Aging and Health in Skilled Nursing Facilities: Exploring Population Characteristics and the Microbiome

by

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Dedication

This dissertation is dedicated to my Grandma Marlene, whose determination and stubbornness I likely inherited, I will follow in your footsteps and continue to fight for science and a better world. To the CC babies, Carson, Caroline, and Cori, you can accomplish anything you set your mind to.

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Table of Contents

Dedication ii

Acknowledgements iii

List of Tables ix

List of Figures xi

Abstract xv

Chapter 1 Introduction 1

1.1 Dissertation Goal 1

1.2 Aging and the need for care facilities 1

1.3 Length of stay and managing care 2

1.4 Frailty and oral health 2

1.5 Nose and throat microbiome 3

1.6 Dissertation Chapters 4

1.7 References 6

Chapter 2 Skilled Nursing Facility Population Characteristics and Length of Stay: A Prospective Cohort Study 8

2.1 Abstract 8

2.2 Introduction 9

2.3 Methods 10

2.3.1 Study Population 10

2.3.2 Data Collection & Storage 10

2.3.3 Participant Characteristics 11

2.3.4 Statistical Analysis 12

2.4 Results 12
2.4.1 Study Population Characteristics 12
2.4.2 Self-Reported Symptom Frequency 13
2.4.3 Visitors & Movement Behaviors 13
2.4.4 Length of Stay 14
2.5 Discussion 15
2.6 Conclusion 17
2.7 Acknowledgements 18
2.8 Tables and Figures 18
2.9 References 38
Chapter 3 Oral Health and Frailty Among Oder Adults: A Prospective Cohort Study of Skilled Nursing Facility Residents 42
3.1 Abstract 42
3.2 Introduction 43
3.3 Methods 44
3.3.1 Study population 44
3.3.2 Data collection 45
3.3.3 Statistical analysis 46
3.4 Results 47
3.4.1 Study population characteristics 47
3.4.2 Association between oral health and frailty 48
3.4.3 Effect modification of the relationship between oral health and frailty 48
3.5 Discussion 49
3.6 Conclusion 50
3.7 Acknowledgements 51
3.8 Tables and Figures 52

3.9 References 58

Chapter 4 Associations of Microbiome Structure and Composition by Health Characteristics Among Older Adults in a Skilled Nursing Facility 60

4.1 Abstract 60

4.2 Introduction 61

4.3 Methods 63

4.3.1 Study population 63

4.3.2 Nose/throat sample collection & storage 64

4.3.3 DNA extraction and sequencing 64

4.3.4 Bioinformatics 64

4.3.5 Staphylococcus aureus and Candida albicans testing 65

4.3.6 Demographic collection 66

4.3.7 Health characteristics 66

4.3.8 Short-term dynamics analysis 67

4.3.9 Microbiome differences associated with health characteristics 69

4.3.10 Functional pathway analysis 69

4.4 Results 70

4.4.1 Study population characteristics 70

4.4.2 Short-term stability of the nose/throat microbiome 70

4.4.3 Microbiome differences associated with health characteristics 72

4.4.4 Impact of health factors on microbial function 75

4.5 Discussion 77

4.6 Conclusion 80

4.7 Acknowledgements 81

4.8 Tables and Figures 81

4.9 References 111

4.10 Supplemental Methods 117
4.10.1 Sequencing 117
4.10.2 DADA2 pipeline 117
Chapter 5 Summary and Closing Thoughts 119
5.1 Public Health Significance 119
5.2 Future Directions 121
5.3 Personal Reflection 121

List of Tables

Table 2.1 Frequency [n (%)] of demographics, health behaviors, health history, external devices, and medication usage by length of stay at a single skilled nursing facility (SNF) in Michigan. 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. 20

Supplemental Table 2.1 Frequency of demographics, health behaviors, health history, external devices, and medication usage at enrollment by gender (women and men) and age category (<70, 70–79, and 80+ years). 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. .. 27

