5-1-2024

Quaternary Ammonium Compound Resistance of Klebsiella Pneumoniae and Klebsiella Quasipneumoniae Strains Isolated from the International Space Station

Natasha Svetlana Sushenko

Repository Citation

Sushenko, Natasha Svetlana, "Quaternary Ammonium Compound Resistance of Klebsiella Pneumoniae and Klebsiella Quasipneumoniae Strains Isolated from the International Space Station" (2024). *UNLV Theses, Dissertations, Professional Papers, and Capstones*. 5086.
http://dx.doi.org/10.34917/37650913

QUATERNARY AMMONIUM COMPOUND RESISTANCE OF *KLEBSIELLA PNEUMONIAE* AND *KLEBSIELLA QUASIPNEUMONIAE* STRAINS ISOLATED FROM THE INTERNATIONAL SPACE STATION

By

Natasha Svetlana Sushenko

Bachelor of Science – Biological Science
University of Nevada, Las Vegas
2019

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science – Biological Science

School of Life Sciences
College of Sciences
The Graduate College

University of Nevada, Las Vegas
December 2023

UNLV | GRADUATE COLLEGE

Thesis Approval

The Graduate College
The University of Nevada, Las Vegas

October 17, 2023

This thesis prepared by

Natasha Svetlana Sushenko

entitled

Quaternary Ammonium Compound Resistance of *Klebsiella Pneumoniae* and *Klebsiella Quasipneumoniae* Strains Isolated from the International Space Station

is approved in partial fulfillment of the requirements for the degree of

Master of Science – Biological Science
School of Life Sciences

Brian Hedlund, Ph.D.
Examination Committee Chair

Duane Moser, Ph.D.
Examination Committee Member

Helen Wing, Ph.D.
Examination Committee Member

Elisabeth Hausrath, Ph.D.
Graduate College Faculty Representative

Alyssa Crittenden, Ph.D.
Vice Provost for Graduate Education &
Dean of the Graduate College

ABSTRACT

QUATERNARY AMMONIUM COMPOUND RESISTANCE OF *KLEBSIELLA PNEUMONIAE* AND *KLEBSIELLA QUASIPNEUMONIAE* STRAINS ISOLATED FROM THE INTERNATIONAL SPACE STATION

By
Natasha Svetlana Sushenko

Dr. Brian P. Hedlund
Graduate Advisory Committee Chair
Professor of Biology
University of Nevada, Las Vegas

Dr. Duane P. Moser
Research Advisor/GAC Member
Assistant Research Professor
Desert Research Institute

The International Space Station (ISS) has been continuously inhabited for twenty-three years and harbors a diverse population of microorganisms under conditions of microgravity, elevated radiation, and relative isolation, including Biosafety Level 2 (BSL-2) opportunistic pathogens. To sanitize surfaces on the ISS, astronauts use a combination of four antimicrobial quaternary ammonium compounds (QACs). As the stresses of space flight are known to stimulate virulence and antimicrobial resistance in bacteria, the frequent use of QAC disinfectants on the ISS is of concern to NASA. This not only poses risks to astronauts during future long-term space travel, but there is also a concern that these hypervirulent and multidrug-resistant strains will be returned to Earth by astronauts.

For my master's thesis, I studied strains of *Klebsiella pneumoniae* and *Klebsiella quasipneumoniae,* opportunistic BSL-2 pathogens that were isolated from the interior surface microbiome of the ISS, with particular emphasis on how these ISS-adapted strains differ from an Earth-origin type strain when exposed to QACs. This thesis consists of four chapters: Chapter 1,

an introduction and literature review; Chapter 2, a description of the complete genome of a strain of *Klebsiella quasipneumoniae* isolated from the ISS, which was published in 2022 in Microbial Resource Announcements; Chapter 3, a study of the responses of both ISS- and Earth-origin strains of *Klebsiella* to QAC exposure; and Chapter 4, a conclusion.

The first research chapter (Chapter 2) consists primarily of *in silico* work to complete the genome of *Klebsiella quasipneumoniae* subsp. *similipneumoniae* strain IF3SW-P1, isolated from the ISS; this included assembly and annotation of the genome based on long-read Oxford Nanopore Technology sequencing data. The completed genome was then analyzed for the presence of putative virulence and antimicrobial resistance genes. The second research chapter (Chapter 3) consists of experimental approaches to study the responses to QAC disinfectants by both ISS- and Earth-origin strains of *Klebsiella.* This work included determining the minimum inhibitory concentration (MIC) of QACs for each strain before conducting viability assays at both sub-lethal and standard QAC concentrations. These viability assays included viability qPCR using the viability dye PMAxx, and fluorescence microscopy conducted using LIVE/DEAD BacLight viability dyes. This work showed significant differences in the response of the two ISS-origin strains of *Klebsiella,* as well as differences between the ISS-origin strains and the Earth-origin type strain, in both the amount of QACs required to inhibit growth of the strains and in the responses of the strains after exposure to lethal QAC concentrations. This suggests the ISS-origin strains, which are frequently exposed to QACs in the ISS environment, may be less susceptible to QACs.

ACKNOWLEDGEMENTS

Few things in life can be called a singular accomplishment, and this thesis is no exception. This work could not have started without the work preceding it at NASA's Jet Propulsion Laboratory (JPL) overseen by principal investigator Dr. Kasthuri Venkateswaran, who also provided me advice and feedback to much of the work in this thesis. Dr. Nitin Singh, also from JPL, assisted with the bioinformatics work, as well as Scott Tighe of UVM, who provided access to long-read sequencing as well as his depth of expertise in the field.

I would like to extend my gratitude to my committee members, Dr. Helen Wing and Dr. Elisabeth Hausrath, for the expertise they provided and assistance they gave shaping this project, and for their patience as I worked my way through this process. I also would like to thank Dr. Boo Shan Tseng, not only for allowing me to use the departmental microscope and training me on how to use it. For my first three semesters, she was my professor for journal club and microbial ecology, and being in her classes had an indelible effect on how I think about science.

And of course, finally, Dr. Duane Moser and Dr. Brian Hedlund. Most students only get one advisor, but I was fortunate enough to have two. To Duane, thank you for providing me with the opportunity and support to work on such a special project in your lab these last few years, and thank you again for sharing all your expertise and stories. I don't think there's anyone else quite like you. To Brian, thank you for your guidance and support since I was an undergraduate in your microbial ecology class. You and your class helped me realize that I especially loved microbiology and that I wanted to pursue it. And I especially thank you for always doing what you can to help me when I need it most.

Of course, I’m also thankful for my two institutions, DRI’s Department of Hydrological Sciences and UNLV’s School of Life Sciences. Most people don’t have two institutions either, and I was grateful to receive support from both when it was needed, via the DHS and VPR graduate student fellowship and through a SoLS graduate teaching assistantship. I’m also grateful to my other funders, particularly Nevada NASA Programs who funded the original project my thesis work was based upon via a NASA EPSCoR R3 grant to Dr. Moser, as well as funded my work directly via the Nevada Space Grant graduate fellowship.

From the Moser lab, I’d like to thank postdoc Dr. Ali Saidi—not just for sharing his broad expertise in microbiology and specifically for guiding me in this work, but for all the times we nerded out about video games when we needed a break. To the grad students in other labs at DRI—Adhee, Zak, Charlotte, and Tejinder—we were a small group, but we always stuck together. And to the grad students at UNLV too, particularly in Dr. Hedlund’s lab, who I wish I could’ve known better if not for social distancing. And of course, to my cohort in the Moser lab, Victoria Wuest and Molly Devlin. We were all we really had during those rough two pandemic years, and we’ve truly been through it all together. I can’t imagine having done it without you two.

As for outside of the lab, I am fortunate to be surrounded by people who love and support me unconditionally, and the feeling is mutual. To my friends—Shaun & Rachel, Maegan & Michael, Chris & Bailey, Kathy, Shaun, Skyler, Lorenzita, and more names I’m surely missing—thank you for your support, through tears and smiles, and especially thank you for all the good times we’ve shared. To my sister Sasha, I don’t think I could’ve been luckier than to have you as a sister. I can’t wait to celebrate with you and Alex soon. And to my mom and dad—you gave the world for us, and hopefully I can give something back to the world in exchange.

Finally, to my fiancé, Sean Breckling, I offer my whole heart, though it can barely compare to what you've done for me. Even as you were dealing with your own struggles, you were always there for me, always telling me I could conquer the world if I wanted to do so. I can't tell you how much your support meant to me, how you helped me see the path forward. I'm so excited that we're starting our life together.

DEDICATION

Nathan Josef Grill

February 22, 1987 – October 11, 2022

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Introduction

1.1 The International Space Station as a built environment

The International Space Station (ISS) occupies a unique position as both a built environment and a nearly closed ecosystem, only opening approximately every 90 days for recrewing and resupplying. Since its launch over twenty years ago, the ISS has been continually occupied by a crew of up to seven astronauts who inhabit the station for an average of 3 to 6 months. Over this time, a total of 266 people from 20 nations have visited the ISS as astronauts or space tourists, with many making repeat visits (1). The unique conditions on the ISS, including microgravity, high radiation, and relative isolation are known to be stressful to humans and microbes alike. These stresses of spaceflight are known to compromise the human immune system, activate latent viral infections in humans, and trigger virulence and antimicrobial resistance (AMR) in bacteria (2–5). As NASA's strategic goals shift beyond low-Earth orbit to both the Moon and to Mars, the safety of astronauts during long-term spaceflight is of increasing importance (6, 7).

While vessels and supplies going to the ISS are assembled in clean rooms to prevent bacterial and fungal contamination, it is not possible to separate crew members from their microbiomes (8–10). As the first astronauts arrived on the ISS, their microbiota began colonizing both the air filtration and water recycling systems as well as surfaces on the interior of the station, the same as any other built environment on Earth (11). It is well-established that human interactions with the various built environments they occupy, from homes to schools to hospitals, lead to a constant exchange of their microbiota with that of the microbiomes of those built environments (12–14). The spread of hypervirulent and multi-drug resistant (MDR) strains of pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) is known to be led by interactions with the built environment of hospitals (15, 16). The potential that pathogens from the ISS built environment could be returned to Earth is another concern that pushes NASA to monitor the ISS microbiome under the tenet of planetary protection (17).

1.2 Spacecraft microbial monitoring

Space agencies have been aware of human microbiota colonizing the built environment of spacecraft since the advent of spaceflight. As early as the 1960s, NASA tracked the microbiota of Apollo astronauts before and after flight to document changes to their microbiomes; less than a decade later, the interior surface microbiome of the space station Skylab was compared to the microbiota of the crew to study the exchange of microbes between host and environment (18, 19). The Soviet Union also monitored the surface microbiomes of their space stations Salyut 6 and 7 throughout the 1970s and 1980s, with particular attention to pathogen virulence and biofilm formation (20–22).

Skylab and Salyut were first-generation space stations designed to be intermittently occupied for short periods, leaving the stations unoccupied for long stretches of time. The first modular space station designed to be continually occupied was Mir, launched by the Soviet Union in 1986. Mir was continually occupied for over ten years from 1989 until its decommissioning in 1999, setting a record that would later be surpassed by the ISS. While the Soviet space program monitored the microbiota of Mir, uncontrolled colonization of bacteria and fungi quickly occurred due to inadequate cleaning procedures (23, 24). This microbiome included technophilic extremophiles, which led to corrosion and degradation of critical hardware on the station (25–27). As a result, a regular microbial monitoring program was implemented, and rigorous cleaning procedures were designed to prevent further biocorrosion (28).

1.3 ISS microbiome

As the ISS was designed as a modular space station to support long-term continually crewed spaceflight, much like Mir, routine microbial monitoring and rigorous cleaning procedures have been in place since its launch in 2000 (29, 30). Samples from the water recycling system, dust from HEPA filters, and interior surfaces have been frequently studied for microbial characterization (31–40). Most of these early studies relied on traditional culture-based or early molecular techniques such as amplicon sequencing to catalog and characterize the ISS microbiome (41, 42). These culture-based techniques, however, miss a large fraction of microbes that are not culturable under standard laboratory conditions, so more molecular technique approaches are needed for ISS microbiome study (43).

Beginning in March 2015, NASA began the Microbial Tracking-1 project, the first study of the ISS microbiome using a combination of both shotgun metagenome sequencing and culture-based techniques. Eight sites across the space station were chosen for monitoring over time, a mixture of high-contact surfaces such as the dining table and the cupola viewing window, and low-contact surfaces such as stowage bays. The surface of each site was swabbed with a dry sterile polyester wipe, which was then returned to Earth for processing. This procedure was repeated across 15 months, with samples taken again in May 2015 and May 2016 (44). Microbial monitoring with these techniques continues on the ISS, with Microbial Tracking-2 beginning in 2017 and Microbial Tracking-3 in 2021, which expanded to include tracking crewmembers' microbiomes alongside the surface microbiome of the ISS (45, 46).

1.4 Pathogens on the ISS

Pathogenic bacteria have been a constant focus of microbial monitoring studies since Apollo and continue to be a prominent focus of microbiome studies of the ISS. As previously described (21, 47), the unique conditions of life on the ISS both suppress the human immune system and promote virulence and antimicrobial resistance (AMR) in microbes. Microbes developing these traits of increased pathogenicity appear to be doing so as survival adaptations to the stresses of spaceflight, such as microgravity and increased background radiation (48–50). Many of these pathogens detected on the ISS are known to form biofilms, a structure consisting of a slimy matrix of extracellular polysaccharides, proteins, and nucleic acids (51, 52). Biofilms are associated with both increased virulence and AMR on Earth and may provide protection to bacteria from the physiological stress of microgravity (53).

The metagenomic sequencing used in Microbial Tracking-1 allowed for the assessment of virulence and antimicrobial resistance genes in the ISS microbiome for the first time. Using both broad functional analysis of the metagenome and specific amplicon-targeted analysis for known genes, hundreds of AMR and virulence genes were detected (54). Additionally, the abundance and diversity virulence and AMR genes, as well as Biosafety Level-2 (BSL-2) pathogen community structure, was analyzed for each site over the three flights sampled. Across the three flights, reads assigned to *Klebsiella pneumoniae*, an opportunistic BSL-2 pathogen, not only increased in number but increased from being detected in just three sites during Flight 1 to being detected in all but one site in Flight 3 (55). Additionally, culture-based studies of the ISS samples resulted in the isolation and genome sequencing of 11 strains of *Klebsiella* spp. (56, 57).

1.5 Introduction to *Klebsiella*

The genus *Klebsiella* is a member of the family *Enterobacteriaceae*, and consists of Gram-negative, facultatively anaerobic, non-motile, rod-shaped bacteria. The genus is commonly found in nature, from environmental microbiomes like soil and water to human and animal microbiomes. *Klebsiella pneumoniae,* the best-studied species of this genus, was first described in 1887 and is a causative agent for pneumonia and urinary tract infections in neonates, the elderly, and the immunocompromised (58). *Klebsiella quasipneumoniae* was previously described as two phylogroups of *K. pneumoniae* before being identified as a novel species in 2014 (59). Both species are known to have hypervirulent, multi-drug resistant (MDR) strains that result in both hospital- and community-acquired infections (60–64).

