Resistance of *Klebsiella* strains from the International Space Station to quaternary ammonium compound disinfectants

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Overview: Opportunistic bacteria on the International Space Station (ISS) such as *Klebsiella* live in an extreme environment, surviving not only under the stresses of spaceflight such as microgravity but also exposure to the quaternary ammonium compound (QAC) disinfectants used to sanitize surfaces on the ISS. To investigate the response of *Klebsiella* strains isolated from the ISS to these QAC disinfectants, we used viability assays with a combination of quantitative and qualitative analyses to determine their physical response and the minimum inhibitory concentration (MIC) at which each strain survives. These data will be used in conjunction with simulated microgravity in future directed evolution studies to determine how these stressors drive genomic changes in virulence and antimicrobial resistance, which will help protect astronauts' health during future long-duration spaceflight.

Introduction

NASA has been monitoring the surface microbiome of the ISS since 2015, using a combination of metagenomic and culture-based techniques (1). Metagenomic analyses have shown high prevalence of the Biosafety Level 2 opportunistic pathogen *Klebsiella pneumoniae,* and culturing has led to the isolation of novel *Klebsiella* sp. strains (2,3). Microorganisms on the ISS experience the same stresses of spaceflight as humans, living under extreme environmental conditions of microgravity, elevated radiation, and isolation. Beyond these common stressors of spaceflight, the impact of the quaternary ammonium compound (QAC) disinfectants employed for environmental hygiene on the ISS microbiome is poorly understood. Here, we examine the responses two *Klebsiella* strains isolated from the ISS, *K. pneumoniae* F2-3P(2*) and *K. quasipneumoniae* IF3SW-P1 (**Figure 1),** to different concentrations of QAC disinfectants used on the ISS with the aim of understanding the ISS strains' physical responses to this stressor and to determine the minimum inhibitory concentration (MIC) for future experiments.

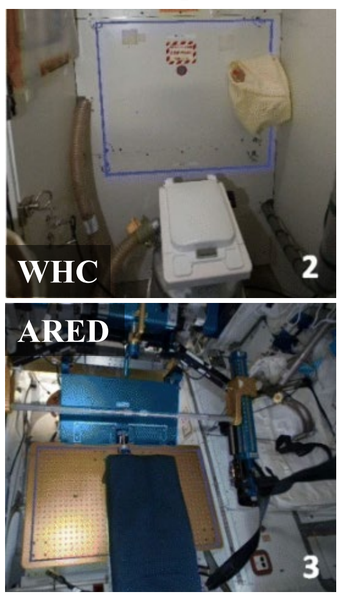


Figure 1. Sampling sites from the ISS. F2-3P(2*) sampled from top, IF3SW-P1 from bottom. From (1).

Methods

Objective 1: Assess responses to full-strength QAC disinfectant

- A mixture of methods, including culturing, growth curves, phase-contrast microscopy, and fluorescence microscopy using LIVE/DEAD® viability dyes.

Objective 2: Determine MIC of QAC disinfectant

- MIC determination protocol from (4) conducted in a plate reader with OD_{600} measurements.

Objective 3: Assess viability over a QAC dilution course

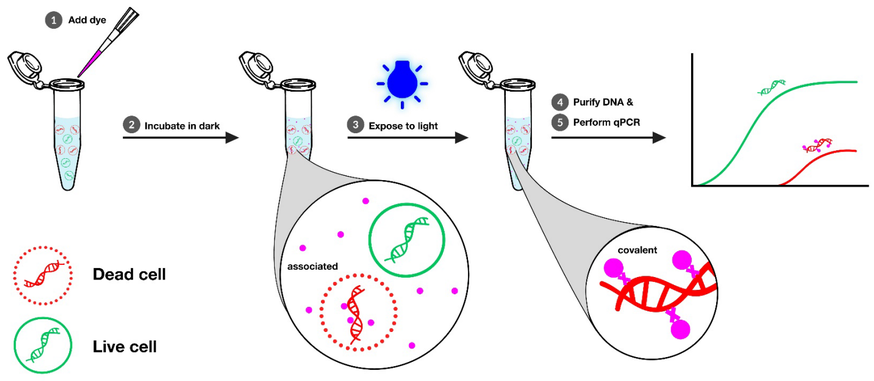
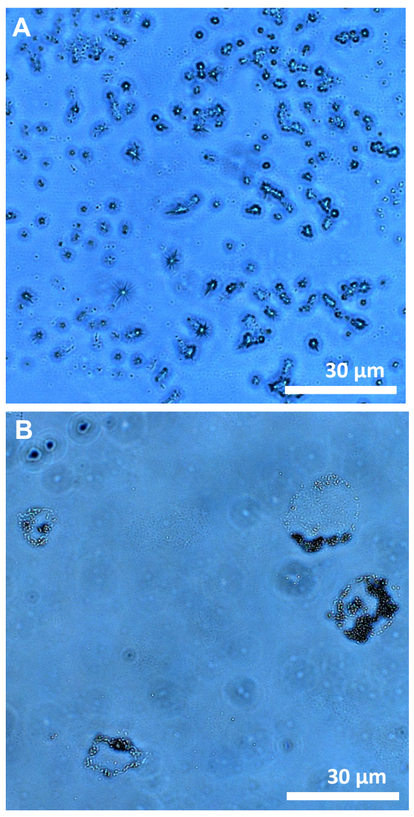


