ADVERSE EFFECTS OF MICROORGANISMS

- Infectious Diseases
- Plant Diseases
- Food Spoilage
- Immune Alteration

- Toxin Production
- Allergies
- Volatile Release
- Material Degradation
- Environmental Contamination
<table>
<thead>
<tr>
<th>Sources</th>
<th>Controls</th>
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<tbody>
<tr>
<td>Crewmembers</td>
<td>Preflight screening, quarantine, vaccination, antimicrobials, antivirals</td>
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<td>Water</td>
<td>Preflight/inflight monitoring, biocides</td>
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<td>Food</td>
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<td>Air</td>
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<td>Payloads</td>
<td>Preflight cleaning, biosafety assessment, disinfection</td>
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PATHOGENS

Public Health

- Mycobacterium tuberculosis
- Corynebacterium diphtheriae
- Bordetella pertussis
- Neisseria meningitidis
- STDs
- Human immunodeficiency virus
- Hepatitis A
- Hepatitis B and C
- Influenza viruses
- Exotic Viruses

Space Flight

- Staphylococcus aureus
- Streptococci spp
- Escherichia coli
- Pseudomonas aeruginosa
- Legionella pneumophila
- Salmonella spp
- Herpes viruses
- Aspergillus spp
- Candida spp
- Penicillium spp

Exotic Viruses

Public Health

- M. tuberculosis
- C. diphtheriae
- B. pertussis
- N. meningitidis
- STDs
- HIV
- Hepatitis A
- Hepatitis B
- C. pneumoniae
- Influenza viruses
- Exotic Viruses

Space Flight

- S. aureus
- Strepococci spp
- E. coli
- P. aeruginosa
- L. pneumophila
- Salmonella spp
- Herpes viruses
- A. spp
- C. spp
- P. spp
FACTORS INCREASING DISEASE RISK

- Crowded Living Conditions
- Closed-Loop Environment (Water/Air)
- Reduced Capability for Personal Hygiene
- Limited Clean-up and Disinfection Capability
- Inability to Isolate Contagious Crewmember
- Limited Treatment Capability and Crew Return
- Altered Immune Response
PREVENTIVE MEASURES

- Crew Physical Examinations
- Immunization
- Health Stabilization Program
- Quarantine
- Preflight Food Testing
- Payload Biosafety Evaluation
- Establishment of Acceptability Limits
- Systems Design
- Environmental Monitoring
- In-Flight Housekeeping
- In-Flight Diagnostic Capabilities
- Antimicrobials
Bacteria Isolated From Shuttle In-Flight Air (n=16)

- **Flavobacterium** sp.
- **Klebsiella** sp.
- **Pseudomonas paucimobilis**
- **Serratia marcescens**
- Unidentified gram negative rods
- **Enterobacter** sp.
- **Acinetobacter** sp.
- **Streptococcus** sp.
- **Staphylococcus aureus**
- **Corynebacterium** sp.
- **Bacillus** sp.
- **Micrococcus** sp.
- **Staphylocooccus** sp.

**Isolation Frequency (%)**

- 0 20 40 60 80 100
Fungi Isolated From Air In The Dining Area (n=24)

- Acremonium sp.
- Hyphomycete
- Yarrowia sp.
- Candida sp.
- Paecilomyces sp.
- Unidentified yeast
- Cladosporium sp.
- Aspergillus niger
- Aspergillus sp.
- Aspergillus flavus
- Penicillium sp.
**Mir Water Data**

**Microbial Total Counts**

- **Dominant Bacteria:**
  - *Corynebacterium* sp.
  - *Xanthomonas* sp.
  - CDC group EF-4
  - *Flavobacterium* sp.

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<tr>
<th>Location</th>
<th>Colony Forming Units/100 mL</th>
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<tr>
<td>SRV-Hot</td>
<td>6.5</td>
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<tr>
<td>SRV-Cold</td>
<td>211</td>
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<td>SVO-ZV</td>
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</table>
Microbial Concentration and Characteristics
Mir Water Isolates - SRV-K “Warm”

✓ Agrobacterium rhizogenes
✓ CDC Group EF-4
✓ CDC Group II-H
✓ Clavibacter michiganense
✓ Corynebacterium aquaticum
✓ Flavobacterium species
✓ Flavobacterium meningosepticum
✓ Flavobacterium indolgenes
✓ Kingella kingae
✓ Kingella species
✓ Kluvera ascorbata
✓ Methylobacterium extorquens
✓ Pseudomonas aeruginosa
✓ Psychrobacter glathei
✓ Ralstonia pickettii
✓ Rhizobium loti
✓ Sphingobacterium thalpophilium
✓ Sphingomonas paucimobilis
✓ Suttonella indolgenes
✓ Xanthomonas campestris
✓ Xanthomonas maltophilia
✓ Xanthomonas species
“... The aging Mir ... is nearly overrun with the stuff (fungi). Visitors have found numerous fungal patches with hues between green and black, feeding behind control panels, slowly digesting the ship’s air conditioner, communications unit, and myriad other surfaces.”

