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MICROBIAL CONTAMINATION OF SPACECRAFT

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ABSTRACT

Spacecraft and space habitats supporting human exploration contain a diverse population of microorganisms. Microorganisms may threaten human habitation in many ways that directly or indirectly impact the health, safety, or performance of astronauts. The ability to produce and maintain spacecraft and space stations with environments suitable for human habitation has been established over 40 years of human spaceflight. An extensive database of environmental microbiological parameters has been provided for short-term (< 20 days) spaceflight by more than 100 missions aboard the Space Shuttle. The NASA Mir Program provided similar data for long-duration missions. Interestingly, the major bacterial and fungal species found in the Space Shuttle are similar to those encountered in the nearly 15-year-old Mir. Lessons learned from both the US and Russian space programs have been incorporated into the habitability plan for the International Space Station. The focus is on preventive measures developed for spacecraft, cargo, and crews. On-orbit regular housekeeping practices complete with visual inspections are essential, along with microbiological monitoring. Risks associated with extended stays on the Moon or a Mars exploration mission will be much greater than previous experiences because of additional unknown variables. The current knowledge base is insufficient for exploration missions, and research is essential to understand the effects of spaceflight on biological functions and population dynamics of microorganisms in spacecraft.

Equally important is a better understanding of the immune response and of human-microorganism-environment interactions during long-term space habitation.

INTRODUCTION

The International Space Station (ISS) is rapidly becoming a reality that holds much promise for the conduct of science but also presents many microbiological challenges to continuous human habitation. Past spaceflight experiences have demonstrated that microorganisms are ubiquitous in space much as they are on Earth (Cioletti et al., 1991; Pierson, 1993; Pierson et al., 1993; Pierson et al., 1994; Mishra and Pierson, 2000). Most microorganisms do not threaten human health, and will likely play essential roles in making long-term human habitation possible in space. These roles may be found in solid waste remediation and water and air purification, and microorganisms may even serve as food sources on long-term missions. We must be mindful, however, that in a closed environment microorganisms may produce adverse effects on the optimal performance of space crews and the integrity of the spacecraft or habitat (Mishra et al., 1992; Burge, 1995; Burge et al., 2000). These effects range from infections, allergies, and toxicities to degradation of air and water supplies. Biodegradation of critical materials may result in system failure, endangering crews (Pierson et al., 1994; Ahearn et al., 1995). Plant pathogens may also affect crew health and performance by destroying plants serving as part of the food supply or the systems recycling air, water, or waste. Some latent viruses may lead to immunosuppression.

The risk of infectious disease is expected to increase as mission duration increases. A number of other factors increase this risk. Living and working in

relatively crowded conditions in an environment with reclaimed water and air are contributory factors. Limitations in diagnostic and treatment technologies and crew return serve to further increase the risks and consequences. Preventive measures exist that have proven effective in past and present spaceflight programs (Ferguson et al., 1975; Pierson, 1993; Pierson et al., 1993). The astronauts are exceedingly healthy, and many infectious disease risks in the general population are not probable risks in astronauts. Examples of unlikely infectious agents include human immunodeficiency virus, tuberculosis, and hepatitis B and C, for which the astronauts are screened. Much more likely are infections from the astronauts' normal microbiological flora. For example, staphylococcal and streptococcal skin infections and urinary tract infections with an *Escherichia coli* etiology are more likely scenarios. Latent viruses will remain a risk to the astronaut crews because of their ubiquity and the ineffectiveness of current preventive practices (e.g., quarantine) (Payne et al., 1999; Mehta et al., 2000; Stowe et al., 2000). Clinically significant decreases in the immune response will result in sharp increases in disease risks (Pierson, 1993; Taylor et al., 1997).