Virulence and AMR in *K. pneumoniae* and *K. quasipneumoniae* is commonly associated with carbapenamases and biofilm production (65, 66). Multi-drug resistant strains of *Klebsiella* have the gene *Klebsiella pneumoniae* carbapenamase (*kpc*), which encodes the KPC carbapenamase that provides active resistance to all beta-lactam antibiotics and is now widely detected among Gram-negative bacteria (67–69). All *Klebsiella* strains produce a thick capsule layer of polysaccharides that are both a protective structure on their own and promote biofilm formation, which contributes to AMR (70). Some hypervirulent strains of *Klebsiella* produce a hypermucoviscous phenotype, where production of the K1 and K2 capsule serotypes are overexpressed, producing a thick, mucoid biofilm that further increases AMR (71).

1.6 Overview of thesis

For my thesis research, I studied strains of two *Klebsiella* species isolated from the surface of the ISS during Microbial Tracking-1: *Klebsiella pneumoniae* subsp. *pneumoniae* strain F3-2P(2*) and *Klebsiella quasipneumoniae* subsp. *similipneumoniae* strain IF3SW-P1 (56, 57). These two strains were isolated from the Waste and Hygiene Compartment wall panel and the Advanced Resistive Exercise Device foot panel during Flight 1 and Flight 3, respectively (44). These strains were of particular interest due to the prominence of metagenomic reads mapping to *Klebsiella* across all three flights (55).

The goal of this work was to investigate putative virulence and AMR genes of these two strains, particularly against the quaternary ammonium compound (QAC) disinfectants used on the ISS. QAC disinfectants work by disrupting cell membranes, leading to lysis, and are widely used in industrial and medical applications due to their favorable safety profile. However, QAC resistance associated with efflux pumps is well-documented (72), and *K. pneumoniae* and *K. quasipneumoniae* are frequently associated with MDR efflux pumps (62-64).

Considering the high prevalence of *Klebsiella* reads in the ISS surface microbiome, this work aims to investigate QAC resistance in these ISS-origin strains of *Klebsiella* compared to Earth-origin strains. To do so, I used both bioinformatic approaches to assemble, annotate, and interpret the genome of strain IF3SW-P1, as described in Chapter 2; and laboratory approaches including minimum inhibitory concentration (MIC) determination, viability assays such as

PMAxx viability qPCR, and LIVE/DEAD fluorescence microscopy, as described in Chapter 3.

Finally, I will discuss the results of this work and continuing questions in this field.

CHAPTER 2

Complete Genome Sequence of *Klebsiella quasipneumoniae* subsp. *similipneumoniae* Strain IF3SW-P1, Isolated from the International Space Station

Published in *Microbiology Resource Announcements*

23 June 2022 (doi: 10.1128/mra.00476-22)

Natasha S. Sushenko, Nitin K. Singh, Daniel L. Vellone, Scott W. Tighe,

Brian P. Hedlund, Kasthuri Venkateswaran, Duane P. Moser

2.1 Graduate student involvement and general background

Chapter 2 represents a manuscript published in *Microbiology Resource Announcements* on work primarily completed by me as a part of my thesis project. I (NSS) extracted the genomic DNA, assembled, circularized, and polished the resulting sequence data, annotated the completed genome, analyzed the annotated genome, and wrote the manuscript. Access to Oxford Nanopore Technology sequencing was provided by SWT and conducted by SWT and DLV. NKS and KV provided guidance on bioinformatic analyses. BPH and DPM supervised the work and assisted with revising the manuscript.

2.2 Abstract

The 5.2-Mb circular genome of *Klebsiella quasipneumoniae* subsp. *similipneumoniae* strain IF3SW-P1, isolated from the International Space Station, was sequenced using Oxford Nanopore Technologies. The genome lacks a megaplasmid typical of hypervirulent and multidrug-resistant Klebsiella strains but does contain a chromosomally encoded OqxAB efflux pump associated with carbapenem resistance.

2.3 Announcement

In 2014, two phylogroups of the opportunistic pathogen *Klebsiella pneumoniae* were described as the novel species *Klebsiella quasipneumoniae* (59) . Since its definition as a species, *K. quasipneumoniae* has emerged as an understudied human pathogen with hypervirulent, multidrug-resistant (MDR), carbapenem-resistant, and hypermucoviscous strains isolated from both hospital-borne and community-acquired infections (44, 60–62). Considering its prevalence on the International Space Station (ISS) (56), the newly recognized pathogenicity of *K. quasipneumoniae* increases concerns about the consequences of this species being exposed to the stresses of spaceflight, which are known to trigger bacterial virulence and antimicrobial resistance (2, 3, 21, 47).

Strain IF3SW-P1 was isolated from the surface of the foot panel of the Advanced Resistive Exercise Device (ARED) on the ISS on 4 March 2015 (44) using a standard spread plate method on Reasoner’s 2A (R2A) agar and archived in glycerol cryostocks (56). For this study, strain IF3SW-P1 was subcultured from cryostock and grown to late exponential phase in

Trypticase soy broth (TSB) at 37°C; genomic DNA was then extracted using the DOE Joint Genome Institute bacterial genomic DNA isolation protocol (73).

Oxford Nanopore Technologies sequencing was performed using a GridION MK1 sequencer on a R10.4 flow cell (FLO-MIN112) with a library synthesized from Q20+ EA (early access) ligation reagents (SQK-LSK112-XL). The raw reads were base called using MinKNOW v29.10.8, with a mean quality score of 16.3 and a mode of 18.03. The genome was assembled, circularized, and polished using Flye v2.9 with the parameters *–nano-hq* and *–read-error* 0.03 for the Q20+ data (74). The Flye-generated assembly contains two contigs, one 5.2-Mb circular chromosome and one 3-kb linear fragment confirmed via BLASTN v2.12.0 to be 99.8% identical to *Escherichia coli* strain Q4552 plasmid pECQ4552_IHU08 (GenBank accession number CP077071.1) (75). Notably, the genome does not encode any virulence- or drug resistance-associated plasmids, such as *bla*KPC and Inc(FII), which are known to occur in Klebsiella species (76).

The genome was identified as *K. quasipneumoniae* subsp. *similipneumoniae* by calculating the average nucleotide identity (ANI) using the EzBioCloud calculator compared to the two subspecies' type strains, *K. quasipneumoniae* subsp. *quasipneumoniae* 01A030^T (ANI, 96.63%) and *K. quasipneumoniae* subsp. *similipneumoniae* 07A044^T (ANI, 99.03%) (77). Strain IF3SW-P1 is also related to but distinct from eight previously published draft genomes of *K. quasipneumoniae* strains isolated from the ISS, with >99% ANI for all (56).

The assembly was annotated using RASTtk v1.3.0 (78) as part of the Pathosystems Resource Integration Center (PATRIC) v3.6.12 (79). Predicted virulence genes on the chromosome include *iutA*, which encodes a ferric aerobactin receptor, although the gene encoding the associated siderophore aerobactin (*iucA*) is not present (80). The IF3SW-P1 genome also contains genes for the multidrug resistance efflux pump OqxAB, associated with carbapenem resistance in *K. pneumoniae* (81, 82). OqxAB is reported to be associated with resistance to benzalkonium chloride, a quaternary ammonium compound used as a disinfectant on the ISS (44). Default parameters were used for all software unless otherwise specified. Additional assembly and annotation information is listed in **Table 1**.

2.4 Data availability

The genomic assembly and raw reads have been deposited at GenBank (accession number CP092121) and the Sequence Read Archive (SRR17974437). These data are also available at NASA GeneLab (GLDS-470).

2.5 Acknowledgements

We thank astronaut Terry Virts for collecting samples aboard the ISS, Aleksandra Checinska-Sielaff for isolating the strain, and the implementation team at NASA Ames Research Center (Fathi Karouia) for coordinating the sampling effort.

Part of this research was performed at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with NASA. This work was supported through 2012 Space Biology (NNH12ZTT001N) grant number 19-12829-26 under task order NNN13D111T awarded to K.V.; by NASA EPSCoR Rapid Response Research Cooperative Agreement (NNH18ZHA005C) award number 80NSSC19M0169 to D.P.M.; and by project MANGO, a 2018 Space Biology (NNH16ZTT001N) grant under task order 80NM0018F0589. Additional support was provided through the Nevada Space Grant Consortium Graduate Research Opportunity Fellowship award (number 13584) to N.S.S. and the UNLV MSI Open Article Fund.

Table 1. Assembly and annotation information

Characteristic	**Data**
Strain name	IF3SW-P1
ISS sampling date	4 March 2015
Location	ARED foot panel
Nearest species	*K. quasipneumoniae* subsp. *similipneumoniae* 07A044^T
ANI (%)	99.03
No. of raw reads	364,351,976
Genome size (bp)	5,238,176
N50 (bp)	5,238,176
No. of contigs	2 (1 chromosomal, 1 linear fragment)
Median coverage (x)	62
G+C content (%)	58.06
No. of coding sequences	4,998
GenBank accession no.	CP092121
SRA accession no.	SRR17974437
GeneLab accession no.	GLDS-470

CHAPTER 3

Sensitivity of *Klebsiella* Strains Isolated from the International Space Station After Exposure to Quaternary Ammonium Compound Disinfectants

3.1 Abstract

Recent metagenome studies of International Space Station (ISS) interior surfaces have shown *Klebsiella pneumoniae* to be the most prevalent biosafety level 2 (BSL-2) organism in the ISS microbiome. Multi-drug resistant (MDR) strains of *Klebsiella* species are known to carry genes encoding efflux pumps, which confer resistance to many antibiotics as well as common disinfectants such as quaternary ammonium compounds (QACs). QACs are used to sanitize surfaces on the ISS, but the effects of QACs on *Klebsiella* are poorly understood. This study evaluated the responses of two *Klebsiella* strains isolated from the ISS, *Klebsiella quasipneumoniae* IF3SW-P1 and *K. pneumoniae* F3-2P(2*), to the QAC disinfectants used on the ISS. Minimum inhibitory concentration (MIC) assays using the QAC mixture showed *K. pneumoniae* F3-2P(2*) was able to grow when exposed to a higher QAC concentration than *K. quasipneumoniae* IF3SW-P1. Measurement of culture turbidity and cell appearance under phase-contrast microscopy also showed stationary-phase cells of *K. quasipneumoniae* IF3SW-P1 to be more sensitive to QAC killing than *K. pneumoniae* F3-2P(2*) cells. Strain IF3SW-P1 lysed after QAC treatment, while the cells of strain F3-2P(2*) clumped together. Fluorescence microscopy using LIVE/DEAD viability dyes identified viable cells inside these clumps; however, these cells were not viable on standard plate assays. Furthermore, a viability qPCR assay using PMAxx at a range of concentrations also showed that strain *K. pneumoniae* F3-2P(2*) remained viable at

higher QAC concentrations than *K. quasipneumoniae* IF3SW-P1, and also its type strain, *K. pneumoniae* 13883^T. This study suggests frequent cleaning of ISS surfaces with QACs is selecting for strains that are resistant to QACs, which may increase risk of infection among astronauts; based on these results, more research should be done to elucidate the prevalence of QAC resistance on the ISS so NASA can determine whether their use should be discontinued.

3.2 Introduction

In 2015, NASA initiated Microbial Tracking-1, a large-scale microbial monitoring project of the ISS in which astronauts sampled eight surfaces over three flights across a 15-month timespan and the resulting samples were returned to Earth for both traditional culture-based studies and molecular techniques such as amplicon-targeted and shotgun metagenome analyses (44, 54–56). Of the 318 total bacterial species identified, 16 were BSL-2 pathogens, and eight were observed in all three flights, with the highest abundance of BSL-2 pathogens at the final time point (44, 55). The dominant BSL-2 pathogen detected on the ISS was *Klebsiella pneumoniae*, an enteric bacterium and common opportunistic pathogen, which accounted for 60% of the total BSL-2 metagenomic reads. The relative abundance of *K. pneumoniae* reads was more than twice as high on Flight 3 compared to Flights 1 and 2. Correspondingly, analysis of the overall metagenome of the ISS, with reads normalized between the three flights, showed 50% higher relative abundance of reads for genes associated with virulence and 100% higher reads for genes associated with antimicrobial resistance (AMR) between Flight 1 and 3 (55).

K. pneumoniae is one of the named ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species), known not only for its risks to human health as an opportunistic pathogen but also as a vessel for AMR genes that can be passed to other bacteria via horizontal gene transfer (58, 66). *Klebsiella* species are known to carry AMR and multi-drug resistance (MDR) genes such as *oqxAB*, which encodes an efflux pump associated with MDR (82). The presence and expression of efflux pumps in Enterobacteria like *K. pneumoniae* is not only a reason for the rise in resistance to many common antibiotics, but efflux pumps have also been shown to confer resistance to QAC disinfectants like benzalkonium chloride (72, 99).

QACs consist of salts of quaternary ammonium cations, which consist of a positively charged nitrogen ion at the center with four substituent arms consisting of alkyl or aryl groups. The alkyl arms often consist of long, bulky chains that are believed to disrupt cell membranes, leading to cell lysis and death (83). These compounds are the active ingredient in many common disinfectants used in industrial and medical settings as well as in household cleansers due to their high safety profile for humans and other animals (84). Beyond disinfectant usage, QACs are also ingredients in other common consumer and industrial goods, leading to the presence of QACs in wastewater effluent (85). Bacterial resistance to QACs is understudied relative to other classes of antimicrobial compounds, but Gram-negative bacteria such as *Klebsiella* are known to have emerging resistance (86). QAC resistance is particularly associated with the overexpression of efflux pumps and reduced expression of membrane porins, as well as associated with strains that have a hypermucoviscous phenotype (72, 87, 88).

In this work, I studied two strains of *Klebsiella* isolated from the ISS during Microbial Tracking-1, *K. pneumoniae* F3-2P(2*), described in Solomon 2020 (57), and *K. quasipneumoniae* IF3SW-P1, described in Chapter 2 of this work (previously published as Sushenko 2022) (56). These strains had not only been exposed to spaceflight conditions such as microgravity and increased radiation, but they were isolated from ISS surfaces that were sanitized at least once weekly with wipes containing a mixture of QACs. My objective was to elucidate the responses of these ISS-origin *Klebsiella* strains to the QAC mixture used on the space station by determining the minimum inhibitory concentration (MIC) for each strain and studying the responses of stationary-phase cells of each strain to QAC exposure. For the latter, I used techniques such as killing curves and phase-contrast microscopy to see if cultures survived after treatment with QACs, in addition to viability assays such as LIVE/DEAD fluorescence microscopy and PMAxx qPCR to determine culture viability at different QAC concentrations.

