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Targeted Amplification of Antibiotic Resistant Genes Associated with the International Space Station Environment

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Objectives

- Subsequent to the utilization of the traditional methods in monitoring the International Space Station (ISS), advanced molecular microbial analyses are required to assure the health and safety of crewmembers.
- National Research Council (NRC) recommended to utilize the ISS as “Microbial Observatory” to measure changes in microbial population due to the microgravity. Subsequently, several locations of ISS surfaces were sampled and studied utilizing state-of-the art molecular technologies.
- The occurrence of antibiotic resistance microorganisms and antibiotic resistance genes in the ISS environment is poorly understood, and the determination of the resistome will allow for further development of countermeasures for crew living in space as well as for the habitat maintenance.
- During two sampling events on the ISS (March and May, 2015), pre-moistened polyester wipes were used to sample eight different locations in the ISS (Table 1).
- Each sample was split to retrieve cultivable microorganisms, and to extract DNA for a downstream analysis. Traditional and molecular techniques were used to isolate, identify, and determine phenotypic and antibiotic resistance properties of the Biosafety Level 2 strains.
- AmpliSeq technology (Life Technologies) was used to determine a pool of antimicrobial resistance genes in the ISS environment and to compare it with the properties of individual strains based on phenotypic susceptibility testing for antibiotics and whole-genome sequencing.
- Whole genome sequences (WGS) of ISS BSL-2 strains were compared with the WGS of the Earth counterparts to measure changes of and laterally acquired antibiotic genes in ISS strains.

Sample collection

Table 1. Description of various ISS location sampled. The samples were obtained from the same locations during both sessions.

Sample #	ISS Module	Surface Sampling Location
1	Port panel next to cupola	In Node 3 next to the cupola, on the way down into the cupola on the port panel.
2	N3_F4	Node 3 “F4” location right in front of the WHC
3	Node 3	ARED Foot platform
4	Node 1	Dining Table
5	N1_O4	Node 1 Overhead 4
6	PMM_P1	PMM Port 1
7	LAB_O3	Lab Overhead 3
8	Node 2	Port crew quarters Bump-Out exterior aft wall

Technical Approach

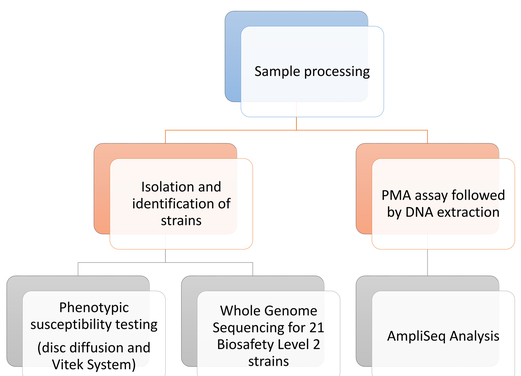


Fig. 1. Sample processing overview. The polyester wipe was transferred to 200 mL of sterile phosphate buffered saline and shaken for 2 min followed by concentration with the concentrating pipette (InnovaPrep, Drexel, MO) to ~4 mL. The sample was split into two parts. One part was used for cultivation, and the second part was treated with propidium monoazide (PMA). DNA extracted from the PMA and non-PMA treated samples and downstream analysis were performed with the AmpliSeq system.

Cultivable microbial diversity

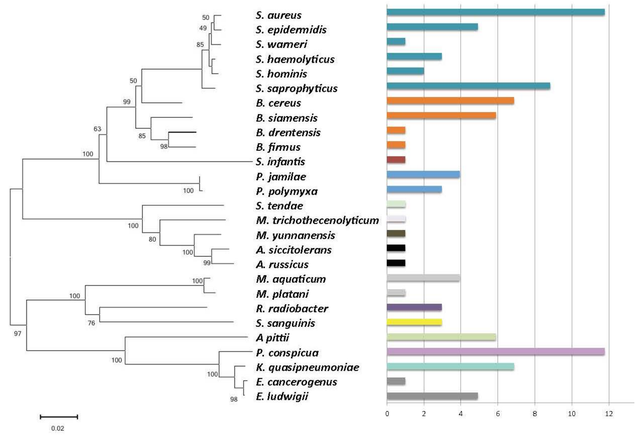


Fig. 2 The cultivable bacterial diversity of the ISS surfaces. The bar represent number of isolates retrieved and the color of the bar indicates the same genus.