SPACE SHUTTLE

Bioaerosols are increasingly recognized as important contributors to degradation of indoor air quality (Burge, 1995; Amman and Burge, 1999). Since the inception of the US space program, the spacecraft environment has been monitored to

ensure a safe environment for the crewmembers. The Apollo spacecraft were monitored before flight, but no in-flight samples were collected (Ferguson et al., 1975). The first in-flight air samples to be analyzed for bacteria and fungi were taken during the Skylab missions (Taylor et al., 1977). The Space Shuttle contains about 65 m^3 of habitable volume, including the flight deck and the mid-deck. When the Spacelab is included, the habitable volume increases by 77 m^3. A small portable, battery-operated centrifugal air sampler (Biotest Diagnostics Corp., Denville, N.J.) is most commonly used to assess the airborne bacterial and fungal content of the Space Shuttle (Mehta et al., 1996; Mehta et al., 2000), as in Figure 1. The airborne bacteria and fungi are impacted onto an agar surface (trypticase soy agar for bacteria, rose bengal for fungi). The data from the STS-58 mission are shown in Figure 2. Bacterial levels tend to increase modestly during flight, whereas fungal levels are usually low and remain low throughout the mission. It is not uncommon for the fungal levels to decrease as the mission continues, probably because of low humidity (generally < 50%) and lack of a continuous source of fungi. *Staphylococcus*, *Micrococcus,* and *Bacillus* are the most common bacterial genera recovered from the air. Most bacterial species cultured, with the exception of *Bacillus*, are commonly associated with humans. *Aspergillus*, *Penicillium*, and *Cladosporium* are the fungal genera most frequently collected from the air.

Bacteria and fungi are cultured from 12 crew compartment surfaces before and after Space Shuttle missions. Average preflight levels of bacteria are 300 colony-forming units (cfu)/100 cm^2 or less. As with the air samples, the genera of bacteria

most commonly cultured from spacecraft surfaces are *Staphylococcus*, *Micrococcus*, *Corynebacterium*, and *Bacillus*. Fungal levels are low, generally under 100 cfu/100 cm^2. *Aspergillus*, *Penicillium*, and *Cladosporium* are the most frequently cultured fungi.

Potable water is generated by the fuel cells, and passage through iodinated resins provides a residual disinfectant of 2 to 3 ppm iodine. Bacterial levels in potable water are very low and *Burkholderia cepacia* is the most commonly cultured bacterial species.

Initially, the Shuttle flight crews were examined twice before flight and again at landing. Swab specimens from the external nares and throat and urine and fecal samples were evaluated. Currently, the crew microbiological sampling schedule for short-term missions has been reduced to one sampling period 10 days before launch. The microbiological profiles are typical of healthy individuals, and no significant changes in microbiota resulting from spaceflight have been detected (Pierson et al., 1993). DNA fingerprinting by restriction fragment length polymorphism (RFLP) technology was used to follow the movement of *Staphylococcus aureus* (Pierson et al., 1996) and *Candida albicans* (Pierson et al., 1995) among the Space Shuttle crewmembers. Contrary to earlier reports (Ferguson et al., 1975; Taylor et al., 1977), we did not find cross-colonization among crewmembers to be a common event. Clearly, cross-contamination is common and unavoidable, but colonization is not a common occurrence among Space Shuttle crewmembers according to our findings. However, we have documented cross-colonization occurrences of *C. albicans* in the

oral cavity among family members. *C. albicans*-positive children were shown to carry the same strain as one or both parents (Mehta et al., 1999).

MIR

Nine Russian space stations have orbited since 1971, and Mir was in operation for nearly 15 years. The NASA Mir Program afforded an historic opportunity for the US to gain knowledge of long-term habitability aboard spacecraft. The dynamics of microbial buildup, selection, and adaptation processes could be studied in a space station that had hosted many international crew members and with which many resupply spacecraft had docked.

The ISS Phase 1 Program resulted in seven US astronauts residing aboard the Russian space station Mir between March 1995 and May 1998. Collaborations between US and Russian scientists consisted of sample collection and analyses from the crewmembers and the Mir and Shuttle environments before, during, and after missions of 75 to 209 days' duration.

Microbiological samples were collected before and after spaceflight from the throat, nose, ears, hands, axilla, groin, and urine. Sampling began approximately five months before launch and continued approximately monthly until launch. Three postflight sampling periods, beginning at landing and extending up to 14 days afterward, were included. Analysis revealed aerobic microbiota of the crewmembers consistent with healthy individuals, and no medically significant changes were found.

