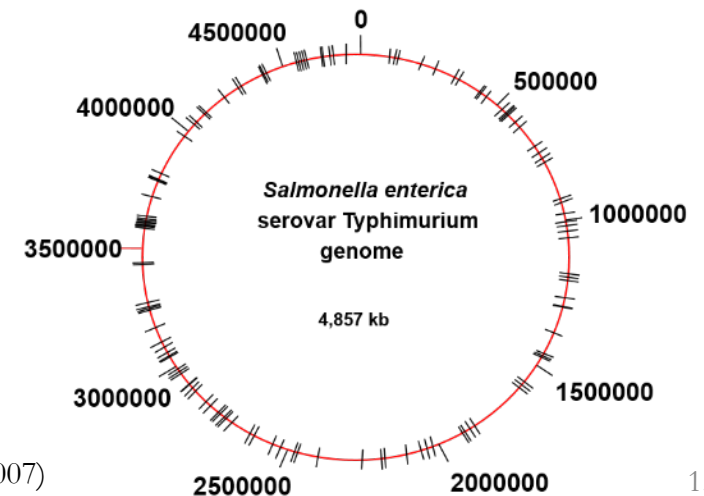
In-flight hardware



Flight Sample



Ground Control





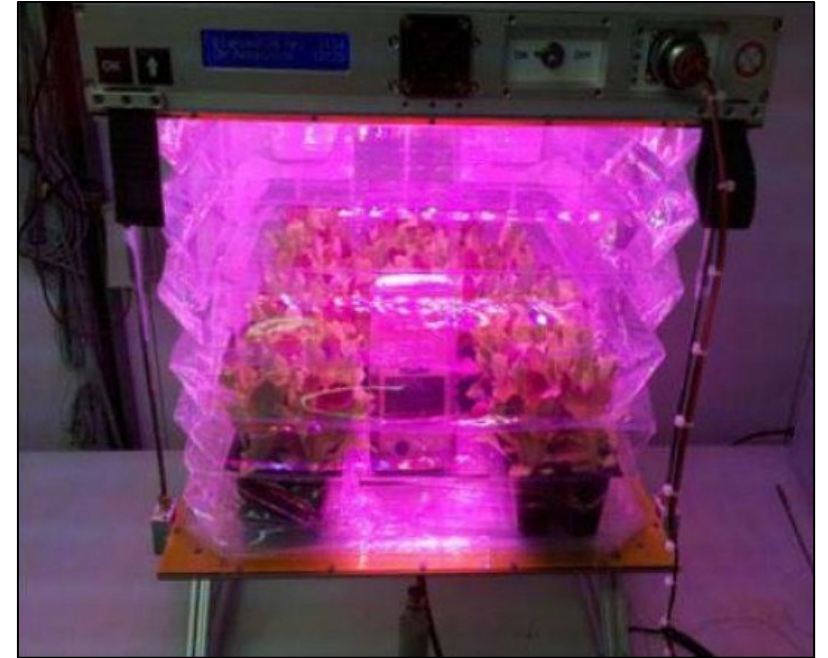
Other microbial responses to spaceflight

- A follow-up study of ***S. Typhimurium*** confirmed the spaceflight-associated increase in virulence and found the changes were affected by media type to a greater extent than terrestrial controls.
- ***Serratia marcescens*** cultured during spaceflight also demonstrated an increase in virulence using a *Drosophila melanogaster* infection model.
- Increased resistance to multiple antibiotics was observed with spaceflight-grown ***S. aureus*** (oxacillin, chloramphenicol, and erythromycin) and ***E. coli*** (colistin and kanamycin).
- ***Pseudomonas aeruginosa*** also responded to spaceflight conditions through differential regulation of 167 genes and 28 proteins, with Hfq as a global transcriptional regulator.
- ***Candida albicans*** cultured in space displayed 452 differentially regulated genes and random budding patterns (as opposed to bipolar budding normally expected on earth).
- Microbial biofilms of ***P. aeruginosa*** grown in space were altered into a novel “column and canopy” structure.



With so many findings...

- What microbiological requirements are needed for future space missions?
 - Do we continue to repeat what we have done in the past?
 - Do we change our monitoring targets and/or sampling frequency?
 - Do we add or change requirements considering new operational activities, such as pick and eat crops and other bioregenerative systems?
- If we aren't sure, what new research is needed to help make these decisions?

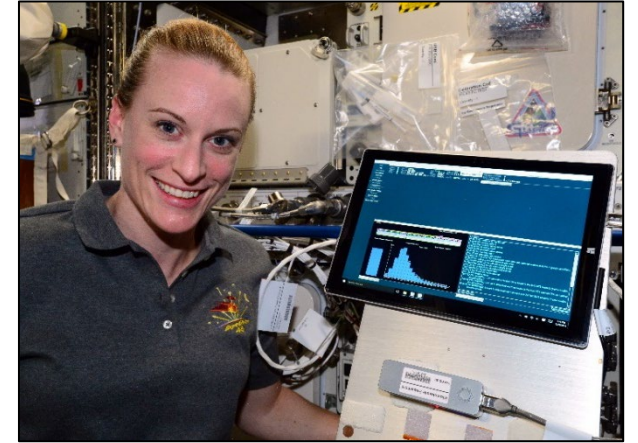


The Veggie plant growth system provides crops for crew consumption.