Enterobacter piersonii resistomes

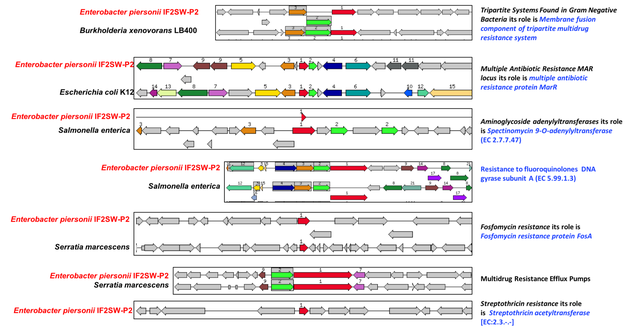


Fig. 3. Antibiotic resistant genes in *Enterobacter piersonii sp. nov.* (Node 3 “F4” location; WHC). Seven specific Antibiotic resistant tuple were analyzed in *Enterobacter piersonii sp. nov.* (Node 3 “F4” location; WHC). These antibiotic resistance tuples were associated with other known pathogens but were not seen in the *Enterobacter* species isolated from Earth whose genomes were published. In our previous studies we have shown the presence of pathogenic microbes (*Acinetobacter* sp.) in ISS with similar tuples, this leads us to conclude that these genes might have laterally transferred and acquired by the ISS *Enterobacter piersonii sp. nov.*

rpoB mutations seen in BSL-2 strains

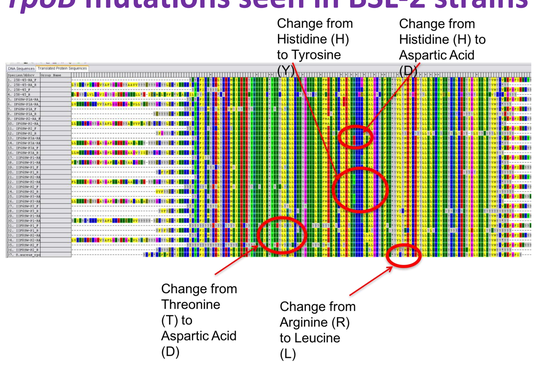


Fig. 4. *rpoB* mutations seen in several ISS BSL-2 strains. Watanabe et al., reported the involvement of an *rpoB* mutation in vancomycin-intermediate *S. aureus* phenotype expression (J. Clin. Microbiol., Vol 49: 2680–2684, 2011).

Resistomes of ISS BSL-2 strains

Staphylococcus aureus	Strain	Ciprofloxacin (CIP-5) (15-20)	Erythromycin (E-15) (>=22)	Gentamycin (GM-10) (13-14)	Oxacillin (OX-1) (11-12)	Penicillin (P-10) (≥28)	Rifampin (RA-5) (17-19)	Tobramycin (NN-10) (13-14)	Vancomycin (VA-5)	Clindamycin (DA-2)	Erythromycin (E-15)
1	IF4SW-P1	19	15	15	21		26		S		
2	IF4SW-P3	36	15	18	20		25	21	S	R	R
3	IF4SW-P5	25	15	15	23		28		S		
4	IF4SW-P1A	27	23	16	16		26 (6)	16	R	S	S
5	IF6SW-P2	25	21	15	11		30 (12)	16	R	S	S
6	IF6SW-P3A	28	27	15	16		28 (9)	15	R	S	S
7	IF7SW-P3	22	15	17	20	30	26	19	S	R	R
8	IF8SW-P1	28	25	16	15		27 (6)	18	R	S	S
9	IF6SW-P2	27	22	14	15		28 (6)	14	R	S	S
10	IF8SW-P3	27	23	15	19		30 (6)	14	R	S	S
11	IF8SW-P5	25	20	15	18		28 (6)	15	R	S	S
12	IF8SW-P2	26	20	15	18		25 (8)	15	R	S	S
13	258-45	26	22	15	19		26 (6)	15	R	S	S
14	258-47	25	20	15	20		26	15	S	R	R