3.3 Materials and methods

Strains and experimental conditions

Strains of *Klebsiella* used in this work are described in **Table 2**. *K. pneumoniae* subsp. *pneumoniae* F3-P2(2*) and *K. quasipn*eumoniae subsp. *similipneumoniae* IF3SW-P1 were isolated from interior surfaces of the ISS during the Microbial Tracking-1 project (44). A draft genome of *K. pneumoniae* F3-2P(2*) was previously published, while a complete genome of *K. quasipneumoniae* IF3SW-P1 was described in Chapter 2 of this thesis (57. 58). The type strain, *K. pneumoniae* subsp. *pneumoniae* (ATCC 13883^T), which has 99.01% average nucleotide identity (ANI) to strain F3-P2(2*) (57), was obtained from ATCC to serve as an Earth-origin control. Unless otherwise stated, all strains were cultivated from freezer stocks in 10 mL of 0.1X tryptic soy broth (TSB) (Sigma-Aldrich) in 125 mL Erlenmeyer flasks with shaking at 150 RPM. This diluted media was taken from an inherited protocol from previous work at JPL, intended to replicate low nutrient availability on ISS surfaces. Strains were incubated at either 37°C (optimal growth temperature) or 23°C (approximate room temperature on ISS). If grown on solid media, strains were grown on 0.1X TSB solidified with 1.5% microbiology-grade agar (TSA, Sigma-Aldrich).

Table 2. Strain information

Strain name	Source	Obtained from	Ref.
Klebsiella quasipneumoniae subsp. *similipneumoniae* strain F3-P2(2*)	Waste and Hygiene Compartment (WHC) wall panel on ISS	JPL	(57)
Klebsiella pneumoniae subsp. *pneumoniae* strain IF3SW-P1	Advanced Resistive Exercise Device (ARED) foot panel on ISS	JPL	(56)
Klebsiella pneumoniae subsp. *pneumoniae* strain 13883^T	Type strain (isolated by Schroeter 1886)	ATCC	(89)

To test *Klebsiella* strains for a hypermucoviscous phenotype, the string test was used (94). Strains were streaked on 5% blood agar hemolysis plates (Hardy Diagnostics) and incubated overnight at 37°C. A sterile loop was then used to touch and lift an individual colony. If a viscous string >5 mm formed, the strain was considered to have a hypermucoviscous phenotype, which is associated with hypervirulent strains of both *K. pneumoniae* and *K. quasipneumoniae* (61, 71, 80).

At the time of Microbial Tracking-1, disinfectant wipes used on the ISS contained a mixture of four QACs at the following concentrations (m/v): alkyl dimethylbenzylammonium chloride (0.0532%), octyl decyl dimethyl ammonium chloride (0.0399%), dioctyl dimethyl ammonium chloride (0.01995%), and dodecyl dimethyl ammonium chloride (0.01995%) (44). These compounds were acquired from Santa Cruz Biotechnology and were reconstituted in 25 mL of MilliQ ultrapure water to create stock solutions that were filter sterilized. Stock solutions were made at either 10X or 100X concentration due to material limitations, and then the separate stock solutions were combined into a 2X working solution of the QAC mixture with MilliQ water (**Table 3**). All experiments consisting of QAC treatment used an equal volume of QAC mixture to the bacterial culture being treated, resulting in a 1X treatment concentration.

After treatment with QACs, cultures were attempted to be resuscitated in fresh media in case the cells had entered a viable but nonculturable state (VBAC) (95). To do this, both spread plates and broth cultures were prepared. 0.1X TSB media was used for both plates and broth, as to not shock potentially stressed cultures with nutritionally rich media. All cultures were then incubated at 37°C for 72 hours, with shaking at 150 rpm for the broth cultures.

Table 3. QAC concentrations

QAC name	Percent m/v as used on ISS (44)	Percent m/v in 2X working solution
Alkyl dimethylbenzylammonium chloride	0.0532	0.10
Dioctyl dimethyl ammonium chloride	0.01995	0.04
Dodecyl dimethyl ammonium chloride	0.01995	0.04
Octyl decyl dimethyl ammonium chloride	0.0399	0.08

Minimum inhibitory concentration (MIC) determination

MIC determination was conducted using the broth microdilution method as described in Wiegand (2008) (90, 91). A modification was made to the assay by using 0.1X TSB to replicate the low-nutrient environment of the ISS, as described above. Ten QAC dilutions were prepared by serially 2-fold diluting the QAC mixture with 0.1X TSB. 50 µL of each dilution was pipetted in triplicate into untreated flat bottom 200 µL 96-well clear polystyrene plates with gas permeable lids (ThermoFisher), one plate for each strain. Perimeter wells of each plate were filled with MilliQ water to reduce evaporation. Cultures of each strain were grown overnight in 0.1X TSB and diluted to 0.5 OD_{600}, and 50 µL was added to each well with QAC dilutions. For a growth control, 50 µL of each culture was added in triplicate with 50 µL 0.1X TSB. 100 µL of 0.1X TSB was added as a sterile control, and 100 µL of MilliQ water added as a blank. The plates were then incubated for 20 hours at 37°C with 150 rpm shaking before being evaluated. The standard evaluation for this assay is a visual assessment of turbidity, where the highest dilution with no turbidity is the MIC. This assay was instead performed in a BioTek Synergy 2 plate reader (Agilent Technologies), with an OD_{600} measurement recorded by the machine at the conclusion of the experiment, with any $OD_{600} < 0.1$ considered not turbid.

Initial serial 2-fold dilutions produced QAC concentrations of 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56%, 0.78%, 0.39%, and 0.20% relative to the stock concentration. Dilutions were prepared at 2X concentration to produce the stated concentration after being added to an equal volume of bacterial culture. After running a preliminary MIC assay with these QAC concentrations, a new dilution range between the last two dilutions was established for the final MIC determination: 0.4%, 0.35%, 0.3%, 0.25%, 0.2%, 0.175%, 0.15%, 0.125%, 0.1%, 0.05%. These dilutions were then used for the MIC determination as shown and described below.

Killing curves

Cultures of each strain were grown to late-exponential phase and adjusted to an OD_{600} of 0.05. 100 µL aliquots of each culture at 0.05 OD_{600} were added in triplicate to an untreated 200 µL flat bottom 96-well clear polystyrene plate with gas-permeable lid (ThermoFisher), with perimeter wells filled with MilliQ water to minimize evaporation. The plate was then incubated for 24 hours in a BioTek Synergy 2 plate reader at 23°C with shaking at 150 RPM until reaching late-exponential phase, approximately 8 hours. At that timepoint, the plate reader was paused for the addition of either the ISS concentration of the QAC mixture or MilliQ water for the negative controls. Incubation then continued for an additional 8 hours with the OD_{600} recorded every 30 minutes. After a total of 16 hours, data were collected and analyzed, including standard deviation, standard error and Student's t-test to evaluate statistical significance.

Microscopy and LIVE/DEAD viability assay

All microscopy was conducted using a Zeiss Axio Imager microscope using a 63x Objective Plan-Apochromat 63/1.40 Oil Ph3 M27 (Zeiss), with images captured using either a Zeiss AxioCam MRc5 camera (phase contrast) or Hamamatsu ORCA Flash 4.0 LT Monochromatic Digital CMOS camera (fluorescence). SYTO-9 dye was visualized using the Zeiss Filter Set 38 HE (BP 470/40 HE) and PI dye was visualized using the Zeiss Filter Set 43 HE (BP 550/25 HE). Microscopy images were captured with Zeiss ZEN 3.3 Pro microscopy software and processed using Fiji/ImageJ.

The LIVE/DEAD *Bac*Light Bacterial Viability Kit (Invitrogen) was used to differentially stain cells based on their viability, allowing both live and dead cells to be visualized via fluorescence microscopy. Prior to beginning the LIVE/DEAD assay, triplicate cultures were incubated at 23°C until late-exponential phase (8 hours), then treated with either MilliQ water or the same concentration of QAC mixture used to sanitize ISS surfaces as described above (44). Treated cultures were incubated for a further 8 hours before the cultures were washed and prepared as described by the manufacturer. Briefly, 20 mL of each culture was transferred to 30 mL Nalgene Oak Ridge High-Speed PPCO Centrifuge Tubes (ThermoFisher) and centrifuged at 10,000 x g for 10 minutes. The supernatant was then removed, and the pellet resuspended in 20 mL of 0.85% NaCl wash buffer, with the exception of the LIVE/DEAD killed control which was resuspended with 20 mL of 70% isopropyl alcohol instead of wash buffer. Samples were left to incubate at room temperature for 60 minutes before centrifuging again at 10,000 x g for 10 minutes, removing the supernatant, and resuspending all samples in 10 mL wash buffer. This last wash step was then repeated for a final time before the cells were prepared for LIVE/DEAD staining.

To stain the cells, the separate LIVE (SYTO-9) and DEAD (propidium iodide/PI) dyes were used at a final concentration of 1.67 mM SYTO-9 and 20 mM PI. 1 mL of each washed sample was aliquoted into microcentrifuge tubes, stained first with 1.5 μL of PI followed by 1.5 μL of SYTO-9, and incubated at room temperature in the dark for 15 minutes. After incubation, 10 μL of the stained sample was pipetted onto a glass microscope slide. A drop of ProLong Gold Antifade Mountant (Invitrogen) was added to each slide to reduce fading before a glass coverslip was added.

PMAxx and viability qPCR

The viability dye PMAxx (propidium monoazide, Biotium) was used in conjunction with viability qPCR to determine the viability of each strain at QAC concentrations around the MIC. Triplicate cultures of each strain were grown at 23°C to late-exponential phase (8 hours), then treated with a QAC dilution or MilliQ water. Treated cultures were incubated for a further 16 hours before being homogenized by vigorously vortexing them and then separated into 800 µL aliquots in microcentrifuge tubes. Each sample was prepared in two sets of triplicates (PMAxx treated and untreated). An additional negative control was prepared to serve as the dead control and was killed via incubation in a 90°C water bath for 5 minutes.

PMAxx treatment was conducted according to the manufacturer's protocol. Briefly, 1 µL of 20 µM PMAxx was added to one set of aliquots while working in the dark for a final concentration of 25 µM, while the other set of aliquots was left untreated. The treated tubes were covered in foil and incubated for 10 minutes on a rocker to ensure the PMAxx was well mixed. To covalently bond PMAxx to free dsDNA, the treated tubes were exposed to LED light (3000 lumens/4000K) for 15 minutes. After photoactivation, tubes were centrifuged at 10,000 x g for 10 minutes to pellet the cells.

Upon completion of the PMAxx treatment, genomic DNA was extracted from the resultant cell pellets using the DNeasy Blood & Tissue extraction kit (Qiagen), using the manufacturer's protocol for Gram-negative bacteria. Any DNA bound to PMAxx was insoluble and lost as waste during the extraction process (103, 104). As a quality control step to confirm successful extraction, the resultant DNA was then quantified via a Qubit 4 Fluorometer (dsDNA HS assay, Invitrogen). Primers 341F (CCT ACG GGA GGC AGC AG) and 518R (ATT ACC

GCG GCT GCT GG) (92, 93), producing a 194 bp amplicon from the V3 hypervariable region of the 16S rRNA gene, were obtained from IDT. To prepare a standard for these primers to be used in qPCR, traditional PCR was performed using DNA from an untreated culture of *K. pneumoniae* 13883^T. The resultant PCR product was visualized on a 1.5% agarose gel. DNA from a band migrating at the expected amplicon size was extracted using the PureLink Quick Gel Extraction Kit (Invitrogen) and quantified using the Qubit dsDNA HS assay. This value was then used for calculating a standard curve for each qPCR run, allowing for quantification of the amplified DNA and calculation of the R^2 statistic.

qPCR was conducted using the QuantStudio 3 Real-Time PCR System (Applied Biosystems). SYBR Green PCR Master Mix (Applied Biosystems), containing AmpliTaq Gold DNA polymerase, was used for all qPCR runs. DNA extracted from each treatment was aliquoted in triplicate into a MicroAmp optical 96-well reaction plate (Applied Biosystems), with the perimeter wells filled with MilliQ water to reduce evaporation, and then the plate was sealed with MicroAmp optical adhesive film (Applied Biosystems). Cycle parameters included a 5-minute initial denaturing step at 95°C before 35 cycles of 15 seconds denaturing at 95°C and 1 minute annealing and extension at 60°C. Upon run completion, qPCR results were analyzed using Design and Analysis Software version 2.6.0 (Applied Biosciences). qPCR statistics and standard curves were calculated by this software. The R^2 for all three runs was close to 1 (0.998, 0.987, 0.997) and the amplification efficiency was 97.176%, 94.545%, and 89.123%.

Viability qPCR results were calculated as described in the PMAxx manufacturer's protocol. Briefly, dC_T (delta cycle threshold) was calculated by subtracting the average C_T of the PMA-treated samples from the average C_T of the non-PMA-treated samples. For the live control, the dC_T is expected to be 0 (+/- 1) and the dead control is expected to have a $dC_T > 4$. As C_T is on a $\log_2$ scale, fold reduction can then be calculated via $2^{(dCt)}$, while the remaining percent viable can be calculated via $100/2^{(dCt)}$. Any negative dC_T values were normalized to 0 to prevent percent viability >100%.

3.4 Results and discussion

Surfaces on the International Space Station are sanitized at least once per week using wipes containing a mixture of QACs, and the strains of *Klebsiella* isolated from the ISS were likely exposed to these compounds via this regular process. Before testing how stationary-phase cells of each strain respond to the QAC mixture in killing and viability assays, I investigated how susceptible the growth of each strain is to the QAC mixture. To do so, I determined the minimum inhibitory concentration (MIC) of the QAC mixture for each strain using the broth microdilution assay as described in the methods. The type strain for *K. pneumoniae*, 13883^T, was also tested to see if there was a difference between ISS-origin and Earth-origin strains within the same species.

The results of the MIC determination assay are shown in **Figure 1**, where the MIC is the lowest concentration with an OD_{600} measurement <0.1. The MIC for *K. quasipneumoniae* IF3SW-P1 was 0.15% (**Fig 1A**), the MIC for *K. pneumoniae* F3-2P(2*) was 0.175% (**Fig 1B**), and the MIC for Earth-origin *K. pneumoniae* 13883^T was 0.125% (**Fig 1C**). Interestingly, the strain with the lowest MIC, and thus the most susceptible to the QAC mixture, was the Earth-origin type strain for *K. pneumoniae* that has 99.01% ANI to *K. pneumoniae* F3-2P(2*). Additionally, the ISS-origin *K. quasipneumoniae* IF3SW-P1 had a MIC that fell in between the ISS-origin *K. pneumoniae* and the Earth-origin type strain, suggesting it is less susceptible than the Earth-origin type strain as well.