Do we need better monitoring approaches?

- DNA sequencing in space
 - First performed in 2016 using Oxford Nanopore’s MinION technology
- In-flight identification of environmental samples
 - Three microbial colonies from a media plate used for ISS surface sampling were processed and sequenced on ISS.
 - The MinION accurately identified the three isolates that were selected.
- Following up with “Swab to Sequencer” direct analysis
 - Technology Demonstration using the flight hardware and process was successful for vehicle surface monitoring.
 - Feasibility of water monitoring is currently being evaluated.



When do we use advanced molecular techniques versus simple assays?



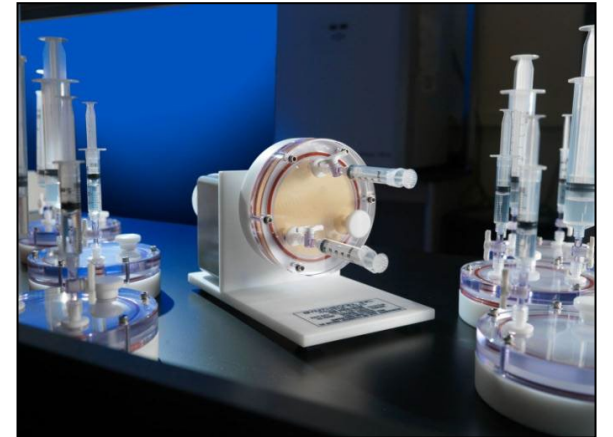
What other questions are we answering?

Do other microorganisms display an increase in virulence?

- PI: Mark Ott, NASA
 - Determine if growth in a spaceflight analogue environment changes stress response, adhesion/invasion of 3-D tissue co-culture models, and virulence of five bacterial species.

Does low dose radiation of host cells synergistically exacerbate the increased virulence observed with spaceflight analogue culture?

- PI: Cheryl Nickerson, Arizona State University
 - Investigate the compound effect of spaceflight analogue culture with low dose radiation to determine if the combination synergistically increases the potential infection risk.



The Rotating Wall Vessel (RWV) bioreactor simulates several aspects of culture in microgravity



What other questions are we answering?

How do bacterial biofilms change when cultured in the spaceflight environment?

- PI: Bob McLean, Texas State University
 - Investigate polymicrobial biofilm development by *P. aeruginosa* and *E. coli* during **spaceflight** to identify changes in biofilm architecture, disinfection, and corrosion potential during spaceflight.

Are we fully utilizing the data that we have?

- PI: Kristyn Hoffman, KBR/NASA
 - Employ machine learning techniques to compare historical environmental monitoring data and astronaut clinical data to determine if there is any correlation.

Do microorganisms build up a tolerance to inflight disinfectants?

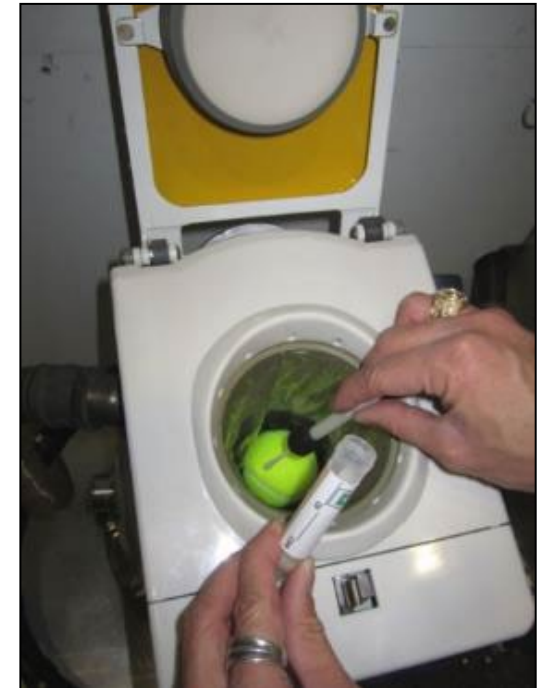
- PI: Erica Hartmann, Northwestern University
 - Characterize benzalkonium chloride (active agent in ISS disinfection wipes) resistance in 73 *Staphylococcus* isolates collected from a variety of surfaces inside the ISS between 2006 and 2015.



What other questions are we answering?

What do we know about astronaut microbiomes?

- PI: Hernan Lorenzi, J. Craig Venter Institute
 - Baseline study of skin sites, nostrils, fecal samples from nine astronauts collected preflight, in-flight, and post-flight.
 - The composition of the intestinal microbiota became more similar across astronauts in space
 - Alterations in the skin microbiome were observed that might contribute to the high frequency of skin rashes/hypersensitivity episodes.
 - Spaceflight studies inherently have a very low sample number but extensive environmental, medical, and dietary information.
 - This study provided a foundation for multiple astronaut microbiome experiments that are currently underway.





Why is it important to review our requirements?

- As NASA missions reach to the moon and Mars, the available space for cleaners, disinfectants, and monitoring hardware will decrease. Understanding how microbiological benefits and risks during spaceflight differ from those on Earth will streamline what we will need to take with us.
- In addition, commercial spaceflights and space stations are becoming commonplace. Identifying the “real” risks and requirements over the next few years will help to establish future occupational health and safety guidelines.



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