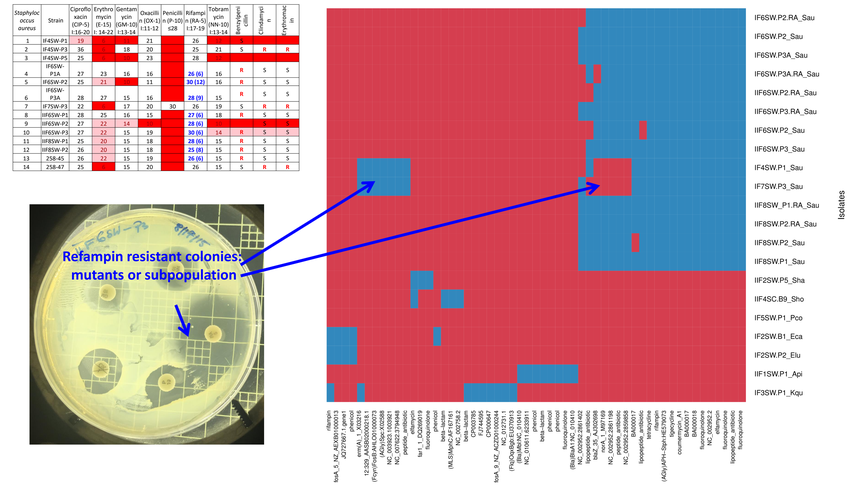
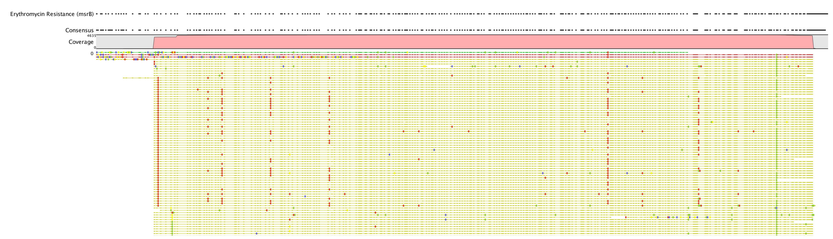


Fig. 5. **Top Left (Table).** Antibiotic resistance profile of *S. aureus* strains by disc diffusion methods and Vitek 2 test with AST-GP67 card. **Bottom (Petridish).** Rifampin resistant mutants of *S. aureus.* The arrows show several mutants and presence (red) and absence (blue) of resistance genes. The heatmap (**right panel**) shows the gene presence (red) absence (blue) profile of antibiotic resistance (AR) genes. Rows represent a sequenced isolate and columns report the AR genes found in the isolate. Genes come from a collection of curated antibiotic resistance databases - CARD, ResFinder, ARG-ANNOT, RED-DB. Genes were retrieved from each assembled genome using Glimmer and each gene was compared against the collated AR database. Genes with at least 90% nucleotide identity and 90% of the query gene aligned were considered to be valid matches. Genes annotated with similar resistance phenotypes were combined into single columns. *Staphylococcus* genomes show a distinct AR gene profile, with individual variation evident among many of the genomes. Sau: *Staphylococcus aureus,* Sha: *Staphylococcus haemolyticus*, Sho: *Staphylococcus hominis*, Pco: *Pantoea conspicua*, Eca: *Enterobacter piersonii*, Elu: *Enterobacter piersonii*, Api: *Acinetobacter pitti*, and Kqu: *Klebsiella quasipneumoniae*.

Resistomes of ISS Environmental Samples



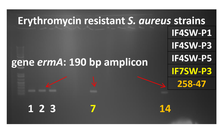


Fig. 6. **Top panel.** Reference Mapping of AmpliSeq reads for the DNA extracted from the sample IFSW4 (Node 1 Dining Table). The reference gene ID: gi|1503050:130-404 which is an erythromycin resistance gene (msrB). This correlates with the isolate phenotypic and WGS data. Coverage depth is indicated on the Y-axis. **Left panel.** The presence of erythromycin resistant genes in the ISS *S. aureus* cultures. The *ermA* gene amplification is seen in 4 strains.

Conclusions

- Multiple antibiotic resistance genes were identified by targeted amplification in DNA samples without culturing for microorganisms.
- The ISS resistome mostly represented genes conferring beta-lactam, macrolide (erythromycin) and tetracycline resistance.
- Concurrently, the WGS analysis of the BSL-2 microorganisms revealed that same genes observed in the AmpliSeq resistome analyses were also identified in their genomes.
- Disc diffusion tests showed that some of the strains are multidrug resistant and exhibited resistance to antibiotics with the identified antimicrobial genes.
- Although the targeted amplification does not allow for a precise identification of the gene origin, it shows the overview of the total gene pool in the ISS environment.
- The knowledge of the antibiotic resistance genes will allow for more efficient use of the antibiotics on board of the ISS.
- Resistance against the currently used antibiotics (ciprofloxacin, erythromycin and clindamycin) in the BSL-2 strains and DNA isolated from the ISS environments were documented.

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