A. *K. quasipneumoniae* IF3SW-P1

	0.4%	0.35%	0.3%	0.25%	0.2%	0.175%	0.15%	0.125%	0.1%	0.05%
1	0.091	0.092	0.091	0.087	0.086	0.086	0.085	0.087	0.086	0.302
2	0.091	0.093	0.089	0.087	0.086	0.087	0.088	0.272	0.273	0.317
3	0.091	0.09	0.089	0.088	0.088	0.087	0.087	0.258	0.281	0.312
H_2O	0.081	0.08	0.081							
SC	0.087	0.086	0.085							
GC	0.316	0.32	0.295							

B. *K. pneumoniae* F3-2P(2*)

	0.4%	0.35%	0.3%	0.25%	0.2%	0.175%	0.15%	0.125%	0.1%	0.05%
1	0.09	0.092	0.093	0.091	0.09	0.093	0.091	0.267	0.269	0.26
2	0.091	0.091	0.091	0.094	0.089	0.092	0.103	0.251	0.264	0.264
3	0.097	0.093	0.095	0.093	0.093	0.096	0.242	0.274	0.231	0.283
H_2O	0.083	0.08	0.08							
SC	0.086	0.087	0.084							
GC	0.291	0.291	0.28							

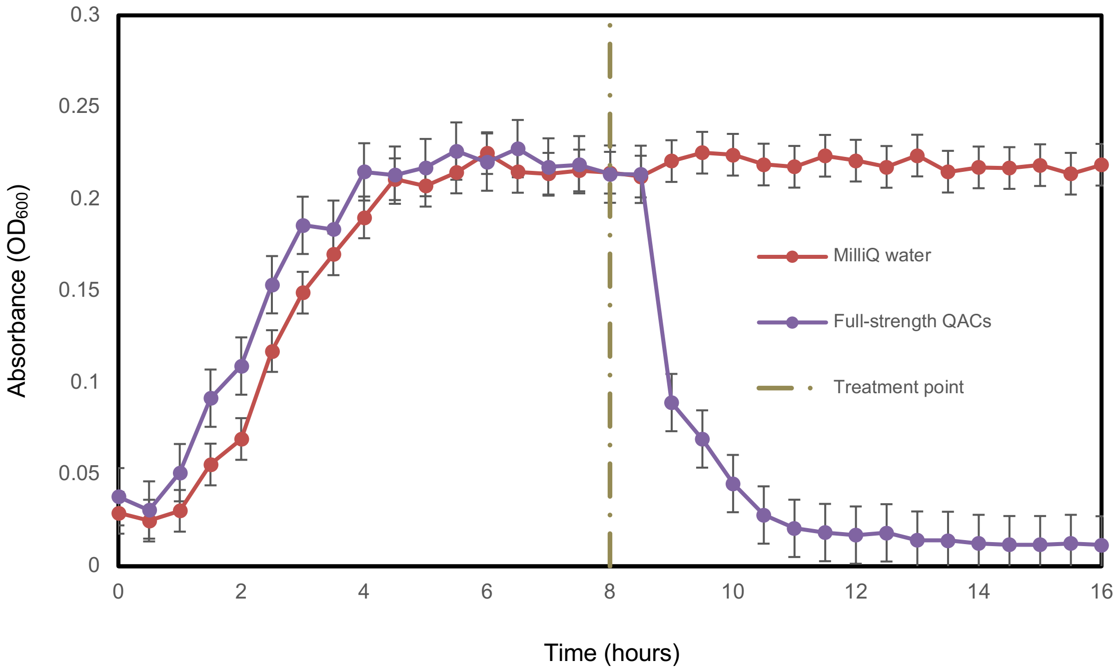
C. *K. pneumoniae* 13883^T

	0.4%	0.35%	0.3%	0.25%	0.2%	0.175%	0.15%	0.125%	0.1%	0.05%
1	0.091	0.09	0.088	0.088	0.089	0.089	0.088	0.087	0.211	0.263
2	0.091	0.088	0.086	0.087	0.087	0.089	0.087	0.091	0.225	0.254
3	0.091	0.094	0.088	0.087	0.089	0.091	0.09	0.088	0.212	0.268
H_2O	0.08	0.078	0.079							
SC	0.085	0.083	0.084							
GC	0.293	0.249	0.248							

Figure 1. QAC MIC determination.

Broth microdilution assay to determine the QAC MIC for each strain; OD_{600} recorded after 20 hours incubation at 37°C in 0.1X TSB. Assay conducted in triplicate with three controls: H_2O: blank (MilliQ water); SC: sterile control (0.1X TSB); GC: growth control (untreated culture). OD_{600} measurements <0.1 are considered not turbid.

A *K. quasipneumoniae* IF3SW-P1



B *K. pneumoniae* F3-P2(2*)

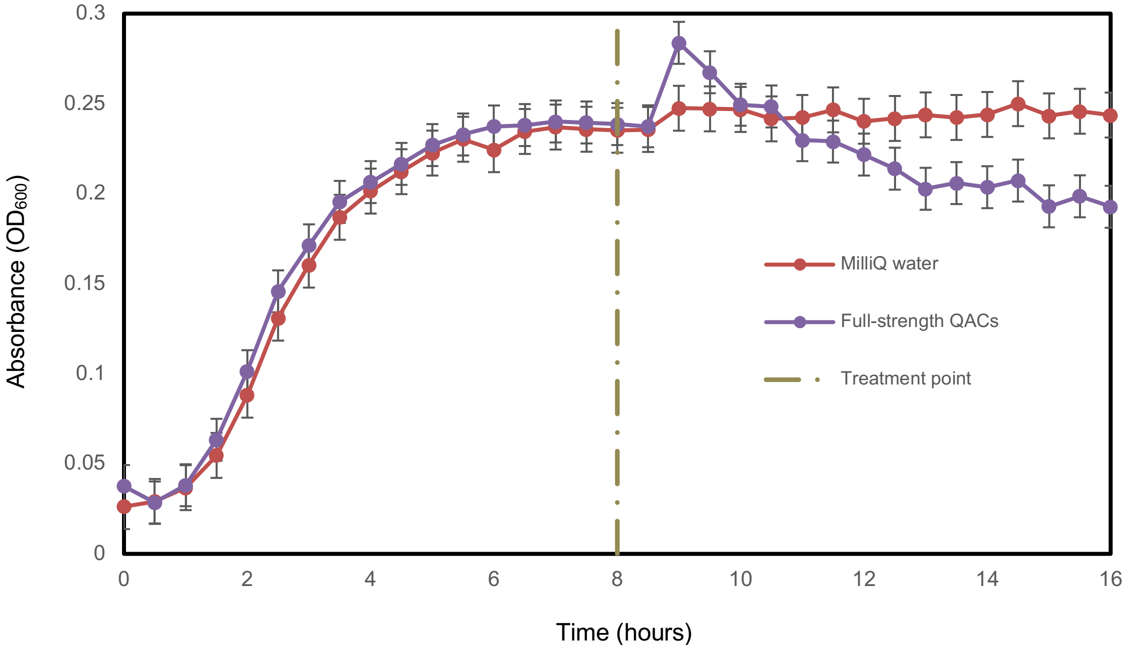


Figure 2. Killing curves after QAC treatment

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 the QAC mixture or MilliQ water was added and the plates were incubated for an additional 8 hours.

To determine the effect of the QAC mixture used to clean the space station on stationary-phase cells, strains of the ISS-origin *Klebsiella* were grown and then treated with QACs and effects were monitored by assessing optical density and microscopy. The two strains showed drastic differences in response to QAC treatment (**Fig 2-4**). Prior to QAC treatment, the OD_{600} of both strains was just above 0.20, while the OD_{600} of QAC-treated *K. pneumoniae* F3-2P(2*) showed a small but significant decrease 8 hours after treatment (**Fig 2A**; unpaired t-test compared to untreated control, $p = 3.16x10^{-11}$), while the OD_{600} of QAC-treated *K. quasipneumoniae* IF3SW-P1 approached 0.00 over the same time period (**Fig 2B;** unpaired t-test compared to untreated control, $p = 2.09x10^{-14}$). This result showed that *K. quasipneumoniae* IF3SW-P1 lysed almost completely within 3 hours of treatment (**Fig 2B**). Lysis after QAC treatment was expected due to its known mechanism of action. However, *K. pneumoniae* F3-2P(2*) remained turbid even 8 hours after exposure to ISS-strength QAC exposure (**Fig 2A**), implying the cells remained intact after treatment.

To confirm that the QAC-treated cells of *K. pneumoniae* F3-2P(2*) remained intact whereas those of *K. quasipneumoniae* IF3SW-P1 lysed, wet mounts of both strains were prepared and the cultures were viewed under 630x total magnification and phase contrast. Wet mounts of the water-treated cultures were also prepared as controls, showing healthy cultures (**Fig 3A, 4A**). In accordance with its OD_{600} reading of nearly 0.00, cells of *K. quasipneumoniae* IF3SW-P1 appeared to be lysed (**Fig 2B**) as only cell fragments were visible under microscopy (**Fig 3B**). *K. pneumoniae* F3-2P(2*) did not appear to be lysed, nor as individual cells, but instead in large clumps of intact cells (**Fig 4B**). As *K. pneumoniae* F3-2P(2*) is known to have a hypermucoviscous phenotype as confirmed by the string test, these structures are possible protective structures formed by *K. pneumoniae* F3-2P(2*) in response to QAC exposure.

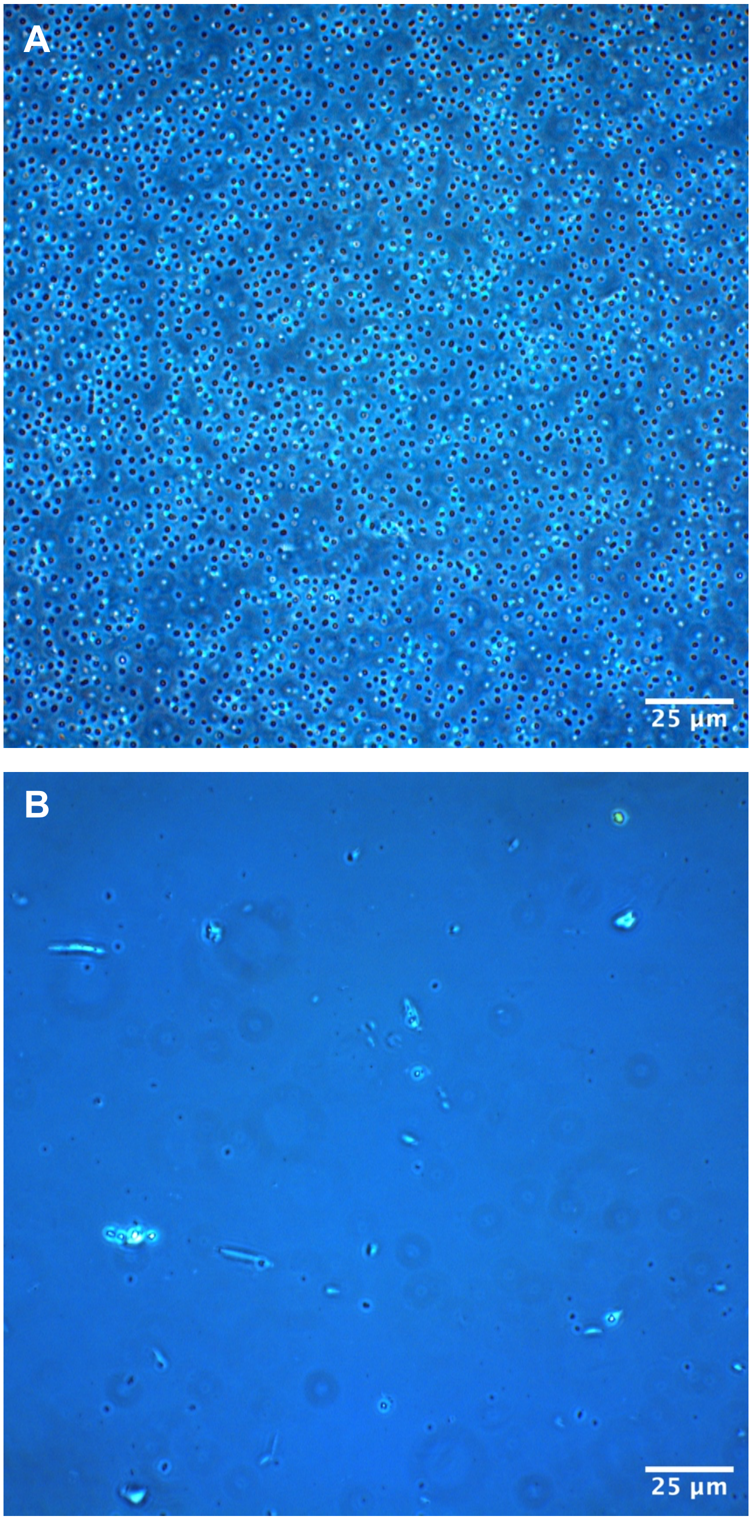


Figure 3. Phase-contrast microscopy of *K. quasipneumoniae* IF3SW-P1 with or without QAC treatment

Overnight cultures grown in 1:10 TSA were treated with either MilliQ water (**A**) or ISS-strength QAC mixture (**B**) and prepared as wet mounts. Slides were visualized at 630x with phase contrast. **A** shows a healthy culture of *K. quasipneumoniae* IF3SW-P1; **B** shows cell fragments, likely from lysis.