Supplemental Table 2.2 Frequency (n[%]) of visitors and facility movement across five study visits overall. 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. 31

Supplemental Table 2.3 Frequency [n (%)] of visitors and facility movement across five study visits by gender (women and men). 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023 32

Supplemental Table 2.4 Frequency [n (%)] of visitors and facility movement across five study visits by age category (<70, 70–79, and 80+ years). 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023 33

Table 3.1 Frequency (n[%]) of demographics, health behaviors, oral history, health history, and medication usage by self-assessed oral health and frailty status among 309 residents at a single skilled nursing facility (SNF) participating in the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. 52

Table 3.2 Results of multivariable logistic regression model showing odds ratios between self-assessed oral health and frailty status obtained through Frailty Short Interview among 309 residents at a single skilled nursing facility (SNF) participating in the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. 55

Table 3.3 Additional analyses using univariate and multivariable logistic regression model showing odds ratios between three independent oral health measures (non-collapsed self-assessed oral health, tooth/gum pain, and tooth/gum worry) and frailty status obtained through Frailty Short Interview among 309 residents at a single skilled nursing facility (SNF)

participating in the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. 56

Table 4.1 Frequency (n[%]) of demographics, health behaviors, and comorbidities by frailty status, device type, antibiotics (AB) use, and Candida albicans colonization among 301 residents at a single skilled nursing facility (SNF) participating in the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. 82

Supplemental Table 4.1 Mean and standard deviation (SD) of alpha diversity measures (Chao1 and Shannon) by frailty status, device type, antibiotic use and by study visit among 301 residents at a single skilled nursing facility (SNF) participating in the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. Kruskal-Wallis tests were performed to identify significant differences by Chao1 or Shannon across each health characteristic subgroup and study visit independently. Bold indicates those that were significantly different. 92

Supplemental Table 4.2 Reference taxa identified by independent ADAPT analysis for each study visit comparing those with no colonization (REF) to any colonization of Candida albicans or Staphylococcus aureus. Table summarizes which taxa were identified as reference for which study visits and details median coefficient of variation (CV) within and across participants for these taxa. Rows in bold are taxa that were identified as reference taxa across all independent analyses for each of the five study visits. 97

Supplemental Table 4.3 Differentially abundant taxa identified by ADAPT for (A) any device use [REF: no devices], (B) respiratory or non-surgical tubing [REF: no devices], and (C) surgical drains or vascular access devices [REF: no devices], (D) any antibiotics use [REF: no antibiotics], (E) only prior antibiotics [REF: no antibiotics], (F) any antibiotics during study [REF: no antibiotics] adjusting for age and *Candida albicans* colonization. The log10 fold change informs the direction of effect and the adjusted p-value informs significance; this adjusts for multiple hypothesis testing using the Benjamini-Hochberg correction with values <0.05 being significant. Species are ordered by significance level beginning with the most significant. 102

Supplemental Table 4.4 Differentially abundant taxa identified by ADAPT for (A) any antibiotics use [REF: no antibiotics], (B) only prior antibiotics [REF: no antibiotics], (C) any antibiotics during study [REF: no antibiotics] adjusting for age and *Candida albicans* colonization. The log10 fold change informs the direction of effect and the adjusted p-value informs significance; this adjusts for multiple hypothesis testing using the Benjamini-Hochberg correction with values <0.05 being significant. Species are ordered by significance level beginning with the most significant. 104

List of Figures

Figure 2.1 Consort diagram of 362 total participants in Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study (December 17, 2019 through May 22, 2023). Includes enrollment and exclusion criteria, detailing the two enrollment periods and the frequency of participants at each study visit. 18

Figure 2.2 Upset plot reporting the frequency of different self-reported respiratory symptom combinations at enrollment collected from 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. Upset plot prepared using the "ComplexUpset" package in R (version 1.3.3).[29,30] 19