Gareth Cook, Boston Globe Staff (10-1-00)
Microbial Concentration and Characteristics
Floating Condensate – Case Study

Wolf spent several hours working with Vinogradov to mop up a basketball-size drop of water...“I didn’t realize I bought myself anywhere from two to six hours per day doing this for the rest of the mission.”

*From DRAGONFLY by Bryan Burrough*
### Microbial Concentration and Characteristics

**MIR Condensate Fungi**

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<th>NASA 6</th>
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<td><strong>“SLIMY” SAMPLE</strong></td>
<td><strong>“FRESH” SAMPLE</strong></td>
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<td><em>Acremonium</em> species</td>
<td><em>Candida guilliermondii</em></td>
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<td><em>Candida krusei</em></td>
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<td><em>Fusarium</em> species</td>
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<td><em>Rhodotorula</em> glutinis</td>
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<tr>
<td><em>Rhodotorula rubra</em></td>
<td><em>Rhodotorula glutinis</em></td>
<td><em>Rhodotorula rubra</em></td>
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“…(fungi) feeding behind control panels, slowly digesting the ship’s air conditioner, communications unit, and myriad other surfaces.”

Gareth Cook, Boston Globe Staff
(10-1-00)
Davey and O’toole, MMBR, 2000

Dunne, Clin Microbiol Rev, 2002
Microbial Concentration and Characteristics

Food Microbiology Acceptability Limits

• Total aerobic count: <20,000 CFU/gram
• *Escherichia coli*: <1 CFU/gram
• Coagulase positive Staphylococci: <1 CFU/gram
• *Salmonella*: <1 CFU/gram
• Yeast and Molds: <1000 CFU/gram
## Disqualified Food Samples

<table>
<thead>
<tr>
<th>Sample description</th>
<th>Disqualifying agent</th>
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<tr>
<td>Freeze dried shrimp</td>
<td><em>Salmonella</em> species serotype &quot;B&quot;</td>
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<tr>
<td>Oatmeal with raisins</td>
<td><em>Aspergillus flavus</em></td>
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<tr>
<td>Miso soup</td>
<td><em>Staphylococcus aureus</em></td>
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<tr>
<td>Berry medley</td>
<td>Total aerobic (TNTC) <em>Bacillus</em> species</td>
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<tr>
<td>Chicken Pineapple salad</td>
<td><em>Enterobacter cloacae</em></td>
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<tr>
<td>Freeze dried chopped pecans</td>
<td><em>Aspergillus fumigatus, Penicillium species</em></td>
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<tr>
<td>Freeze dried corn</td>
<td><em>Klebsiella pneumoniae, Enterobacter cloacae</em></td>
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<tr>
<td>San Francisco seasoning</td>
<td>Total aerobic (TNTC) <em>Bacillus</em> species</td>
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<tr>
<td>Chicken salad</td>
<td><em>Pseudomonas aeruginosa</em></td>
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<tr>
<td>Onion medley seasoning</td>
<td>Total aerobic (TNTC) <em>Bacillus</em> species</td>
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<tr>
<td>Almond M&amp;Ms</td>
<td><em>Yeast species</em></td>
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<td>Japanese sugar candy</td>
<td><em>Yeast species</em></td>
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<td>Trail mix</td>
<td><em>Aspergillus niger, Aspergillus fumigatus</em></td>
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<tr>
<td>Chicken salad</td>
<td><em>Enterobacter cloacae, Enterobacter intermedius, Pantoea agglomerans</em></td>
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</tbody>
</table>
Microbial Monitoring – Environment

- Air assessments
- Surface assessments
  - Multiple locations within the vehicles
- Hardware assessments
  - Random samples to ensure quality control
- Payload assessments
  - Biosafety Review Board
IN-FLIGHT MICROBIAL SPECIFICATIONS AND MONITORING REQUIREMENTS FOR AIR AND SURFACES

Acceptability Limits

Surfaces:
- Bacteria: 10,000 CFU/100 cm²
- Fungi: 100 CFU/100 cm²

Air:
- Bacteria: 1000 CFU/m³
- Fungi: 100 CFU/m³

Sampling Frequency
Once per 3 months

Sampling Locations
Surfaces: 6 locations
Air: 3 locations

Modules
- FGB Node 1 Service Module
- U.S. Lab Node 2 Research Module 1
- Node 3 Research Module 2 U.S. Hab
### Microbial Concentration and Characteristics

