Evidence Report:

Risk of Adverse Health Effects Due to Alterations in Host-Microorganism Interactions

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II. Executive Summary

While preventative measures limit the presence of many medically significant microorganisms during spaceflight missions, microbial infection of crewmembers cannot be completely prevented. Spaceflight experiments over the past 50 years have demonstrated a unique microbial response to spaceflight culture, although the mechanisms behind those responses and their operational relevance were unclear. Thus, clearly defining and addressing the impact of spaceflight-associated alterations on host-microorganism interactions have not been addressed. In 2007, the operational importance of these microbial responses was emphasized, as the results of an experiment aboard STS-115 demonstrated that the enteric pathogen *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) increased in virulence in a murine model of infection. The experiment was reproduced in 2008 aboard STS-123, confirming this finding. In response to these findings, the Institute of Medicine of the National Academies recommended that NASA investigate this Risk and its potential impact on the health of the crew during spaceflight. NASA assigned this risk to the Advanced Environmental Health Portfolio of the Human Research Program. To better understand this risk, evidence has been collected and reported from both spaceflight analogue systems and true spaceflight. Although the performance of virulence studies during spaceflight are challenging and often impractical, additional information has been and continues to be collected to better understand the risk to crew health. Still, the uncertainty concerning the extent and severity of these alterations in host-microorganism interactions is very large and requires more investigation.

III. Introduction

The human body serves as host to some 100 trillion microbes that exist in the oral cavity, skin and the gut. Transfer of microorganisms from person to person are common in closed habitats such as spacecrafts and indeed, crewmembers from Apollo 7 through Apollo 11 tested positive for microbes post-flight, that were absent pre-flight [4]. Moreover, sites within the body that tested negative for certain microorganisms before flight, were found to test positive post-flight, signifying the growth of opportunistic organisms and an overall risk to astronaut health during spaceflight missions of extended duration.

Current spaceflight data clearly demonstrates alterations in aspects of the crew immune system during spaceflight. In addition, microorganisms have been demonstrated to increase virulence and/or virulence characteristics in true spaceflight. Taken together, it makes a strong argument to investigate the risk to crew health and develop adequate preventive strategies to maintain astronaut health and performance during spaceflight missions. In this review, we identify evidence of molecular genetic and phenotypic

alterations in microorganisms during true spaceflight and ground-based spaceflight analogue models.

A. **Identifying the need for investigation.** In 2008 the Institute of Medicine (IOM) of the National Academies reviewed the Human Research Program Evidence Book of the *"Risk of Crew Adverse Health Event Due to Altered Immune Response."* The IOM cited research from a flight experiment by Dr. Cheryl Nickerson aboard STS-115, which indicated that the enteric pathogen, *Salmonella enterica* serovar Typhimurium, had become more virulent when cultured during spaceflight. The IOM recommended NASA "Develop evidence books on additional risks, including alterations in microbe and host interactions…" In November 2008, a Risk entitled, "*Risk of Adverse Health Effects Due to Alterations in Host-Microorganism Interactions,*" was added to the Human Research Program Integrated Research Plan to determine the likelihood and consequences of alterations in microbial interactions with the crew and their environment that could impact their health and performance.

B. **Flight experiments used to study host-microbe interactions.** While several experiments have been performed in spaceflight to assess the effects of this unique environment on microbes, there are several factors that complicate the evaluation and comparison of the resulting data. Some of these confounding elements include (a) the wide variety of organisms that have been studied including motile versus non-motile bacteria; (b) the different spaceflight parameters that have been used (e.g. differences in lengths of missions, sample handling – fixed or frozen, in-flight centrifuged 1x*g* controls versus ground 1x*g* controls); and (c) differences in growth media used (e.g. minimal versus rich media or liquid versus solid media). These factors will be discussed in the Evidence Book Report where appropriate. It is also clear that in spite of these differences, the space environment affects microbes differently than the earth environment, and these changes must be understood in order to ensure the safety of humans during long duration space missions.