Fecal samples were collected during the preflight period and analyzed for aerobic bacteria, selected anaerobic bacteria, fungi, and parasites. Unfortunately, fecal samples were rarely available after the missions, and no conclusions on effects of spaceflight on enteric microorganisms were possible. Overall, the Mir crew microbiological results were similar to findings from Shuttle crews (Pierson et al., 1993).

Spacecraft air samples were collected using a Burkard impaction air sampler, as shown in Figure 3. Figure 4 presents the levels of bacteria and fungi at four different locations on Mir. Mean bacterial levels at the four sites ranged from approximately 200 to 425 cfu/m^3 of air (the Russian acceptability limit was 500); the mean of the fungal levels ranged from 175 to 325 cfu/m^3 (the Russian acceptability limit was 100). *Staphylococcus*, *Bacillus*, and *Corynebacterium* were the bacterial genera cultured most frequently from Mir air. *Penicillium*, *Aspergillus*, and *Cladosporium* were the fungal species most frequently cultured from air samples. *Aspergillus flavus* was recovered from approximately 50 % of the samples.

Mir surfaces were sampled using a swab and inoculating solid media, as shown in Figure 5. Mean bacterial levels were about 2700 cfu/100 cm^2 or less; fungal levels were 500 cfu/100 cm^2 or less. *Staphylococcus*, *Bacillus*, and *Micrococcus* were the most frequently isolated bacteria, and *Penicillium*, *Candida*, and *Aspergillus* were the most frequently isolated fungal genera from Mir surfaces.

The Mir potable water sources were analyzed during flight, and samples were archived and returned to Earth for analysis. Humidity condensate was reclaimed

through a water processor system that included a terminal heat step. Bacterial levels in this water source were very low. Some water was brought from ground sources and stored aboard Mir for potable use. This water tended to have higher bacterial counts. Analyses indicated that bacterial levels in potable water on board the Mir were within Russian acceptability criteria.

INTERNATIONAL SPACE STATION

Our experiences with the Mir increased our understanding of microbial population dynamics during long stays in space, preparing us for the ISS era. While construction is underway, small international crews inhabit the ISS on a continuous basis. Prevention has been the cornerstone of our approach to microbiological risks associated with living and working aboard the ISS. Experience has taught us it is much easier to prevent problems of microbial origin than to attempt in-flight solutions. Our efforts began in the design phase. Examples include the addition of HEPA (or equivalent) filters in the air regeneration system to remove particulates and airborne microorganisms. Restrictions on accumulation of moisture (e.g., air handlers), relative humidity, and materials selections are but a few of the measures taken during the design phase to mitigate the adverse effects of microorganisms. Preflight analysis of the spacecraft environment and the food and water to be used on the ISS are additional safeguards. US-provided hardware and cargo for the ISS are maintained under "visibly clean" conditions and reviewed for biosafety risks. Risk mitigation will also

include in-flight monitoring of the environment (air, surfaces, and water), and routine housekeeping and periodic detailed inspections are essential components of ensuring habitability with minimal adverse effects on the crew.

Current plans require monitoring of ISS air and surfaces for viable bacterial and fungal contaminants every 90 days. A battery-operated, portable air impact sampler is used by crew members for air quality assessment. The acceptability limits for airborne bacteria and fungi are 10,000 and 100 cfu/m^3 of air, respectively. Selected surfaces are sampled by swab and transferred to solid media for bacterial and fungal determinations. The bacterial acceptability limit is 10,000 cfu/100 cm^2 whereas the acceptability limit for fungi is 100 cfu/100 cm^2. There are several sources of potable water aboard the ISS. Some of it is provided by the reclamation of humidity condensate; eventually hygiene water and urine will be reclaimed to potable standards. Ground-supplied potable water is transported to the ISS and stored in tanks for use as needed. Silver, at approximately 500 ppb, is used as the residual disinfectant in Russian water supplies whereas US water supplies use 2 to 4 ppm iodine for water disinfection. Water processed through the US water processor must be free of coliforms, with a total heterotrophic content not to exceed 100 cfu/100 mL of water. The Russian acceptability limit for heterotrophic bacteria is 10,000 cfu/100 mL.