#### ISS Bacterial Isolates - Air

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<td>Staphylococcus hominis</td>
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<td>Staphylococcus saprophyticus</td>
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<td>Staphylococcus simulans</td>
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<td>Staphylococcus xylosis</td>
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<td>Bacillus licheniformis</td>
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<td>Staphylococcus aureus</td>
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<td>Staphylococcus capitis</td>
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<td>Streptococcus species</td>
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<td>Corynebacterium afermentans</td>
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<td>Corynebacterium riegelii</td>
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<td>Staphylococcus species</td>
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<td>Micrococcus species</td>
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<td>Staphylococcus pumilis</td>
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<td>Acinetobacter lwoffii</td>
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*IBMP; Castro, et al, 2004*
SURFACE ANALYSIS

• 40 sampling sessions performed
• Bacterial samples = 353
  15 samples have exceeded acceptability limit (10,000 colony forming units/100 cm²)
  Most common isolates – *Staphylococcus and Bacillus* spp.
• Fungal samples = 311
  33 samples have exceeded acceptability limit (1000 colony forming units/100 cm²)
  Most common isolates – *Aspergillus* and *Penicillium* spp.
• Disinfection using Fungistat wipes performed
Microbial Concentration and Characteristics

Staphylococcus Sources - Crew
Microbial Concentration and Characteristics

DNA Fingerprinting - S. aureus

- **Blue** - isolated from the crew of ISS-5, the crew of ISS-4, and in-flight environmental isolates
- **Green** - isolated from the crews of ISS-1, ISS-4, and ISS-5
- **Orange** - isolated from crewmembers of ISS-1 and ISS-4 and from an in-flight environmental surface
• Microorganisms play a key role in the biodestruction of materials and can contribute to equipment and hardware malfunctions
IN-FLIGHT MICROBIAL SPECIFICATIONS AND MONITORING REQUIREMENTS FOR WATER

Acceptability Limits

Total Bacteria: 50 CFU/ml
Coliforms: Non-detectable/100 ml

Sampling Locations
Russian Segment:
SRV-K/Hot
SRV-K/Ambient
SVO-ZV

Sampling Frequency
Once every 3 months
Microbial Concentration and Characteristics

Water Microbiology Kit
Organisms Isolated from ISS Water

**SRV K Hot**
- Acidovorax temperans
- Acinetobacter radioresistens
- Caulobacter vibrioides
- Methylobacterium species
- Non-viable organisms
- Ralstonia eutropha
- Sphingomonas paucimobilis
- Sphingomonas stygialis
- Staphylococcus species

**SRV K Warm**
- Acidovorax temperans
- Corynebacterium species
- Dechlorosoma suillum
- Flexibacter species
- Methylobacterium species
- Microbacterium species
- Pseudomonas aeruginosa
- Ralstonia eutropha
- Ralstonia metallidurans
- Ralstonia pickettii
- Sphingomonas paucimobilis
- Sphingomonas stygialis
- Sphingomonas xenophaga
- Staphylococcus species

**SV0 ZV**
- Leifsonia xyli
- Methylobacterium fugisawaense
- Methylobacterium podarium
- Methylobacterium species
- Pseudomonas fluorescens
- Pseudomonas species
- Ralstonia pickettii
- Sphingomonas species
- Sphingomonas stygialis
- Sphingomonas yanoikuyae
- Stenotrophomonas maltophilia
- unidentified Gram negative rods
- unidentified Gram negative rods
Microbiology Water Analysis Kit (MWAK)

- Capability was initiated in May 2004 to perform analysis for in-flight detection of coliforms in ISS potable and stored water
- Dry media is contained in Coliform Detection Bag
- Direct bag to bag transfer of water
- 44 +/- 4 hours incubation period
- Presence/Absence Test
Engineering/Design Solutions

- HEPA air filters
- Moisture Control
- Antimicrobial materials
- In-line water filters
- Surface disinfectants
- Water biocides
- Trash containment
- Solid waste containment
LESSONS LEARNED

PREFLIGHT CREW MONITORING

ACCEPTABILITY LIMITS

IN-FLIGHT MONITORING

LIFE SUPPORT SYSTEMS

PREFLIGHT MONITORING of WATER

PREFLIGHT MONITORING of FOODS

PREFLIGHT MONITORING of PAYLOADS

HOUSEKEEPING

PREVENTION IS BETTER THAN REMEDIATION