C. **Earth based cell culture systems used to study host-microbe interactions.** While spaceflight is the ultimate platform for performing experiments to determine alterations in microbial responses and host-pathogen interactions, spaceflight research is constrained by high costs, inconsistent flight availability (up-mass and down-mass), minimal in-flight analytical equipment, as well as limitations in power usage, payload weight and volume, and crew time. Thus, ground-based analogues have been developed to evaluate alterations in microbial responses to these conditions [7]. These analogues do not remove gravity from the system, but instead develop an environment, which reflects many of the secondary effects observed in microgravity (decreased mass transfer, lower fluid shear, etc.). Most all of these analogues rely on the continuous sedimentation of microbial cultures in a growth medium. The simplest system is the clinostat, which is a cylindrical tube completely filled with media (no bubbles, i.e., "zero headspace"), that is rotated perpendicular to the gravitational force vector [8].

Likewise, a more complex system, designed by NASA, called the rotating wall vessel (RWV) has been used extensively since the mid 1990s. The RWV is also an optimized form of suspension culture and consists of a hollow disk or cylinder that is completely filled with medium and rotates on an axis perpendicular to the gravitational force vector. Under these culture conditions, the cells are maintained in suspension as the RWV is rotated and a sustained low-shear environment for cell growth is achieved [7]. Exchange of nutrients and localized "mixing" of the microenvironment is facilitated by the constant falling of the cells through the local fluid environment and the gentle rotation of the culture medium. Unlike the clinostat, a gas-permeable membrane on one side of the RWV allows constant air exchange during growth. Data from previous research on *S.* Typhimurium indicated that the enhanced virulence observed during spaceflight was also observed at a similar trend and magnitude to virulence changes imparted by culture in the RWV [9, 10]. Similar trends in gene expression and regulation were also observed [9, 11].

Other microbial culture spaceflight analogues have been reported, such as the random positioning machine (RPM) and the use of diamagnetic levitation [12]. The RPM also suspends microorganisms in growth media; however, this suspension is maintained by randomly adjusting the movement of the bioreactor. Diamagnetic levitation relies on a strong magnetic field to levitate microbial cultures, and thus reproduce aspects of microgravity. As with all spaceflight analogues the fidelity of these and other culture devices to reproduce culture during spaceflight is not completely known, as the mechanisms driving the alterations in microbial response are unclear.

D. **The need for human surrogate animal models.** The need for having animal models of microbial infection is based on the necessity of having an experimental species whose inflammatory and pathological response closely resembles the human host. In addition, animal models which can be manipulated genetically provide a tremendous advantage to dissect out the underlying molecular mechanisms. Additional requirements of an excellent animal model are reproducibility of the pathological response and availability of a wide range of molecular/biological targets that can be used to thwart or aggravate the response or design effective countermeasures. Depending on the infection and type of study, animal models (mammalian) that have proven to be useful in terrestrial experiments include the rabbit, rat, guinea pig, pig, dog, monkey and mouse. In particular, with the emergence of genetic and conditional knockouts and RNA interference strategies, mouse models of systemic and local inflammatory infectious diseases are more popular than others. Moreover, their small size and short gestation period makes them ideal for biomedical research. Much of our present knowledge about the immune system in space comes from studies conducted on space-flown mice [13-17]. Moreover, to test the pathologic potential of spaceflight conditions, bacteria grown in space have been injected in mice. Such studies have looked at survival, local and systemic inflammation, and pathophysiology of organs [10]. Hind limb unloading is a widely used ground-

based model of simulated microgravity in mice and has been utilized to investigate some of the effects of spaceflight on microbial infection [18, 19].

Even though mice are relatively small, the number of mice that could be infected during spaceflight is extremely limited. As such, other models enabling a greater sample size are being investigated. For example, *Caenorhabditis elegans* (nematode) as a human surrogate model of infection has been used in research aboard the ISS under the commercial flight experiment designated as "National Laboratory Pathfinder, Vaccine". While multiple flight experiments have been accomplished no findings have been released. A future flight experiment using *C. elegans,* designated as "Micro-5," will investigate *Salmonella* virulence and is manifested for implementation on the ISS. In addition, *Drosophila melanogaster* (fruit fly) has been used successfully to investigate host-pathogen interaction while using the *Drosophila* as the host after spaceflight on STS 121. Results are published [20] and are discussed in greater detail in this report's section, *Alterations in specific host-microorganism interactions*, on page 11.