Figure 2. Viability qPCR procedure using PMAxx. From (5).

Results



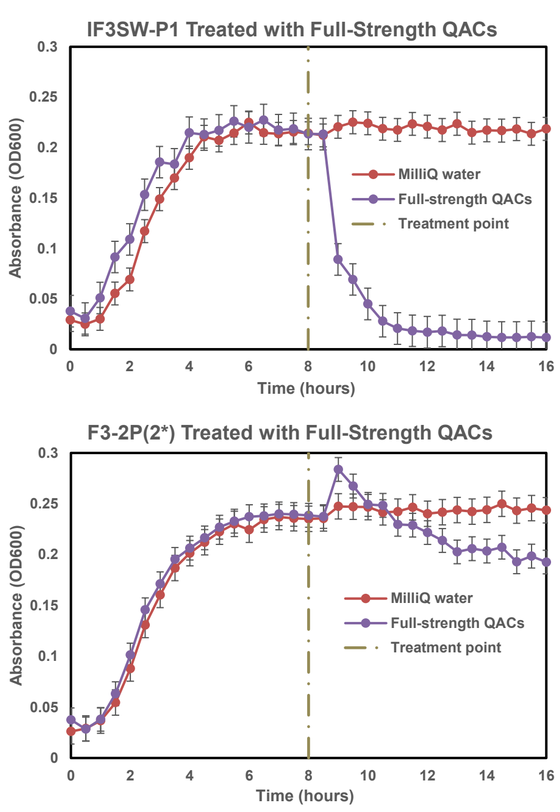


Figure 3. Microscopy and growth curves after treatment with full-strength QAC disinfectant. Overnight cultures were diluted to an OD_{600} of 0.05 in 1:10 TSB and added to a 96-well plate in triplicate. Plates were grown at 23°C with shaking for 8 hours , at which point QAC mixture or MilliQ water was added and continued incubating for 16 total hours. Cultures treated with QAC mixture were then viewed under 400x magnification and phase-contrast. **A.** Strain IF3SW-P1 lyses from QAC exposure (L, starburst shapes) and its OD_{600} goes to 0, further implying full lysis (R). **B.** Strain F3-2P(2*) forms clumps after QAC exposure (L, rings of cells) and its OD_{600} slightly decreases from 0.25 to 0.2 (R).

	0.200%	0.175%	0.150%	0.125%	0.100%	0.050%	Negative Control	Positive Control
IF3SW-P1	0.086	0.086	0.085	0.087	0.086	0.302	0.087	0.316
F2-3P(2*)	0.09	0.093	0.091	0.267	0.269	0.26	0.086	0.291

Table 1. Table represents part of a 96-well plate containing cultures of each strain inoculated with a different QAC dilution grown in a plate reader in TSB at 37°C. Numbers represent OD_{600} measurements, with shading signifying turbidity. MIC is above the highest dilution showing turbidity.