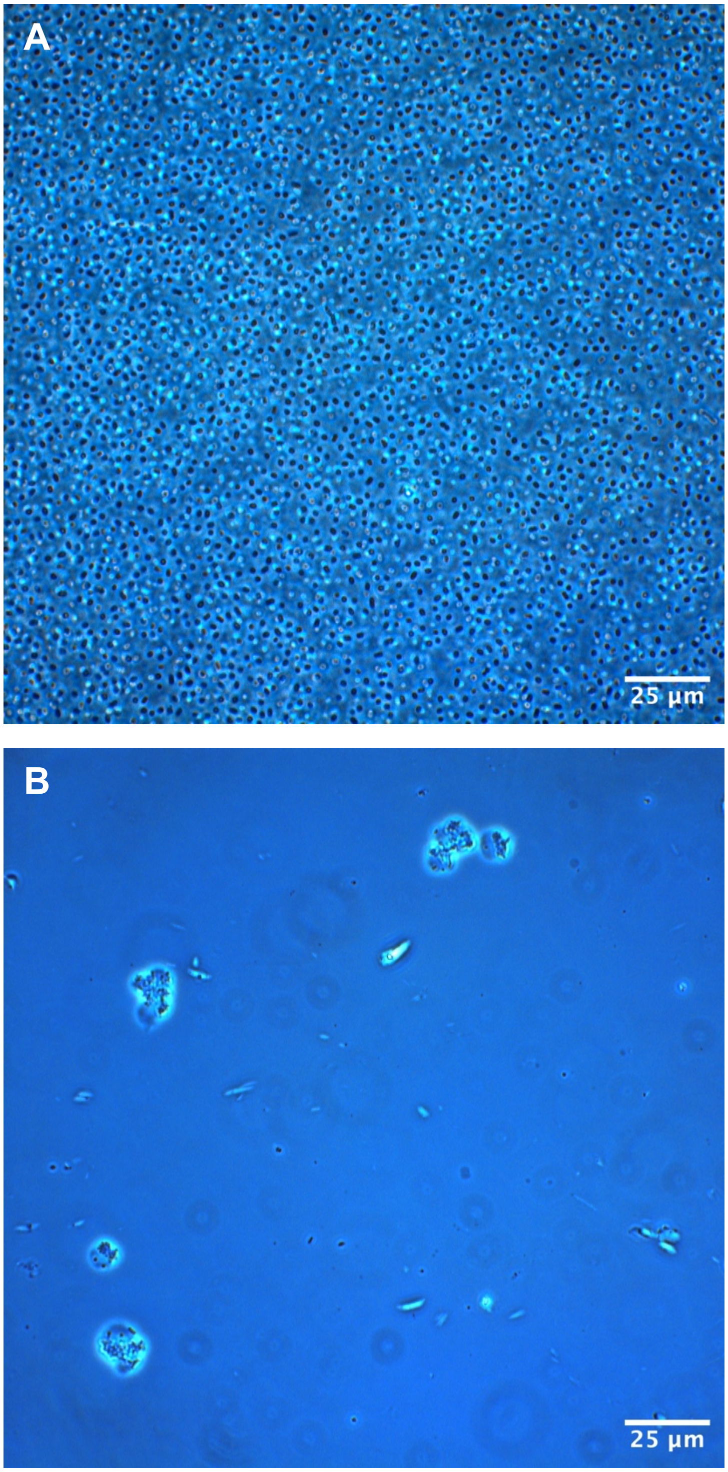
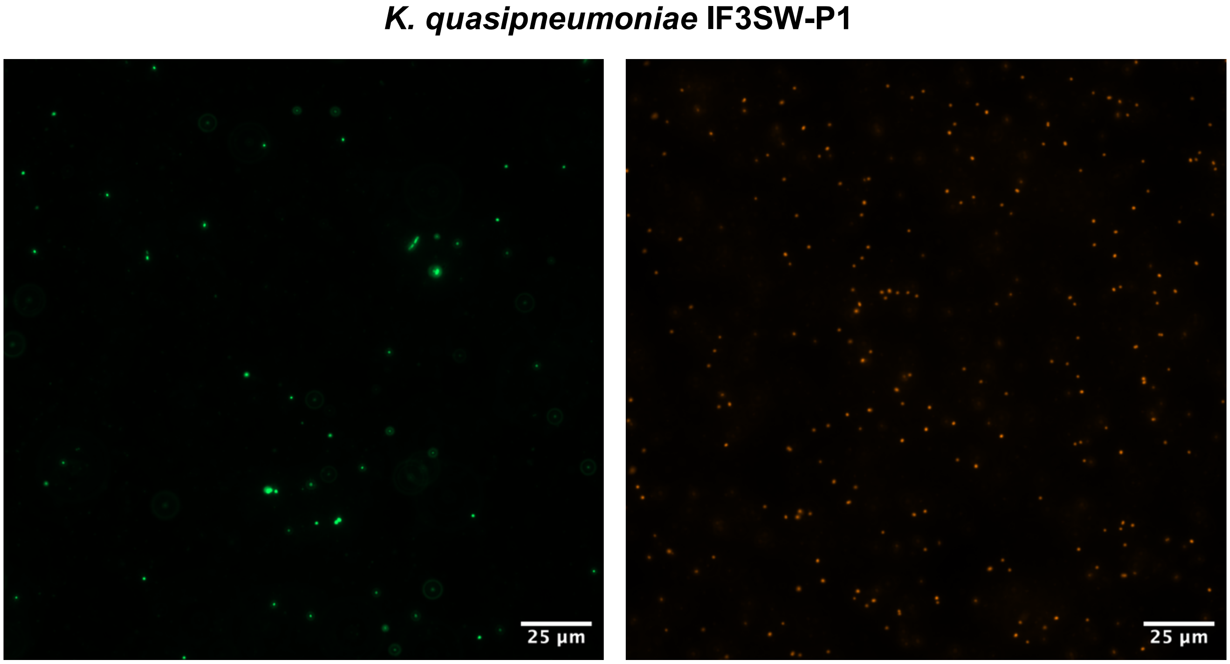


Figure 4. Phase-contrast microscopy of *K. pneumoniae* F3-2P(2*) with or without QAC treatment

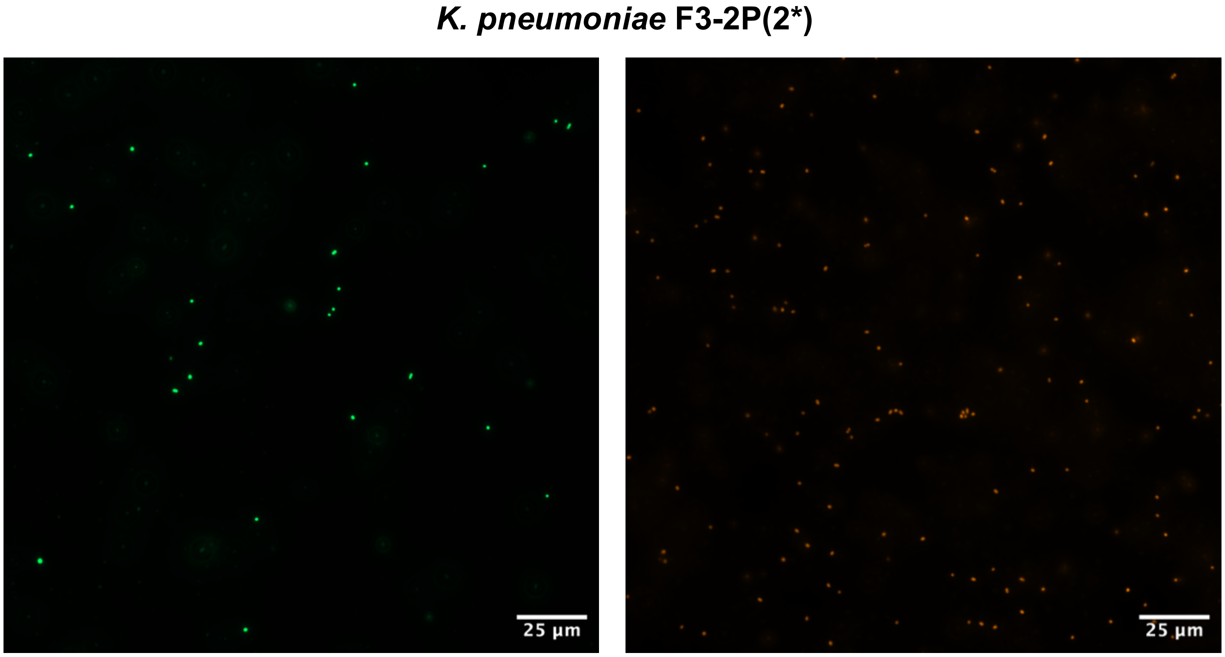
Overnight cultures grown in 1:10 were treated with either MilliQ water (**A**) or ISS-strength QAC mixture (**B**) and prepared as wet mounts. Slides were visualized at 630x with phase contrast. **A** shows a healthy culture of *K. pneumoniae* F3-2P(2*); **B** shows clumps of cells post-QAC treatment.

As shown in **Figure 4B**, the center of the *K. pneumoniae* F3-2P(2*) clump structures appear to be densely packed with cells. To determine whether the center of these clumps contain viable cells, the full-strength QAC treatment was repeated for the purpose of performing a LIVE/DEAD *Bac*Light viability assay. Controls of both live and dead cultures for each strain can be seen in **Figure 5.** As shown in the previous assay, *K. quasipneumoniae* IF3SW-P1 lysed during treatment with the full-strength QAC mixture. This resulted in poor staining with the LIVE/DEAD dyes due to low cell concentration, with cell debris from lysis seen instead (**Fig 6A**). However, as in the previous assay, *K. pneumoniae* F3-2P(2*) cells did not lyse after QAC treatment allowing them to be stained and observed under fluorescence microscopy (**Fig 6B**).

Few individual cells of *K. pneumoniae* F3-2P(2*) were visible in either of the QAC-treated culture when compared to a killed control treated with 70% isopropyl alcohol (**Fig 5, 6B**). Similar to the phase-contrast microscopy in **Figure 4B**, clumps of cells of QAC-treated *K. pneumoniae* F3-2P(2*) were observed in **Figure 6B**. However, these clumps show staining from both SYTO-9 and PI, resulting in inconclusive results as to whether there were viable cells in the center of these clumps (**Fig 6B**). To see if the cells in these clumps were viable, 10-fold serial dilution spread plates of the QAC-treated strains resulted in no growth after 72 hours of incubation at 37°C (data not shown).



Live control **Dead control**

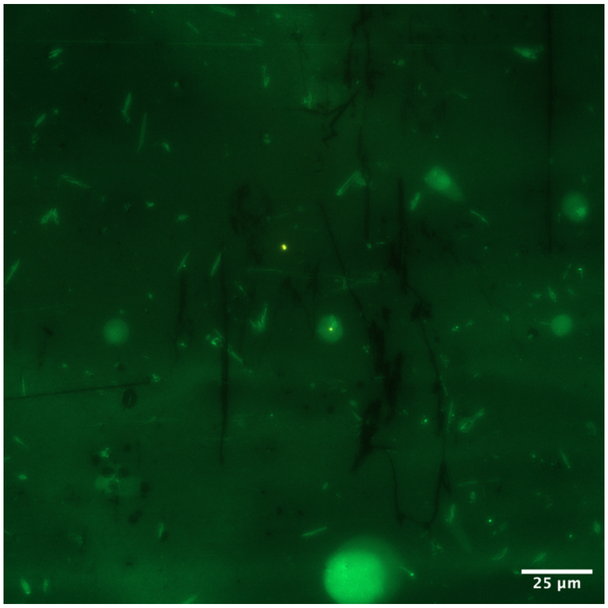


Live control **Dead control**

Figure 5. LIVE/DEAD fluorescence microscopy controls

Fluorescence microscopy images of *K. quasinpneumoniae* IF3SW-P1 taken at 630x total magnification. Live controls show individual cells stained with SYTO-9. Dead controls (killed by treatment of 70% isopropyl alcohol) show individual cells stained with PI.

A ***K. quasipneumoniae*** **IF3SW-P1**



B ***K. pneumoniae*** **F3-2P(2*)**

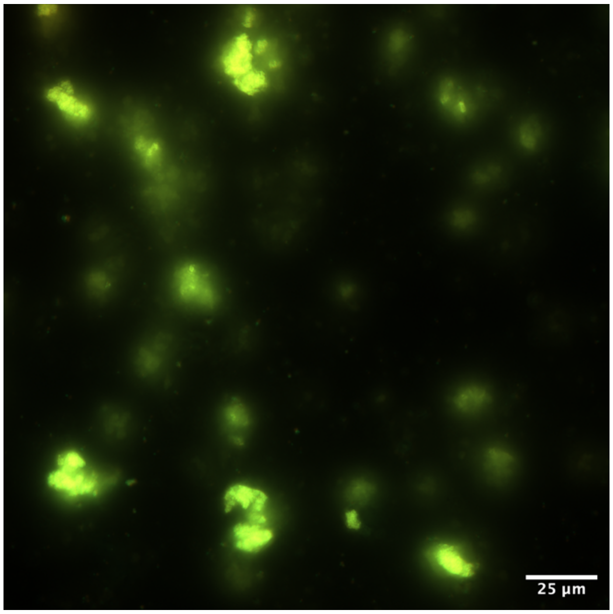


Figure 6. LIVE/DEAD fluorescence microscopy after QAC treatment

Fluorescence microscopy images of QAC-treated *K. quasipneumoniae* IF3SW-P1 (**A**) and *K. pneumoniae* F3-2P(2*) (**B**) taken at 630x total magnification. **A** shows cell debris consistent with this strain lysing. **B** shows clumps of cells stained with both SYTO-9 and PI.

The incongruous result shown in **Figure 6B** remained despite troubleshooting different ratios of the concentrations of the two dyes. A review of the literature for possible dye interaction between SYTO-9 and PI showed these two dyes interact due to the emissions spectrum of SYTO-9 (480/500 nm) overlapping with the excitation spectrum of PI (490/635 nm) (97). This crosstalk has been shown to be the greatest when the ratio of live to dead cells in the sample being stained is skewed in either direction (98). While the ratio of dead to live cells in the QAC-treated *K. pneumoniae* F3-2P(2*) sample is unknown, due to their inability to be resuscitated by plating on 0.1X TSA, it is likely there is greater than 75% in the sample, which is in the range where this crosstalk effect was shown to be most prominent. Due to this known dye interaction, SYTO-9/PI as a LIVE/DEAD dye combination appears to be unsuitable for assessing viability after a killing assay such as QAC treatment. If possible, different viability dyes with emission spectra that do not overlap should be used instead, or a viability assay that only uses a "dead" dye to measure viability, such as PI or SYTOX Blue (101).

Beyond the above-described issues with LIVE/DEAD dye interactions at high dead cell concentrations, there are some caveats to how the dyes in the LIVE/DEAD assay work on cells physiologically. SYTO-9, the "live" dye, permeates all cells, viable or not, by an unknown mechanism. The "dead" dye, PI, works by entering cells with permeable membranes and binding to intracellular DNA. Therefore, the LIVE/DEAD assay uses membrane permeability as its sole marker of viability, meaning any cell with an intact membrane will appear viable in this assay. Certain bacteria, including *Klebsiella*, are known to have a viable but nonculturable state (VBAC) in response to stress where they are metabolically active but cannot grow (95). Cells in this condition have intact membranes and so would appear viable in assays such as LIVE/DEAD. If QAC-treated *K. pneumoniae* F3-2P(2*) strains are indeed under this VBNC state, they may be able to be resuscitated, but were unable to be resuscitated under the attempted laboratory conditions (96).

Additionally, it should be considered whether these dyes can reach the cell membranes of these cells to successfully stain them. Both *K. pneumoniae* F3-2P(2*) and *K. quasipneumoniae* IF3SW-P1 produce capsules, which is one of the main virulence factors of *Klebsiella* spp (61, 71). The hypermucoviscous phenotype of *K. pneumoniae* F3-2P(2*) is due to overexpression of capsule genes (80). The SYTO-9 and PI stained clumps in **Figure 6B** appears to not just show cells being stained, but also the extracellular material holding the clumps together. This capsule or other biofilm components may be preventing the LIVE/DEAD dyes from entering the center of these clumps and staining the cells inside (100). At the same time, if the bacterial capsule can keep SYTO-9 and PI from reaching the cell membrane of these clumped cells, it is reasonable the same capsule could also prevent the QAC disinfectants from reaching these clumped cells as it has previously been shown that the QAC concentrations needed to kill biofilm-producing isolates of common hospital-associated bacteria need to be 10- to 1000-fold greater than concentrations needed to kill non-biofilm-producing strains of the same bacterial species (102). Due to these caveats, LIVE/DEAD staining with SYTO-9 and PI may not be not a suitable assay to investigate this question.