Human tissue culture models have also been investigated for use as infection models during spaceflight. In 2010, the flight experiment designated "Space Tissue Loss, IMMUNE" flew aboard STS-131 and was the first infection of human tissue culture cells by a pathogen. The potential of this model is intriguing as mammalian cells cultured during spaceflight have been demonstrated to develop a three dimensional architecture that reproduces many *in vivo* characteristics [21].

IV. Knowledge Gaps

The Human Research Program has defined four areas of study with levels of uncertainty that could impact the proper assessment and mitigation of this risk. These include:

- AEH 7: What changes are occurring to microorganisms during human exploration of space that could affect crew health?
- AEH 8: What changes are occurring to host susceptibility during human exploration of space that could affect crew health?
- AEH 9: What changes are occurring to specific host-microorganism interactions during human exploration of space that could affect crew health?
- AEH 10: What changes are occurring to the efficiency of current countermeasures?

V. Evidence

A. Alterations in microbial response

a. *Microbial ecology*. Stringent microbiological monitoring of spacecraft has been performed operationally aboard NASA spacecraft throughout the human spaceflight program [22, 23]. Additional spaceflight experiments have also provided greater detailed information by investigating specific niches aboard spacecraft or utilizing alternative methodologies beyond the culture-based isolation historically used [24]. Generally, the data indicate that the potable water, air, and surfaces to which the crew are exposed are free of obligate pathogens; however, opportunistic pathogens such as *Pseudomonas aeruginosa, Stenotrophomonas maltophilia,* and *Staphylococcus aureus* are not uncommon [22, 25]. In addition, identification of microorganisms collected from free-floating water behind panels indicated several potentially medically significant organisms not commonly isolated during standard operational monitoring, including *Legionella* species, and *Serratia marcescens*, and *Escherichia coli* [26]. Further microscopic examination of these samples revealed the presence of amoeba resembling *Acanthamoeba* or *Hartmanella* species and ciliated protozoa resembling *Stylonychia* species [26].

Spaceflight food is currently provided for missions in a shelf stable form for storage at ambient temperature [27]. As such, microbiological contamination control, including stringent microbial monitoring, is maintained. While the incidence of contamination is low, preflight analyses of food samples have indicated the presence of organisms such as *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium), *Staphylococcus aureus*, *Enterobacter cloacae* and *Enterobacter sakazakii* (unpublished data). Contaminated lots are removed before shipment for flight; however, these findings suggest a potential route of infection to the crew. Future spaceflight missions may also provide food with potentially high levels of microorganisms, such as freshly grown crops or foods with probiotic organisms to promote astronaut health. The production and monitoring requirements of these foods are only beginning to be evaluated; initial findings can be found in the HRP report, *Development of Spaceflight Foods with High Microbial Concentrations* (http://www.nasa.gov/centers/johnson/slsd/about/divisions/hefd/about/publications.html).

For spaceflight missions, the primary source of microorganisms is the crew. Selected preflight microbiological monitoring is performed prior to launch, with testing based on the mission design. One key aspect of preflight operations is the Flight Crew Health Stabilization Program, which was established during the Apollo Program in response to problems with incidences of infectious illness [28]. The focus of the program involves

reducing the exposure of flight crews to groups and individuals that are at high risk of harboring infectious disease (*e.g.*, large crowds, small children) beginning approximately 10 days prior to launch.

b. *Microbial growth*. A number of studies have revealed altered growth kinetics of microorganisms in true spaceflight and spaceflight analogue conditions [29, 30]; however, determining general conclusions is complicated by the wide variety of organisms studied and differences in experimental parameters. As example organisms, the differences in growth observed in several spaceflight experiments using the microorganisms, *Escherichia coli* and *Bacillus subtilis*, are summarized in Table 1. While these data are indicative of multiple historical observations with many organisms, the trends from these spaceflight experiments are difficult to establish. Interpretation of the results is complicated as the media composition, apparatus and strain of organism is not necessarily consistent between different experiments.