FUTURE CONSIDERATIONS

Previous spaceflight experience has demonstrated the ability to provide a microbiologically safe space environment. These successes resulted primarily from a sound design and the implementation of appropriate preventive measures. Permanent habitation of the ISS presents new challenges to maintaining healthy and productive crewmembers, but these challenges will pale compared to the challenges of a Mars exploration mission or extended stays on the Moon. Provision of healthy air, potable water, and food for two to three years will be among the most fundamental of challenges of a Mars mission. Such long missions with limited onboard monitoring, diagnostic, and treatment technologies, and the inability to return to earth rapidly, will require more relevant data for risk assessment. Environmental data obtained from Space Shuttle and Mir studies indicate that environments consistent with healthy habitation can be achieved. However, the effects of radiation levels outside of the protection of low-earth orbit may have many adverse effects not yet experienced, including increasing mutation rates and decreasing human immune capabilities. Limited resources and operational constraints have slowed fundamental studies on the effects of spaceflight on microbial population dynamics, selection, adaptation, and other characteristics. Equally important, and yet to be adequately investigated, are the effects of spaceflight upon microbe-human interaction and the immune response. The ISS should serve as the platform to study the microbiology of a permanently inhabited spacecraft over a long time. A comprehensive understanding of microorganisms in space habitats and their interactions with the environment and humans is essential for safe, successful missions.

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FIGURE LEGENDS

Figure 1. Astronaut David Leestma Taking Air Samples on the Space Shuttle for Microbial Analysis.

Figure 2. Bacterial and Fungal Content of Shuttle Air. *Samples (about 84 liters) were taken from three locations on the Space Shuttle, on three days during flight. Bacterial and fungal colonies were counted after appropriate incubation. CFU, colony-forming units.*

Figure 3. Astronaut Jerry Linenger Taking Air Samples on Mir for Microbial Analysis.

Figure 4. Bacterial and Fungal Content of Mir Air. *Samples were taken from four locations on Mir during flight. Bacterial and fungal colonies were counted after appropriate incubation. CFU, colony forming units.*

Figure 5. Shuttle Mir Astronaut Jerry Linenger Taking Surface Samples on Mir for Microbial Analysis.