Table 4. *K. quasipneumoniae* IF3SW-P1 viability qPCR

	dCt	**Fold reduction**	**% viable**
Live control	0	0	100
Dead control	10.6083094	1561.05905	0.06405908
0.2%	10.6083094	1561.05905	0.06405908
0.175%	8.67210006	407.908019	0.24515331
0.15%	16.3706364	84733.0063	0.00118018
0.125%	16.332794	82539.3236	0.00121154
0.1%	18.4132786	349099.464	0.00028645
0.075%	15.4918497	46079.893	0.00217014

Table 5. *K. pneumoniae* F2-3P(2*) viability qPCR

	dCt	**Fold reduction**	**% viable**
Live control	0	0	100
Dead control	6.80417133	111.753122	0.89482958
0.2%	12.5952773	6188.08483	0.01616009
0.175%	10.567494	1517.51394	0.06589725
0.15%	0.13304943	1.09660916	91.1901918
0.125%	0	0	100
0.1%	0	0	100
0.075%	0	0	100

Table 6. K. pneumoniae 13883^{T} viability qPCR

	dCt	**Fold reduction**	**% viable**
Live control	0	0	100
Dead control	10.9510313	1979.65223	0.05051392
0.2%	10.6719561	1631.46925	0.06129444
0.175%	12.4394451	5554.51456	0.01800337
0.15%	11.7471281	3437.46212	0.02909123
0.125%	11.9245243	3887.22351	0.0257253
0.1%	12.4461431	5580.36249	0.01791998
0.075%	11.8647358	3729.4206	0.02681382

Previous killing experiments were conducted with a full-strength QAC disinfectant mixture and resulted in either non-viable lysed cultures (*K. quasipneumoniae* IF3SW-P1) or what appeared to potentially be viable cultures, but they could not be resuscitated under standard growth conditions (*K. pneumoniae* F3-2P(2*)). To further explore the response of these *Klebsiella* strains to QAC exposure, viability qPCR was performed using the PMAxx viability dye. This viability dye enters cells with compromised membranes and binds to dsDNA similar to PI. When DNA is extracted from PMAxx-treated samples, the bound dsDNA is washed away from the final DNA eluant, leading to a purified DNA from only the viable cells in a sample. After the extracted DNA was amplified via qPCR, PMAxx-treated samples were then compared to untreated samples to see the proportion of DNA from viable cells in the original culture.

A range of QAC dilutions was chosen for viability qPCR: 0.20%, 0.175%, 0.15%, 0.125%, 0.1%, and 0.075%. Strains were prepared as per previous assays, where cultures were grown at 23°C until late-exponential phase (8 hours) and then treated with QACs or MilliQ water and incubated for an additional 8 hours. Samples were then treated with PMAxx and then the DNA extracted and analyzed via qPCR as described in the methods. Viability was calculated using the difference between the C_T (cycle threshold) from PMA-treated and untreated samples. The resultant dC_T, fold reduction, and percent viablility is shown in **Tables 4-6**, with viable results highlighted in green. In **Table 4**, the treatment with the greatest viability for *K. quasipneumoniae* IF3SW-P1 is 0.175%, with a viability of 0.241%. While this is higher than the 2% treatment, the dCT is ~8, which is significantly above the dead cutoff of 4. **Table 5** shows *K. pneumoniae* F3-2P(2*), which has 100% viability for treatments 0.125-0.075%, and 91.19% viability for treatment 0.15%. The control strain *K. pneumoniae* 13883^T is shown in **Table 6**, where the treatment with the greatest viability was 0.2%, with a viability of 0.06%.

When comparing the results of the three strains, only *K. pneumoniae* F3-2P(2*) shows any viability at any of the tested dilutions, becoming viable at ≤0.15% (**Table 5**). Both *K. quasipneumoniae* IF3SW-P1 and *K. pneumoniae* 13883^T showed no viability at any dilution between 0.2%-0.075%, only showing viability with the live control (**Tables 4, 6**). This result is consistent with the previous killing experiments, as *K. pneumoniae* F3-2P(2*) was the strain that did not lyse when exposed to ISS-strength QACs and formed the clump structures when exposed to QACs, this result is consistent. However, the response of the type strain *K. pneumoniae* 13883^T being closer to *K. quasipneumoniae* IF3SW-P1 than to the strain of the same species is interesting. This may be due to the hypermucoviscous phenotype of *K. pneumoniae* F3-2P(2*), which *K. pneumoniae* 13883^T does not share (89).

Compared to the poor result from the LIVE/DEAD viability assay, the PMAxx viability qPCR appears to be much better suited as a viability assay for QAC-treated samples, especially in biofilm-producing strains such as the hypermucoviscous *K. pneumoniae* F3-2P(2*). While the mechanism of action of PMAxx is similar to PI, needing to enter cells through a compromised membrane to bind DNA, the different parameters of the assay are able to counteract some the issues seen in LIVE/DEAD staining. After treatment with PMAxx, samples are incubated for 15 minutes with vigorous shaking, which would break up the clumps of cells and allows the dye greater access for staining. While it would be possible to incubate cells stained with LIVE/DEAD to allow for better dye staining, this shaking would break up the clumps of cells that are intended to be visualized under the microscope to learn about whether viability is associated with a particular position in the clumps. Additionally, PMAxx is one dye and is compared to an untreated control, which eliminates any concern about potential dye interaction.

While it is important to control bacterial colonization on spacecraft, not only to protect the health of astronauts (2-6, 41-43) but also as shown by the history of corrosion and degradation of equipment on the Soviet space stations Salyut and Mir (20-27), NASA is committed to the concept of planetary protection (17). This concept goes both ways, protecting not only alien planets from forward contamination but also the Earth from back contamination. QACs are widely used for disinfecting surfaces on Earth, and it is known that bacteria (including *Klebsiella*) can and are developing resistance to these compounds due to long-term exposure, particularly via efflux pump systems (99). As bacteria exposed to the stress of spaceflight are known to develop increased virulence and antibiotic resistance (21, 47-50), frequent cleaning of ISS surface with these compounds may lead to strains developing QAC resistance. When astronauts return to Earth after months on the ISS, or after planned multiple-year trips to Mars, they could bring back these QAC-resistant strains with them in their microbiota (5, 14-16).

Further research should be done to determine the current prevalence of QAC resistance within the ISS surface microbiome, both on strains of *Klebsiella* such as those studied in this thesis as well as on other opportunistic pathogens isolated from the ISS that are known to develop QAC resistance such as *Pseudomonas aeruginosa* (44, 99). As shown in this chapter, the ISS-origin *K. pneumoniae* F3-2P(2*) differed from its 99.01% ANI type strain, *K. pneumoniae* 13883^T, in both growth inhibition assays (**Fig 1B/C**) and in viability assays post-killing with QACs (**Table 5/6**). Comparison of the genomes of the two strains should be conducted to see if the genome of *K. pneumoniae* F3-2P(2*) contains efflux pumps associated with QAC resistance that the genome of the type strain does not have. However, the presence or absence of efflux pump genes alone is not sufficient for an AMR phenotype, as the expression profile of all putative virulence and AMR genes must also be considered. On that end, future work should combine both killing and viability assays as performed in this chapter as well as studying the genomes and transcriptomes of ISS-origin strains for putative QAC resistance genes.

3.5 Limitations of work

The above-described results of this work must be placed in context of the known limitations, both in methodology and the results above. When preparing the samples prior to LIVE/DEAD staining and visualization via fluorescence microscopy, the bacterial cultures were washed as described in the manufacturer's protocol. Each sample was centrifuged 10,000 x g for 10 minutes, which was repeated for 3 total wash cycles. However, it has been shown that the pressure and centrifugal force at 10,000 x g can cause significant damage to cell membranes, particularly in Gram-negative bacteria (105). As LIVE/DEAD viability staining is differentiated by the ability of PI to stain membrane-permeable cells, this physical damage introduced by such a high g force during washing may have resulted in more cells stained as dead. Future wash steps should include a centrifugation at a lower g force such as 6,500 x g.

Additionally, there was an incongruous result in the killing curve experiments as shown in Figure 2. Briefly, 100 µL of each culture was grown for 8 hours before being treated with an equal volume of either the QAC mixture or MilliQ water for the negative control. At the next post-treatment timepoint, the OD_{600} of all treatment arms does not decrease despite the dilution (Figure 2). This result was consistent across triplicates and repeated killing curve experiments. The OD_{600} of the QAC-treated *K. pneumoniae* F3-2P(2*) actually increases immediately after treatment (Fig 2B), which was also observed in every treatment throughout this work. Although this result was consistent in my work, it would be expected for the OD_{600} to drop after dilution with an equal volume, particularly for the MilliQ treatment. As the first measurement post-treatment is 30 minutes after, a timepoint experiment could be done where the cultures are treated and then the OD_{600} measured every 5 minutes for an hour to determine if the turbidity does actually drop after treatment.

CHAPTER 4

Conclusion

In my thesis research, I was provided a rare opportunity to study strains of an opportunistic pathogen isolated from one of the most unusual environments possible, the International Space Station (ISS). While it has long been known that exposure to the stresses of spaceflight selects for virulence and antimicrobial resistance in bacteria, the response of ISS-origin strains to the disinfectants used to clean the ISS, quaternary ammonium compounds (QAC), is understudied. Overall, research on bacterial strains isolated from the ISS is still an emerging field due to the relatively recent shift to a combination of metagenomic and culture-based techniques of monitoring the ISS microbiome.

The biggest remaining question from this work ties to the central question in Chapter 3—what is happening with the response of these *Klebsiella* strains after exposure to the QAC disinfectants used on the ISS? The two ISS-origin strains, *K. pneumoniae* F3-2P(2*) and *K. quasipneumoniae* IF3SW-P1, appear to have opposite responses to QAC exposure, ranging from the formation of clumps of cells for the former and the culture completely lysing for the latter. As *K. pneumoniae* F3-2P(2*) is known to have a hypermucoviscous phenotype, it is possible that the thicker capsule and biofilm produced by this strain is why *K. pneumoniae* F3-2P(2*) forms clumps after QAC exposure, while non-hypermucoviscous *K. quasipneumoniae* IF3SW-P1 does not. However, when the clumps of cells of QAC-treated *K. pneumoniae* F3-2P(2*) were streak plated, they remained unculturable under standard cultivation conditions.

Upon viewing the clumps under fluorescence microscopy with LIVE/DEAD viability dyes, there was dye interaction that made it difficult to distinguish whether there were viable cells inside these clumps. Due to this crosstalk between the dyes, and potential inability for the dyes to bind cells in a clump containing bacterial capsule and other biofilm components, LIVE/DEAD fluorescence microscopy was determined to be an unsuitable assay under these conditions. A different viability assay, viability qPCR with PMAxx, produced results that consistently showed strain *K. pneumoniae* F3-2P(2*) remaining viable post-QAC exposure compared to *K. quasipneumoniae* IF3SW-P1 and the Earth-origin type strain *K. pneumoniae* 13883^T. However, it must be considered that the mechanism of action of QAC disinfectants is bactericidal, lysing the cells, rather than bacteriostatic. *K. pneumoniae* F3-2P(2*) repeatedly did not lyse, even when exposed to ISS-strength QAC mixture. Again, as *K. pneumoniae* F3-2P(2*) has a hypermucoviscous phenotype, the clumps formed by this strain upon exposure to QACs may be the reason this strain is less susceptible to QAC treatment than *K. pneumoniae* IF3SW-P1 and the Earth-origin type strain *K. pneumoniae* 13883^T.

Beyond knowing *K. pneumoniae* F3-2P(2*) has a hypermucoviscous phenotype, the exact nature of the clumps of cells remains unknown. To rule out a physiological response, a heat-killed culture should be treated with the QAC mixture to determine whether the same clumping response occurs. If it is physiological, then to investigate this question differences in gene expression of known biofilm genes could be studied by doing a transcriptome of the two strains immediately after treatment with a sublethal dose of QACs. To specifically identify the components of *K. pneumoniae* F3-2P(2*)'s clumps, a proteomics study could be conducted after exposure to QACs. The combination of these two experiments would not only identify the extracellular components of these clumps of cells, but also identify which genes are being overexpressed when the strains undergo the stress of QAC exposure.

Beyond studying the responses of these strains to QAC exposure, this thesis work produced a complete genome of *K. quasipneumoniae* subsp. *similipneumoniae* strain IF3SW-P1, sequenced using high-accuracy long-read sequencing and assembled into a closed circular chromosome. Most genomes of ISS-origin bacterial strains have been published as drafts, so producing a complete genome for this strain provides a high-quality genome resource for future genomic analyses of ISS-origin strains.

APPENDIX

Copyright Approval for Previously Published Material

Chapter 2 was previously published in Microbiology Resource Announcements under a Creative Commons CC-BY 4.0 license, which allows for unrestricted reuse of the material with proper attribution. Full text of license is available at https://creativecommons.org/licenses/by/4.0/.

REFERENCES

1. National Aeronautics and Space Administration. 2023. Visitors to the station by country. https://www.nasa.gov/feature/visitors-to-the-station-by-country/.
2. Crucian B, Sams C. 2009. Immune system dysregulation during spaceflight: clinical risk for exploration-class missions. J Leukoc Biol 86(5):1017-8.
3. Crucian B, Babiak-Vazquez A, Johnston S, Pierson DL, Ott CM, Sams C. 2016. Incidence of clinical symptoms during long-duration orbital spaceflight. Int J Gen Med 9:383–391.
4. Mehta SK, Laudenslager ML, Stowe RP, Crucian BE, Sams CF, Pierson DL. 2014. Multiple latent viruses reactivate in astronauts during Space Shuttle missions. Brain Behav Immun 41: 210-217.
5. Voorhies AA, Mark Ott C, Mehta S, Pierson DL, Crucian BE, Feiveson A, Oubre CM, Torralba M, Moncera K, Zhang Y, Zurek E, Lorenzi HA. 2019. Study of the impact of long-duration space missions at the International Space Station on the astronaut microbiome. Sci Rep 9:9911.
6. Mermel LA. 2013. Infection prevention and control during prolonged human space travel. Clinical Infectious Diseases 56:123–130.
7. Lauber BA, Bolshakova O, Ullrich O. 2016. Spacecraft Microbiology, p 77-86. *In* Choukèr A(ed), Ullrich O(ed), The Immune System in Space: Are We Prepared? 1st ed, Springer, Cham, Switzerland.

8. Mahnert A, Vaishampayan P, Probst AJ, Auerbach A, Moissl-Eichinger C, Venkateswaran K, Berg G. 2015. Cleanroom maintenance significantly reduces abundance but not diversity of indoor microbiomes. PLoS One 10(8):e0134848.

9. Roberts MS, Garland JL, Mills AL. 2004. Microbial astronauts: assembling microbial communities for advanced life support systems. Microb Ecol 47:137–149.

10. Avila-Herrera A, Thissen J, Urbaniak C, Be NA, Smith DJ, Karouia F, Mehta S, Venkateswaran K, Jaing C. 2020. Crewmember microbiome may influence microbial composition of ISS habitable surfaces. PLoS One 15(4), p.e0231838.