Table 1. Summary of reported alterations in growth characteristics in *Escherichia coli* and *Bacillus subtilis* in true spaceflight

Organism	Observation	Reference
Bacillus subtilis	• Shorter *lag* phase • Increase in the rate of cell division and biomass	[1]
	• Growth Decreased 4-11% below control for 3of 4 separate shuttle missions; the other mission growth increased by 26%	[2]
	• Shortened lag phase; greater final cell concentration	[3]
Escherichia coli	• No change in final cell densities	[5]
	• Minimal glucose media • Doubling time increased to 46 min compared to 59 min for the static ground controls • No increase in cell size • General conclusion: no significant change in growth kinetics	[6]
	• No growth change compared to controls on 3 of 4 separate shuttle missions; the other mission growth increased by 57%	[2]
	• Shortened lag phase; greater final cell concentration	[3]

c. *Microbial virulence, virulence characteristics, and gene expression. S.* Typhimurium is an obligate enteric pathogen with a potential to infect the crew during a spaceflight mission through the spaceflight food system. Extensive initial studies of the response of *S.* Typhimurium to the spaceflight analogue environment in the RWV indicated an increase in microbial virulence using a murine model of infection [10]. The microorganisms also displayed altered stress responses, gene expression, and survival in macrophage cells [10, 11]. Building upon this information, the MICROBE flight experiment was performed by Dr. Cheryl

Nickerson in 2006 aboard the Space Shuttle during mission STS-115. In this experiment, *S.* Typhimurium was grown during flight and compared to identically cultured ground controls [9]. The cultures were either placed in an RNA fixative during flight or returned as live cultures for virulence testing. The cultures grown aboard the Space Shuttle displayed an extracellular matrix that was not seen in the ground controls. Evaluation of the gene expression indicated 167 genes and 73 proteins were differentially regulated compared to ground controls, with the conserved RNA-binding protein Hfq identified as a likely global regulator involved in the response to this environment. Subsequent experiments using the RWV bioreactor supported the necessity of Hfq in the spaceflight/spaceflight-analogue response [9]. In addition, cultures grown in a Lennox Broth medium during flight displayed a 2.7 fold lower LD_{50} in a murine model when compared to inoculation with ground control cultures. This experiment produced several key findings including: (1) the experiment clearly indicated alterations in the expected dose-response curves with implications for the microbial risk assessment of infection potential for the crew during a mission; (2) the experiment provided the first insight into a molecular mechanism behind the alterations of microorganisms during spaceflight culture; and (3) the virulence and gene expression results from the spaceflight experiment paralleled the trends observed with the RWV spaceflight analogue [10], supporting this bioreactor as an indicator of potential microbial alterations during spaceflight.

In 2008, Nickerson and her colleagues reproduced the evaluation of virulence changes using *S.* Typhimurium cultured aboard the Space Shuttle during mission STS-123 [31]. Cultures grown in a Lennox Broth medium during flight displayed a 6.9 fold lower LD_{50} in a murine model when compared to inoculation with ground control cultures. Interestingly, the change in virulence was not observed when spaceflight cultures were grown in a simple salt, M9 medium or in Lennox Broth supplemented with 5 key inorganic salts used in the M9 formulation. Subsequent experiments using the RWV bioreactor suggested that high concentrations of inorganic phosphate may be the salt influencing the spaceflight/spaceflight-analogue response [31].

During the MICROBE experiment, the global transcriptional responses of *Pseudomonas aeruginosa* to spaceflight culture were also investigated [32]. *P. aeruginosa* responded to spaceflight conditions through differential regulation of 167 genes and 28 proteins, with Hfq as a global transcriptional regulator. Key virulence-related genes that were differentially regulated included the lectin genes, *lecA* and *lecB*, and the gene for rhamnosyltransferase (*rhlA*), which is involved in rhamnolipid production. As with *S*. Typhimurium, the transcriptional response of

spaceflight-grown *P. aeruginosa* displayed many similarities to trends observed during culture of *P. aeruginosa* in the RWV bioreactor [33, 34].