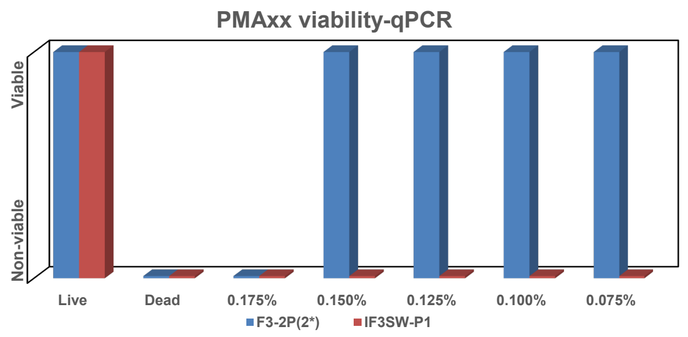


Figure 5. Viability qPCR using PMAxx. Cultures of both strains were grown to late stationary phase in 1:10 TSB at 23°C, and then QAC dilutions (or MilliQ water) was added. Cultures continued incubating for 16 total hours before PMAxx treatment and DNA extraction. Dead control was heat-killed prior to PMAxx treatment. DNA of PMA- and non-PMA-treated samples were analyzed via qPCR with 341F/518R V3-V4 region primers and SYBR Green dye. Viability was calculated by determining the ΔCt between PMA and non-PMA samples, which is on a $\log_2$ scale. This is then used to calculate to fold change, and fold change used to calculate to percent viability.

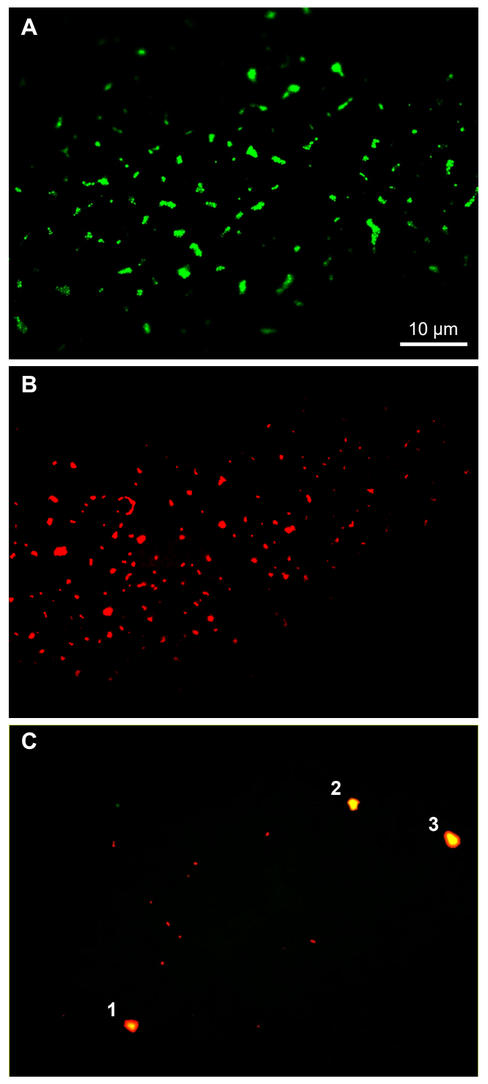


Figure 4. Fluorescence microscopy images of strain F3-P2(2*) treated with LIVE/DEAD® fluorescent dyes. **A.** Live cells (untreated). **B.** Dead cells (treated with isopropyl alcohol). **C.** Overlay of same field of view with both FITC and Rhod filters showing cells treated with QAC disinfectant mixture. Note clumps of cells marked 1-3 as well as almost no individual live cells, suggesting a protective effect from clumping.

Conclusions & Future Directions

Objective 1: *K. quasipneumoniae* strain IF3SW-P1 and *K. pneumoniae* strain F3-2P(2*) are shown to respond differently to full-strength QACs **(Figure 3).** Strain IF3SW-P1 appears to lyse completely, and under phase-contrast shows characteristic lysed "starburst" patterns. Conversely, strain F2-3P(2*) does not lyse and instead shows cells "clumped" together. Further investigation of these clumps using viability dyes shows potential viable cells inside the larger non-viable "clumps," suggesting a putative protective effect **(Figure 4)**.

Objective 2: Both strains were determined to have MICs of under 1%, showing the strong killing effect of the QAC mixture **(Table 1).** Strain IF3SW-P1 was shown to have a MIC of above 0.05%, and strain F2-3P(2*) was shown to have a MIC above 0.125%. These MIC values will be used in future directed evolution studies in conjunction with the stress of simulated microgravity,

Objective 3: Strain F3-2P(2*) continues to show a greater putative resistance to the QAC disinfectant, even at low dilutions near the MIC. At concentrations below 0.175%, this strain has full viability, implying an MIC value nearer to 0.15% than to 0.125%. Strain IF3SW-P1 is much less resistant, showing no viability 0.175% to 0.075%, implying its MIC may be 0.05%.

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