11. Mora M, Mahnert A, Koskinen K, Pausan MR, Oberauner-Wappis L, Krause R, Perras AK, Gorkiewicz G, Berg G, Moissl-Eichinger C. 2016. Microorganisms in confined habitats: microbial monitoring and control of intensive care units, operating rooms, cleanrooms and the International Space Station. Front Microbiol 7:1573.

12. Kembel SW, Jones E, Kline J, Northcutt D, Stenson J, Womack AM, Bohannan BJ, Brown GZ, Green JL. 2012. Architectural design influences the diversity and structure of the built environment microbiome. ISME J 6:1469–1479.

13. Savage AM, Hills J, Driscoll K, Fergus DJ, Grunden AM, Dunn RR. 2016. Microbial diversity of extreme habitats in human homes. PeerJ 4:e2376; DOI 10.7717/peerj.2376

14. Lax S, Smith DP, Hampton-Marcell J, Owens SM, Handley KM, Scott NM, Gibbons SM, Larsen P, Shogan BD, Weiss S, Metcalf JL, Ursell LK, Vázquez-Baeza Y, Van Treuren W, Hasan NA, Gibson MK, Colwell R, Dantas G, Knight R, Gilbert JA. 2014. Longitudinal analysis of microbial interaction between humans and the indoor environment. Science 345:1048-1052.

15. Hu H, Johani K, Gosbell IB, Jacombs ASW, Almatroudi A, Whiteley GS, Deva AK, Jensen S, Vickery K. 2015. Intensive care unit environmental surfaces are contaminated by multidrug-resistant bacteria in biofilms: combined results of conventional culture, pyrosequencing, scanning electron microscopy, and confocal laser microscopy. Journal of Hospital Infection 91:35–44.

16. Huang SS, Datta R, Platt R. 2006. Risk of acquiring antibiotic-resistant bacteria from prior room occupants. Arch Intern Med 171:491-494.

17. Nicholson WL, Schuerger AC, Race MS. 2009. Migrating microbes and planetary protection. Trends Microbiol 17:389–392.

18. Johnston RS, Dietlein LF, Berry CA, Parker JF, West V, Jones WL. 1975. Biomedical results of Apollo. National Aeronautics and Space Administration. https://ntrs.nasa.gov/citations/19760005580.

19. Johnston RS, Dietlein LF. 1977. Biomedical results from Skylab. National Aeronautics and Space Administration. https://ntrs.nasa.gov/citations/19770026836.

20. Ilyin VK. 2005. Microbiological status of cosmonauts during orbital spaceflights on Salyut and Mir orbital stations. Acta Astronaut 56:839–850.

21. Klaus DM, Howard HN. 2006. Antibiotic efficacy and microbial virulence during space flight. Trends Biotechnol 24(3):131-136.

22. Zea L, Nisar Z, Rubin P, Cortesão M, Luo J, McBride SA, Moeller R, Klaus D, Müller D, Varanasi KK, Muecklich F, Stodieck L. 2018. Design of a spaceflight biofilm experiment. Acta Astronaut 148:294–300.

23. Novikova ND, Polikarpov NA, Poddubko S V., Deshevaya EA. 2001. The results of microbiological research of environmental microflora of orbital station Mir. SAE Technical Paper 2001-01-2310.

24. Novikova ND. 2004. Review of the knowledge of microbial contamination of the Russian manned spacecraft. Microb Ecol 47:127–132.

25. Klintworth R, Reher HJ, Viktorov AN, Bohle D. 1999. Biological induced corrosion of materials II: new test methods and experiences from Mir station. Acta Astronaut 44:569–578.

26. Alekhova TA, Aleksandrova AA, Novozhilova TY, Lysak L V., Zagustina NA, Bezborodov AM. 2005. Monitoring of microbial degraders in manned space stations. Appl Biochem Microbiol 41:435-443.

27. Ott CM, Bruce RJ, Pierson DL. 2004. Microbial characterization of free floating condensate aboard the Mir Space Station. Microb Ecol 47:133–136.

28. Kawamura Y, Li Y, Liu H, Huang X, Li Z, Ezaki T. 2001. Bacterial population in Russian space station “Mir.” Microbiol Immunol 45:819-928.

29. Castro VA, Thrasher AN, Healy M, Ott CM, Pierson DL. 2004. Microbial characterization during the early habitation of the international space station. Microb Ecol 47:119-126.

30. Novikova N, De Boever P, Poddubko S, Deshevaya E, Polikarpov N, Rakova N, Coninx I, Mergeay M. 2006. Survey of environmental biocontamination on board the International Space Station. Res Microbiol 157:5-12.

31. La Duc MT, Sumner R, Pierson D, Venkat P, Venkateswaran K. 2004. Evidence of pathogenic microbes in the International Space Station drinking water: reason for concern? Habitation 10:39-48.

32. Checinska A, Probst AJ, Vaishampayan P, White JR, Kumar D, Stepanov VG, Fox GE, Nilsson HR, Pierson DL, Perry J, Venkateswaran K. 2015. Microbiomes of the dust particles collected from the International Space Station and Spacecraft Assembly Facilities. Microbiome 3(50).

33. Mora M, Perras A, Alekhova TA, Wink L, Krause R, Aleksandrova A, Novozhilova T, Moissl-Eichinger C. 2016. Resilient microorganisms in dust samples of the international space station—survival of the adaptation specialists. Microbiome 4(65).

34. Venkateswaran K, Vaishampayan P, Cisneros J, Pierson DL, Rogers SO, Perry J. 2014. International Space Station environmental microbiome - Microbial inventories of ISS filter debris. Appl Microbiol Biotechnol 98:6453-6466.

35. Be NA, Avila-Herrera A, Allen JE, Singh N, Checinska Sielaff A, Jaing C, Venkateswaran K. 2017. Whole metagenome profiles of particulates collected from the International Space Station. Microbiome 5(81).

36. Ichijo T, Yamaguchi N, Tanigaki F, Shirakawa M, Nasu M. 2016. Four-year bacterial monitoring in the international space station—Japanese experiment module “Kibo” with culture-independent approach. NPJ Microgravity 2:1-6.

37. Yamaguchi N, Ichijo T, Nasu M. 2016. Bacterial monitoring in the International Space Station “Kibo” based on rRNA gene sequence. Trans Jpn Soc Aeronaut Space Sci 14:1–4.

38. Lang JM, Coil DA, Neches RY, Brown WE, Cavalier D, Severance M, Hampton-Marcell JT, Gilbert JA, Eisen JA. 2017. A microbial survey of the International Space Station (ISS). PeerJ 12:e4029.

39. Mora M, Wink L, Kögler I, Mahnert A, Rettberg P, Schwendner P, Demets R, Cockell C, Alekhova T, Klingl A, Krause R, Zolotariof A, Alexandrova A, Moissl-Eichinger C. 2019. Space Station conditions are selective but do not alter microbial characteristics relevant to human health. Nat Commun 10:3990.

40. Coil DA, Neches RY, Lang JM, Brown WE, Severance M, Cavalier D, Eisen JA. 2016. Growth of 48 built environment bacterial isolates on board the International Space Station (ISS). PeerJ 4:e1842.

41. La Duc MT, Kern R, Venkateswaran K. 2004. Microbial monitoring of spacecraft and associated environments. Microb Ecol 47:150–158.

42. Van Houdt R, Mijnendonckx K, Leys N. 2012. Microbial contamination monitoring and control during human space missions. Planet Space Sci 60:115–120.

43. Yamaguchi N, Roberts M, Castro S, Oubre C, Makimura K, Leys N, Grohmann E, Sugita T, Ichijo T, Nasu M. 2014. Microbial monitoring of crewed habitats in space—current status and future perspectives. Microbes Environ 29:250-260.

44. Checinska Sielaff A, Urbaniak C, Mohan GBM, Stepanov VG, Tran Q, Wood JM, Minich J, McDonald D, Mayer T, Knight R, Karouia F, Fox GE, Venkateswaran K. 2019. Characterization of the total and viable bacterial and fungal communities associated with the International Space Station surfaces. Microbiome 7(50).

45. Urbaniak C, Morrison MD, Thissen JB, Karouia F, Smith DJ, Mehta S, Jaing C, Venkateswaran K. 2022. Microbial Tracking-2, a metagenomics analysis of bacteria and fungi onboard the International Space Station. Microbiome 10(100).

46. National Aeronautics and Space Administration. 2022. Quantifying selection for pathogenicity and antibiotic resistance in bacteria and fungi on the ISS – a Microbial Tracking study. https://www.nasa.gov/mission_pages/station/research/experiments/explorer/Investigation.html?#id=8333.

47. Taylor PW. 2015. Impact of space flight on bacterial virulence and antibiotic susceptibility. Infect Drug Resist 8:249-262.

48. Wilson JW, Ott CM, Höner Zu Bentrup K, Ramamurthy R, Quick L, Porwollik S, Cheng P, McClelland M, Tsaprailis G, Radabaugh T, Hunt A, Fernandez D, Richter E, Shah M, Kilcoyne M, Joshi L, Nelman-Gonzalez M, Hing S, Parra M, Dumars P, Norwood K, Bober R, Devich J, Ruggles A, Goulart C, Rupert M, Stodieck L, Stafford P, Catella L, Schurr MJ, Buchananb K, Morici L, McCracken J, Allen P, Baker-Coleman C, Hammond T, Vogel J, Nelson R, Pierson DL, Stefanyshyn-Piper HM, Nickerson CA. 2007. Space flight alters bacterial gene expression and virulence and reveals a role for global regulator Hfq. Proc Natl Acad Sci USA 104:16229-16304.

49. Beceiro A, Tomás M, Bou G. 2013. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clin Microbiol Rev 26:185–230.

50. Venkateswaran K, La Duc MT, Horneck G. 2014. Microbial existence in controlled habitats and their resistance to space conditions. Microbes Environ 29:243–249.

51. Kim W, Tengra FK, Young Z, Shong J, Marchand N, Chan HK, Pangule RC, Parra M, Dordick JS, Plawsky JL, Collins CH. 2013. Spaceflight promotes biofilm formation by *Pseudomonas aeruginosa*. PLoS One 8(4):e62437.

52. Burmølle M, Webb JS, Rao D, Hansen LH, Sørensen SJ, Kjelleberg S. 2006. Enhanced biofilm formation and increased resistance to antimicrobial agents and bacterial invasion are caused by synergistic interactions in multispecies biofilms. Appl Environ Microbiol 72:3916–3923.

53. Fux CA, Costerton JW, Stewart PS, Stoodley P. 2005. Survival strategies of infectious biofilms. Trends Microbiol 13:34–40.

54. Urbaniak C, Sielaff AC, Frey KG, Allen JE, Singh N, Jaing C, Wheeler K, Venkateswaran K. 2018. Detection of antimicrobial resistance genes associated with the International Space Station environmental surfaces. Sci Rep 8:814.

55. Singh NK, Wood JM, Karouia F, Venkateswaran K. 2018. Succession and persistence of microbial communities and antimicrobial resistance genes associated with International Space Station environmental surfaces. Microbiome 6(204).

56. Solomon SA, Bharadwaj AR, Singh NK, Wood JM, Debieu M, O'Hara NB, Mason CE, Venkateswaran K. 2020. Draft genome sequences of *Klebsiella* species isolated from the International Space Station. Microbiol Resour Announc 9:e00923-20.

57. Sushenko NS, Singh NK, Vellone DL, Tighe SW, Hedlund BP, Venkateswaran K, Moser DP. 2022. Complete genome sequence of *Klebsiella quasipneumoniae* subsp. *similipneumoniae* strain IF3SW-P1, isolated from the International Space Station. Microbiol Resour Announc 11:e0047622.

58. Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA, Dance D, Jenney A, Connor TR, Hsu LY, Severin J, Brisse S, Cao H, Wilksch J, Gorrie C, Schultz MB, Edwards DJ, Van Nguyen K, Nguyen TV, Dao TT, Mensink M, Le Minh V, Nhu NTK, Schultsz C, Kuntaman K, Newton PN, Moore CE, Strugnell RA, Thomson NR. 2015. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. Proc Natl Acad Sci USA 112:E3574–E3581.

59. Brisse S, Passet V, Grimont PAD. 2014. Description of Klebsiella quasipneumoniae sp. nov., Isolated from human infections, With two subspecies, Klebsiella quasipneumoniae subsp. quasipneumoniae subsp. nov. and Klebsiella quasipneumoniae subsp. similipneumoniae subsp. nov., And demonstration th. Int J Syst Evol Microbiol 64.

60. Arabaghian H, Salloum T, Alousi S, Panossian B, Araj GF, Tokajian S. 2019. Molecular characterization of carbapenem resistant *Klebsiella pneumoniae* and *Klebsiella quasipneumoniae* isolated from Lebanon. Sci Rep 9:531.

61. Arena F, Henrici De Angelis L, Pieralli F, Di Pilato V, Giani T, Torricelli F, D’Andrea MM, Rossolini GM. 2015. Draft genome sequence of the first hypermucoviscous *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* isolate from a bloodstream infection. Genome Announc 3: e00952-15. doi: 10.1128/genomeA.00952-15.

62. Breurec S, Melot B, Hoen B, Passet V, Schepers K, Bastian S, Brisse S. 2016. Liver abscess caused by infection with community-acquired *Klebsiella quasipneumoniae* subsp. *quasipneumoniae*. Emerg Infect Dis 22:529–531.

63. Mbelle NM, Feldman C, Sekyere JO, Maningi NE, Modipane L, Essack SY. 2020. Pathogenomics and evolutionary epidemiology of multi-drug resistant clinical *Klebsiella pneumoniae* isolated from Pretoria, South Africa. Sci Rep 10:1232.

64. Long SW, Linson SE, Ojeda Saavedra M, Cantu C, Davis JJ, Brettin T, Olsen RJ. 2017. Whole-genome sequencing of human clinical *Klebsiella pneumoniae* isolates reveals misidentification and misunderstandings of *Klebsiella pneumoniae, Klebsiella variicola,* and *Klebsiella quasipneumoniae*. mSphere 2:e00290-17.

65. Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ V. 2015. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 13:42–51.

66. Navon-Venezia S, Kondratyeva K, Carattoli A. 2017. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev 41:252–275.

67. Papp-Wallace KM, Bethel CR, Distler AM, Kasuboski C, Taracila M, Bonomo RA. 2010. Inhibitor resistance in the KPC-2 β-lactamase, a preeminent property of this class A β-lactamase. Antimicrob Agents Chemother 54:890–897.

68. Papp-Wallace KM, Taracila MA, Smith KM, Xu Y, Bonomo RA. 2012. Understanding the molecular determinants of substrate and inhibitor specificities in the carbapenemase KPC-2: exploring the roles of Arg220 and Glu276. Antimicrob Agents Chemother 56:4428–4438.