The degree of similarities between the microbial responses during culture in the spaceflight analogue RWV bioreactor and true spaceflight have important implications in understanding the microbiologically associated risk during spaceflight missions. Indeed, *P. aeruginosa* cultured in the RWV displayed distinct changes in its biofilm architecture compared to controls [33], which could impact its virulence and antibiotic resistance. In addition, RWV culture of *P. aeruginosa* appears to influence the *rhl N*-butanoyl-L-homoserine lactone (C4-HSL) directed quorum sensing (QS) system, increasing the production of rhamnolipids, and potentially having an impact on the virulence of the organism [33].

Numerous strains of *E. coli* have been cultured in the RWV. Investigations with *E. coli* MG1655 cultured in Luria Broth displayed decreased growth, the down-regulation of 14 genes, and no discernable changes to environmental stressors, such as resistance to acid and osmotic stress when compared to controls [35]. When this same strain was cultured in a minimal salts media, no difference in growth was observed and 35 genes were differentially expressed [35]. Conversely, culture of *E. coli* AMS6 in minimal media demonstrated an increased resistance to acid and osmotic stress in response to the low-shear conditions [36]. Interestingly, culture of this strain in the RWV displayed significantly higher biofilm production on glass microcarrier beads placed in the reactor [37]. Investigation of the response of adherent-invasive *E. coli* O83:H1to culture in the RWV indicated this organism did not change growth, acid or osmotic resistance; however it did display an increased resistance to thermal and oxidative stress in minimal media [38]. Interestingly, low-shear-cultured *E. coli* O83:H1 displayed increased adherence to epithelial cells although invasion rates were unchanged as compared to controls [38].

Other organisms beyond Gram-negative pathogens have been evaluated using the RWV. The response of *Staphylococcus aureus* to RWV culture has been the most thoroughly studied Gram-positive microorganism. Interestingly, while gene expression appears to be regulated by *Hfq* [39], as seen with *S*. Typhimurium and *P. aeruginosa*, virulence characteristics, such as staphyloxanthin production and hemolytic activity appear to be repressed [39, 40]. Culture of *Streptococcus pneumoniae* in the RWV has also been studied as 41 genes were reported to be differentially regulated [41]. The pathogenic yeast *Candida albicans* displayed random budding patterns and enhanced filamentous growth when cultured in the RWV, suggesting a more pathogenic phenotype [42].

d. *Heritable changes in the microbial genome.* The environmental conditions during spaceflight missions, especially those beyond low Earth orbit, could

impact the selective pressure to increase and stabilize heritable mutations in the microbial genomes. These environmental conditions include changes in the intensity and type of radiation as well as gravity compared to terrestrial conditions. Spaceflight studies exploring this possibility have been limited in part due to the resources necessary to perform long-duration growth experiments. However, some evidence suggests a change in the normally expected mutation rate may occur. Ciferri, *et. al.* evaluated changes in the conjugation, transduction, and transformation using *E. coli* cultures [43]. While the rate of pairing did not appear to be affected during conjugation in spaceflight cultures, they did note that the pairs were being held longer, which they attributed to the absence of external disruptive forces. No differences were reported for transduction, and the results for transformation were inconclusive.

The extent of heritable changes in the microbial genome that are induced by spaceflight radiation and microgravity is unclear. While several spaceflight experiments have investigated aspects of this topic [44-46], no general trend or mechanism has been defined.

e. *Secondary metabolite production.* Alterations in the biochemical pathways of microorganisms have been investigated in multiple spaceflight and ground-based studies. For example, alterations in the production of the secondary metabolite, Actinomycin D, were measured by Benoit, *et. al.* from *Streptomyces plicatus* grown in gas-permeable culture bags aboard the International Space Station [47]. Unfortunately, all cell concentrations over time were not available, and the authors speculated that these changes may have been the result of differences in growth profiles of spaceflight and ground-based cultures that had been previously reported by Mennigmann, *et. al.* in previous studies [1].