FIGURE 1
PIERSON

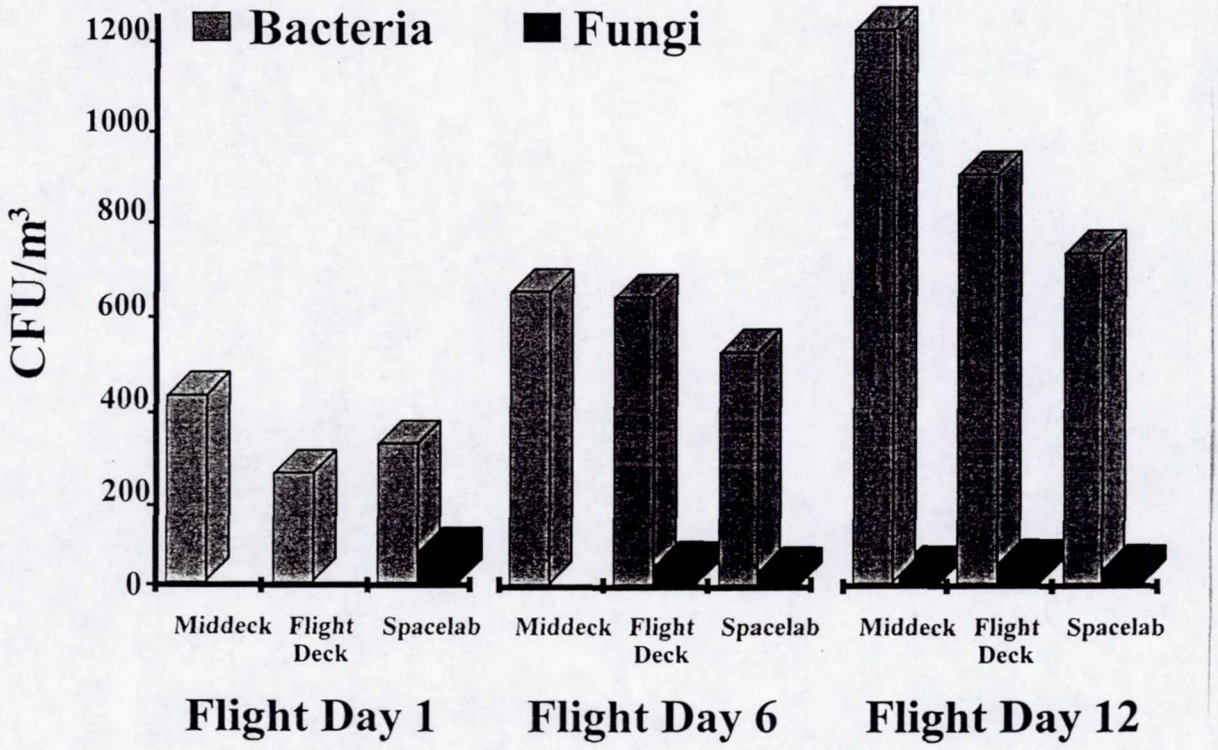

FIGURE 2 PIERSON

FIGURE 3
PIERSON

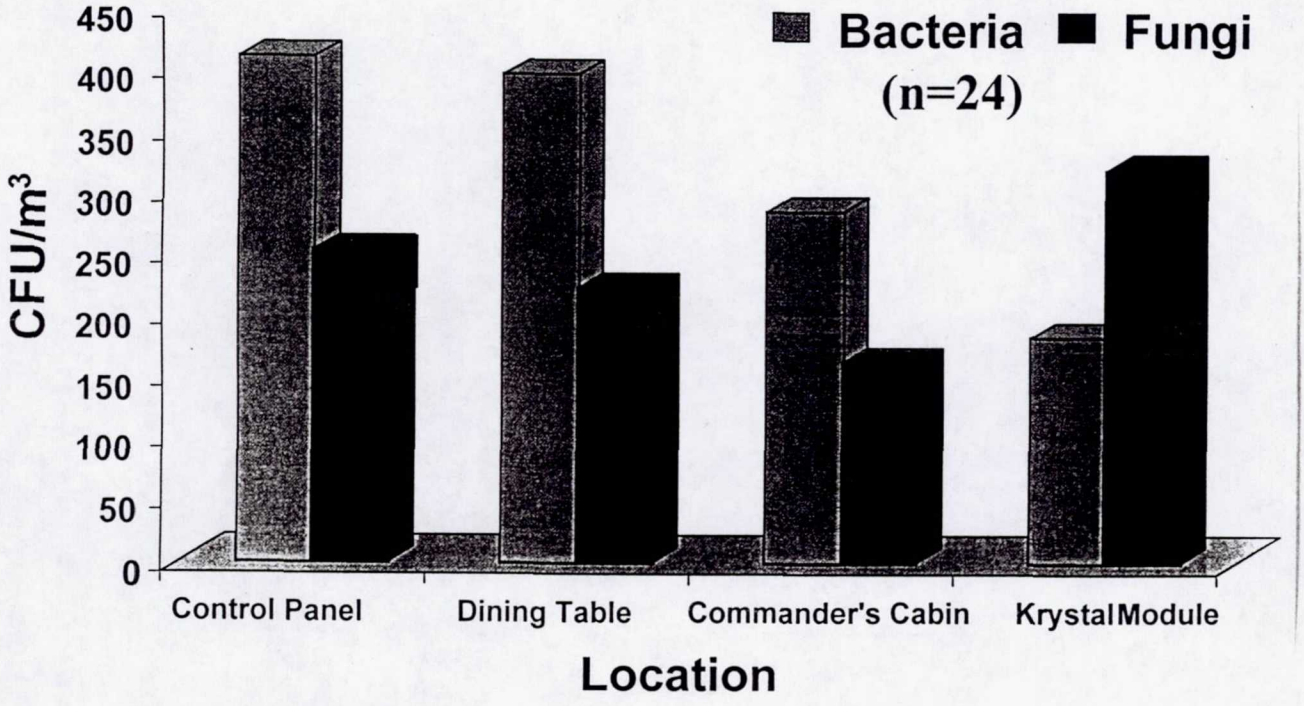

FIGURE 4 PIERSON

FIGURE 5
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