69. Barnes MD, Winkler ML, Taracila MA, Page MG, Desarbre E, Kreiswirth BN, Shields RK, Nguyen MH, Clancy C, Spellberg B, Papp-Wallace KM, Bonomo RA. 2017. *Klebsiella pneumoniae* carbapenemase-2 (KPC-2), substitutions at ambler position Asp179, and resistance to ceftazidime-avibactam: Unique antibiotic-resistant phenotypes emerge from β-lactamase protein engineering. mBio 8:e00528-17.

70. Balcázar JL, Subirats J, Borrego CM. 2015. The role of biofilms as environmental reservoirs of antibiotic resistance. Front Microbiol 6:126.

71. Sánchez-López J, García-Caballero A, Navarro-San Francisco C, Quereda C, Ruiz-Garbajosa P, Navas E, Dronda F, Morosini MI, Cantón R, Diez-Aguilar M. 2019. Hypermucoviscous *Klebsiella pneumoniae*: A challenge in community acquired infection. IDCases 17:e00547.

72. Buffet-Bataillon S, Tattevin P, Bonnaure-Mallet M, Jolivet-Gougeon A. 2012. Emergence of resistance to antibacterial agents: the role of quaternary ammonium compounds—a critical review. Int J Antimicrob Agents 39:381–389.

73. Department of Energy Joint Genome Institute. 2012. Bacterial genomic DNA isolation using CTAB. https://jgi.doe.gov/wp-content/uploads/2014/02/JGI-Bacterial-DNA-isolation-CTAB-Protocol-2012.pdf.

74. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long, error-prone reads using repeat graphs. Nat Biotechnol 37:540–546.

75. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. BMC Bioinformatics 10:421.

76. Nicolás MF, Ramos PIP, de Carvalho FM, Camargo DRA, Alves C de FM, de Morais GL, Almeida LGP, Souza RC, Ciapina LP, Vicente ACP, Coimbra RS, de Vasconcelos ATR. 2018. Comparative genomic analysis of a clinical isolate of *Klebsiella quasipneumoniae* subsp. *similipneumoniae*, a KPC-2 and OKP-B-6 beta-lactamases producer harboring two drug-resistance plasmids from southeast Brazil. Front Microbiol 9:220.

77. Yoon S-H, Ha S, Lim J, Kwon S, Chun J. 2017. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110:1281–1286.

78. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365.

79. Davis JJ, Wattam AR, Aziz RK, Brettin T, Butler R, Butler RM, Chlenski P, Conrad N, Dickerman A, Dietrich EM, Gabbard JL, Gerdes S, Guard A, Kenyon RW, Machi D, Mao C, Murphy-Olson D, Nguyen M, Nordberg EK, Olsen GJ, Olson RD, Overbeek JC, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomas C, VanOeffelen M, Vonstein V, Warren AS, Xia F, Xie D, Yoo H, Stevens R. 2019. The PATRIC Bioinformatics Resource Center: expanding data and analysis capabilities. Nucleic Acids Res 48:D606-D612.

80. Russo TA, Olson R, MacDonald U, Metzger D, Maltese LM, Drake EJ, Gulick AM. 2014. Aerobactin mediates virulence and accounts for increased siderophore production under iron-limiting conditions by hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*. Infect Immun 82:2356–2367.

81. Klontz EH, Tomich AD, Günther S, Lemkul JA, Deredge D, Silverstein Z, Shaw JF, McElheny C, Doi Y, Wintrode PL, MacKerell AD, Sluis-Cremer N, Sundberg EJ. 2017. Structure and dynamics of FosA-mediated fosfomycin resistance in *Klebsiella pneumoniae* and *Escherichia coli*. Antimicrob Agents Chemother 61:10-1128.

82. Rodriguez-Martinez JM, Diaz de Alba P, Briales A, Machuca J, Lossa M, Fernandez-Cuenca F, Rodriguez Bano J, Martinez-Martinez L, Pascual A. 2013. Contribution of OqxAB efflux pumps to quinolone resistance in extended-spectrum-β-lactamase-producing *Klebsiella pneumoniae*. Journal of Antimicrobial Chemotherapy 68:68–73.

83. Gerba CP. 2015. Quaternary ammonium biocides: Efficacy in application. Appl Environ Microbiol 81:464–469.

84. Hora PI, Pati SG, McNamara PJ, Arnold WA. 2020. Increased use of quaternary ammonium compounds during the SARS-CoV-2 pandemic and beyond: Consideration of environmental implications. Environ Sci Technol Lett 7:622–631.

85. Li X, Brownawell BJ. 2010. Quaternary ammonium compounds in urban estuarine sediment environments--a class of contaminants in need of increased attention? Environ Sci Technol 44:7561–7568.

86. Jennings MC, Minbiole KPC, Wuest WM. 2016. Quaternary ammonium compounds: An antimicrobial mainstay and platform for innovation to address bacterial resistance. ACS Infect Dis 1:288–303.

87. Bragg R, Jansen A, Coetzee M, van der Westhuizen W, Boucher C. 2014. Bacterial resistance to quaternary ammonium compounds (QAC) disinfectants. Adv Exp Med Biol 808:1–13.

88. Buffet-Bataillon S, Tattevin P, Bonnaure-Mallet M, Jolivet-Gougeon A. 2012. Emergence of resistance to antibacterial agents: the role of quaternary ammonium compounds—a critical review. Int J Antimicrob Agents 39:381–389.

89. Daligault HE, Davenport KW, Minogue TD, Bishop-Lilly KA, Bruce DC, Chain PS, Coyne SR, Frey KG, Jaissle J, Koroleva GI, Ladner JT, Lo CC, Meincke L, Munk AC,

Palacios GF, Redden CL, Johnson SL. 2014. Draft genome assembly of *Klebsiella pneumoniae* type strain ATCC 13883. Genome Announc 2:10-1128.

90. Wiegand I, Hilpert K, Hancock REW. 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc 3(2):163–175.

91. Belanger CR, Hancock REW. 2021. Testing physiologically relevant conditions in minimal inhibitory concentration assays. Nat Protoc 16(8):3761–3774.

92. Muyzer G, De Waal EC, Uitterlinden AG. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700.

93. Li H, Zhang Y, Li DS, Xu H, Chen GX, Zhang CG. 2009. Comparisons of different hypervariable regions of *rrs* genes for fingerprinting of microbial communities in paddy soils. Soil Biol Biochem 41:954–968.

94. Hagiya H, Watanabe N, Maki M, Murase T, Otsuka F. 2014. Clinical utility of string test as a screening method for hypermucoviscosity-phenotype *Klebsiella pneumoniae*. Acute Med Surg. 1:245-246.

95. Alvear-Daza JJ, Garcia-Barco A, Osorio-Vargas P, Guiterrez-Zapata HM, Sanabria J, Rengifo-Herrera JA. 2021. Resistance and induction of viable but non culturable states (VBNC) during inactivation of *E. coli* and *Klebsiella pneumoniae* by addition of H_2O_2 to natural well water under simulated solar irradiation. Water Res 188:116499.

96. Ramamurthy T, Ghosh A, Pazhani GP, Shinoda S. 2014. Current perspectives on viable but non-culturable (VNBC) bacteria. Front Public Health 2:103.

97. Ou F, McGoverin C, Swift S, Vanholsbeek F. 2016. Rapid evaluation of bacterial viability using the optrode – a near real time portable fluorimeter. Photonics and Fiber Tech Technical Paper AW3C-6.

98. Robertson J, McGoverin C, Vanholsbeek F, Swift S. 2019. Optimisation of the protocol for the LIVE/DEAD BacLight Bacterial Viability Kit for rapid determination of bacterial load. Front Microbiol 10:801.

99. Kim M, Weigand MR, Oh S, Hatt JK, Krishnan R, Tezel U, Pavlostathis SG, Konstantinidis KT. 2018. Widely used benzalkonium chloride disinfectants can promote antibiotic resistance. Appl Environ Microbiol. 84:17.

100. Rosenberg M, Azevedo NF, Ivask A. 2019. Propidium iodide staining underestimates the viability of adherent bacterial cells. Sci Rep 9:6483.

101. Gregorchuk BSJ, Reimer SL, Beniac DR, Hiebert SL, Booth TF, Wuzinski M, Funk BE, Milner KA, Cartwright NH, Doucet AN, Mulvey MR, Khajehpour M, Zhanel GG, Bay DC. 2020. Antiseptic quaternary ammonium compound tolerance by Gram-negative bacteria can be rapidly detected using an impermeant fluorescent dye-based assay. Sci Rep 10:20543.

102. Smith K, Hunter IS. 2008. Efficacy of common hospital biocides with biofilms of multi-drug resistant clinical isolates. J Med Microbiol. 57:966-973.

103. Nocker A, Cheung CY, Camper AK. 2006. Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells. J Microbiol Methods. 67(2):310-20.

104. Nocker A, Camper AK. 2006. Selective removal of DNA from dead cells of mixed bacterial communities by use of ethidium monoazide. Appl Environ Microbiol. 72(3):1997-2004.

105. Peterson BW, Sharma PK, van der Mei HC, Busscher HJ. 2012. Bacterial cell surface damage due to centrifugal compaction. Appl Environ Microbiol. 78(1):120-5.

CURRICULUM VITAE

NATASHA SVETLANA SUSHENKO

natasha.sushenko@unlv.edu | natashasushenko@gmail.com

EDUCATION

M.S., Biological Sciences, Microbiology Concentration, School of Life Sciences, University of Nevada, Las Vegas

Advisors: Brian Hedlund (academic) and Duane Moser (research)

December 2023

B.S., Biological Sciences, Microbiology Concentration, School of Life Sciences, University of Nevada, Las Vegas

Honors College, Cohen Scholar

May 2019

RESEARCH EXPERIENCE

Graduate Research Assistant, Desert Research Institute, Environmental Microbiology Laboratory, Las Vegas, Nevada, 2020-2023

Project: Metagenome data mining to elucidate prevalence and virulence potentials of biosafety-level-2 microorganisms from ISS environments

PI: Duane Moser

Undergraduate Research Assistant, University of Nevada, Las Vegas, School of Life Sciences, Arid Lands Soil-Plant-Water Stress Interactions Lab, 2013-2014

Project: Effect of ultraviolet radiation levels on desiccation tolerance and recovery in a desert moss, *Syntrichia caninervis*

PI: Lloyd Stark

TEACHING EXPERIENCE

Graduate Teaching Assistant, University of Nevada, Las Vegas, School of Life Sciences, Spring 2023-present

Courses Taught: BIOL 351L, 251L (Microbiology Laboratory)

FELLOWSHIPS AND AWARDS

Nevada NASA Space Grant Consortium Graduate Fellowship, 2021-2022

Project: Transcriptional responses of an International Space Station pathogen, *Klebsiella pneumoniae*, to microgravity and disinfectant exposure

Award: $20,000

DRI Department of Hydrological Sciences Graduate Student Fellowship, 2022

Project: Quarternary ammonium compound resistance of *Klebiella pneumoniae* and *Klebsiella quasipneumoniae* strains isolated from the International Space Station

Award: $12,000

UNLV Graduate & Professional Student Association Sponsorship Travel Award, 2022

Award: $1,000 to attend and present at ASGSR 2022 in Houston, TX

ASGSR Student Travel Award, 2022

Award: $500 to attend and present at ASGSR 2022 in Houston, TX

PUBLICATIONS

1. **Zeidman A.B., Moon J., <u>Sushenko N.S.</u>, Son Y, Wuest V.R., Moser D.P., Bandala E.R.** (2022). Catalytic decomposition of sulfamethoxazole using peroxymonosulfate with zero-valent iron nanoparticles immobilized on SBA-15. In preparation for submission to *Chemical Engineering.*
2. **<u>Sushenko N.S.,</u> Singh N.K., Vellone D.L., Tighe S.W., Hedlund B.P., Venkateswaran K., Moser D.P.** (2022). Complete genome sequence of *Klebsiella quasipneumoniae* subsp. *similipneumoniae* strain IF3SW-P1 isolated from the International Space Station, *Microbiology Resource Announcements,* 11(7):e0047622.

CONFERENCE PRESENTATIONS

1. **<u>*Sushenko N.S.,</u> Saidi-Mehrabad A., Vellone D.L., Singh N.K., Tighe S.W., Hedlund B.P., Venkateswaran K., Moser D.P.**, (2022, November 9-12). Resistance of *Klebsiella* Strains from the International Space Station to Quaternary Ammonium Compound Disinfectants, ASGSR 2022 Annual Meeting: Houston, TX. Lightning talk.
2. **<u>*Sushenko N.S.,</u> Saidi-Mehrabad A., Vellone D.L., Singh N.K., Tighe S.W., Hedlund B.P., Venkateswaran K., Moser, D.P.**, (2022, November 9-12). Resistance of *Klebsiella* Strains from the International Space Station to Quaternary Ammonium Compound Disinfectants, ASGSR 2022 Annual Meeting: Houston, TX. Poster presentation.
3. **<u>*Sushenko N.S.,</u> Saidi-Mehrabad A., Hedlund B.P., Singh N.K., Venkateswaran K., Moser D.P.**, (2022, May 13). Resistance of *Klebsiella* Strains from the

International Space Station to Quaternary Ammonium Compound Disinfectants, 2022 Nevada NASA Programs Virtual Poster Competition: Online. Poster presentation.

4. **Hedlund B.P., Nou N., Covington J., Cook A., Torosian N., Palmer M., Sushenko N.S., Singh N.K., Moser D.P., Venkateswaran K.** (2022, April 29). Biomass Recycling Using Novel Thermophilic Enzymes and Importance for Sustainable Space Exploration, 2022 Nevada NASA Programs Statewide Meeting: Las Vegas, NV. Oral presentation.
5. **Moser D.P., Sushenko N.S., Devlin M.G., Saidi-Mehrabad A., Northup D., Hedlund B.P., Singh N.K., Blank J., Venkateswaran K.** (2022, April 29). Deep Biosphere to Deep Space: Microbiome Insights for Life on Rocky Planets and Human Health during Long-Duration Spaceflight, 2022 Nevada NASA Programs Statewide Meeting: Las Vegas, NV. Oral presentation.
6. ***Sushenko N.S., Saidi-Mehrabad A., Hedlund B.P., Singh N.K., Venkateswaran K., Moser D.P.**, (2022, April 29). Resistance of *Klebsiella* Strains from the International Space Station to Quaternary Ammonium Compound Disinfectants, 2022 Nevada NASA Programs Statewide Meeting: Las Vegas, NV. Poster presentation.
7. ***Sushenko N.S., Gans J., Arambula-Quintero C.A., Singh N.K., Venkateswaran K., Moser D.P.**, (2020, November 5-6). Disinfectant resistance of *Klebsiella pneumoniae* strains isolated from the International Space Station, ASGSR 2020 Virtual Meeting: Online. Lightning talk.

*(*Presenter)*

PROFESSIONAL ASSOCIATIONS

American Society of Microbiology (ASM), *Arizona/Southern Nevada Branch*

American Society for Gravitational and Space Research (ASGSR)