The potential of spaceflight-associated changes in secondary metabolite production has been studied in greater detail using RWV culture of microorganisms. In a series of publications, Fang, *et. al.* reported that culture in the RWV resulted in the reduction of β-lactam antibiotics by *Streptomyces clavuligerus* [48], reduction of microcin B17 (MccB17) production by *E.coli* [49], but no change in Gramicidin S by *Bacillus brevis* [50].

f. *Potential mechanisms behind altered microbial response.* The stimulus and corresponding mechanism responsible for the reported microbial responses is not fully understood and thus limits the application of this data for medical operations. Some evidence was provided by investigating the responses of *E. coli* and *B. subtilis* when cultured on semi-solid agar during spaceflight to determine if alteration in fluid dynamics would impact growth profiles [2]. When cultured on this substrate, neither organism displayed the differences in cell concentrations between spaceflight

cultures and controls normally expected when cultured in only a liquid medium. This finding suggested that alterations in gravity alone were not the determining factor, and that fluid dynamics may play a role in the previously observed differences. The concept that fluid dynamics, specifically fluid shear, is a contributing factor of this response was supported by spaceflight-analogue studies of *S.* Typhimurium cultured in the RWV [51]. In these experiments, a correlation was observed between the progressive addition of shear into the system and a decrease in microbial responses associated with culture in the RWV. The potential of a spaceflight-associated mechanotransductive response, which is the product of changes in physical forces on the cell membrane would not be without precedence, as shear forces have been demonstrated to impact microbial responses [52, 53]. Indeed, a number of bacterial cytoskeletal structures, such as MreB (actin homolog) and FtsZ (tubulin homolog) have been identified [54]. Taken together, this evidence suggests the responses, such as altered growth, observed with microorganisms resulting from spaceflight culture may be the result of the secondary effects found in liquid culture during spaceflight, such as very low fluid shear.

Insight into the molecular mechanisms responsible for the microbial response of microorganisms to spaceflight was provided during the investigation of *S.* Typhimurium during the MICROBE experiment aboard STS-115 [9]. An evaluation of differentially regulated genes identified by comparing spaceflight and otherwise identically cultured organisms revealed an association of many of the genes with the conserved RNA-binding regulatory protein Hfq. Culture of the same *S.* Typhimurium strain with an *hfq* deletion in the RWV indicated the gene was necessary to produce the spaceflight-associated responses. Upregulation of the *Hfq* gene has been associated with increased virulence [55]. Interestingly, while *S.* Typhimurium grown in the Lennox Broth during spaceflight culture increased in virulence, *Hfq* was down-regulated [9]. Since this study, the down-regulation of *Hfq* has been associated with spaceflight associated response in both spaceflight cultured *P. aeruginosa* [32] and RWV cultured *S. aureus* [39]. Collectively, these reports suggest the response may be evolutionarily conserved across multiple species.

The follow-up experiment to MICROBE flown aboard STS-123 demonstrated that changes in the ion concentrations impact the spaceflight associated response [31]. As mechanosensitive ion channels exist in bacteria that trigger ion transport [56], the potential that mass transfer during spaceflight or alterations in ion permeability at the cell memebrane are also potential factors that could impact the spaceflight-associated response.

B. Alterations in host immune response

A large body of evidence indicates dysfunction of aspects of the crewmember's immune system during spaceflight missions. This evidence is described in the HRP evidence book addressing "Risk of Crew Adverse Health Event Due to Altered Immune Response."

C. Alterations in specific host-microorganism interactions

Infection studies during flight in which the host and pathogen are both in microgravity during spaceflight are difficult, and no data has been reported to date. As previously mentioned, infection of human tissue culture with *S.* Typhimurium was performed on STS-131 with results expected to be published shortly. Infection of *C. elegans,* during the Micro-5 spaceflight experiment will investigate *Salmonella* virulence and is manifested for implementation on the ISS during late 2012.

Key evidence providing evidence on potential changes in the host response was obtained by using *E. coli* to infect *Drosophila melanogaster* (fruit fly) after return from a 12-day spaceflight mission on STS 121 [20]. In this experiment, five containers each with 10 female and 5 male adult flies were flown and bred on the space shuttle with more than 3000 animals in them that were used for subsequent analyses. The study reported that the *Drosophila* larval innate immunity was depressed after spaceflight. In addition, there was a reduction in phagocytosis efficiency of larval plasmatocytes and of plasmatocyte counts with a concurrent decrease in the expression of genes related to hemocyte maturation. A decrease in gene expression of many of the key humoral immunity genes was also observed. The adult flies were able to clear *E.coli* infection post-flight but showed differences in the kinetics and levels of antimicrobial peptide (AMP) gene expression when compared to the matched ground control flies. This experiment provided insight into immunological alterations that could occur during spaceflight and provides a model to look at various aspects of host-microbe interaction to complement data obtained from other models.

D. Alterations in efficiency of antibiotics

The primary post-infection countermeasure during spaceflight is the use of antibiotics; however, several spaceflight experiments have provided evidence suggesting alterations in antibiotic resistance when microorganisms are cultured during spaceflight. 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 *E. coli* were compared to those of ground controls [57]. These early results indicated an increased resistance of both *S. aureus* and *E. coli* to all antibiotics used in this experiment [57]. However, the observed alterations in microbial antibiotic resistance during spaceflight may be transient, as attempts to reproduce these

changes after return to Earth have been unsuccessful [58]. Spaceflight experiments culturing *E. coli* during STS-69 and STS-73 suggested gentamicin on agar slants that were flown was as effective as and possibly more effective than the antibiotic on ground-based control cultures[59]. In 1999, 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 [60]. While these experiments suggest spaceflight-associated changes in microbial response to antibiotics, the information is not adequate to be predictive about reproducibility with the selected microorganisms, the impact of antibiotics on other microorganisms, or the actual microbial response during exposure in a human host.

VI. RISK IN CONTEXT OF EXPLORATION MISSION OPERATIONAL SCENARIOS

Current medical operations do not incorporate potential alterations in host-microorganism interactions, *per se*; however, the risk of infection is greatly minimized through current vehicle design and operational requirements. Vehicles and their systems are designed to maintain microbial concentrations at very conservative levels (*e.g.*, potable water below 50 CFU per ml). Operational activities are also designed to limit crew exposure, including preflight crew quarantine and stringent preflight/in-flight monitoring.

As the risk of infectious disease is a function of the presence and characteristics of the agents, the dose-response of those agents, and the crew exposure to those agents, the risk of infectious disease during different mission scenarios varies depending on several potential factors, including mission duration, design of the environmental life support system, and continued/repetitive use of the facility. Any change in the risk of infectious disease attributed to spaceflight would have corresponding change in the vehicle design or operational activities. For example, if spaceflight induces changes in the concentration or virulence of opportunistic pathogens during a mission, appropriate adjustments in allowable microbial concentrations, housekeeping, or antibiotic provision may need to occur.

VII. CONCLUSION

Numerous spaceflight experiments have been conducted to investigate alterations in microbial responses resulting from culture during spaceflight and spaceflight-analogues. However, recent studies investigating spaceflight-associated alterations in microbial virulence have initiated the review and production of evidence to better understand the impact these alterations would have on the incidence of infectious disease during a spaceflight exploration mission. The preponderance of evidence indicates that alterations in microbial gene expression and phenotype (including virulence) are occurring; however, the clinical implications of such changes are still unclear. Greater knowledge is required including a better understanding of the mechanism behind unique spaceflight-associated microbial responses to determine how this environmental stimulus impacts various microorganisms, their diversity and

concentration in the spacecraft and crew microbiome, their impact on the vehicle and crew, and their resistance to current mitigation and antibiotic regimens.

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X. LIST OF ACRONYMS

IOM – Institute of Medicine

ISS – International Space Station

HRP – Human Research Program

HSL – Homoserine Lactone

NASA – National Aeronautics and Space Administration

PRD – Program Requirements Document

QS – Quorum Sensing

RNA – Ribonucleic Acid

RPM – Random Positioning Machine

RWV – Rotating Wall Vessel

STS – Space Transportation System