Figure 2.3 Visitors and movement to in-facility rehabilitation center by age and gender (A) Percentage of 313 participants that had visitors at each study visit by gender (women and men) and age category (<70, 70–79, and 80+ years) (B) Percentage of participants that traveled to the in-facility rehabilitation center during each study visit by gender (women and men) and age category (<70, 70–79, and 80+ years). 23

Figure 2.4 Cumulative survival curves estimating mean length of stay at skilled nursing facility (SNF) from 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. Tables calculate absolute difference in mean length of stay at 75th, 50th, and 25th percentiles (shown with dots) by (A) risk group and (B) ADL status from the lognormal survival model. For the risk survival curve, low-risk included being 80 years and older, having heart disease, no antibiotics, independent or partially dependent for ADL, and having no devices while high-risk included being less than 70 years of age, not having heart disease, having any antibiotics during the study, being dependent for ADL, and having a vascular access and/or surgical drain device. For the ADL survival curve, all other variables are held constant in the model at the reference: less than 70 years of age, not having heart disease, not taking any antibiotics, and having no devices. Confidence intervals were calculated using non-parametric bootstrapping with 1,000 iterations..... 24

Supplemental Figure 2.1 Study protocol outlining study visit frequency, biological samples, and information collected from 362 participants in the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study from December 17, 2019 through May 22, 2023..... 25

Supplemental Figure 2.2 List of the different types of devices present at entry to the Skilled Nursing Facility (SNF) and how each device was categorized for modeling. 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study. 26

Supplemental Figure 2.3 Upset plot reporting the frequency of different device combinations at enrollment collected from 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. Upset plot prepared using the "ComplexUpset" package in R (version 1.3.3)[29,30]..... 34

Supplemental Figure 2.4 Kaplan Meier curves reporting length of stay at skilled nursing facility by covariates (A) age, (B), activities of daily living [ADL], (C) antibiotic usage, (D), device type, from 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023..... 35

Supplemental Figure 2.5 Cumulative survival curves estimating mean length of stay at skilled nursing facility (SNF) from 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. Tables calculate absolute difference in mean length of stay at 75th, 50th, and 25th percentiles (shown with dots) by covariates [(A) age category, (B) device type included in the lognormal survival model. For each covariate survival curve, all other variables are held constant in the model at the reference: less than 70 years of age, having no devices, not having heart disease, not taking any antibiotics, and being independent or partially dependent for ADL. Confidence intervals were calculated using non-parametric bootstrapping with 1,000 iterations..... 36

Supplemental Figure 2.6 Cumulative survival curves estimating mean length of stay at skilled nursing facility (SNF) from 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. Tables calculate absolute difference in mean length of stay at 75th, 50th, and 25th percentiles (shown with dots) by covariates [(A) heart disease, (B) antibiotics usage included in the lognormal survival model. For each covariate survival curve, all other variables are held constant in the model at the reference: less than 70 years of age, having no devices, not having heart disease, not taking any antibiotics, and being independent or partially dependent for ADL. Confidence intervals were calculated using non-parametric bootstrapping with 1,000 iterations..... 37

Figure 3.1 Plots visualizing how the predicted probability of frailty changes with self-assessed oral health across each level of a single covariate, (A) age category (ref <70), (B) chronic respiratory disease (ref no), (C) activities of daily living [ADL] (ref independent), (D) antidepressant usage (ref no), while keeping all other variables constant at their reference value. Confidence intervals were calculated using non-parametric bootstrapping with 1,000 iterations..... 57

Figure 4.1 Differentially abundant species identified using ADAPT, comparing participants who did not have a device (reference group) to participants with at least one device (collapsed categories), adjusting for age and Candida albicans colonization. Significance was determined based on an adjusted p-value < 0.05 correcting for multiple hypothesis testing using Benjamini-Hochberg correction; however, species are displayed by their -log10(p-value) for easier interpretability, with non-significant species shown in gray. Labeled points reflect species with p-values < 0.001. Log10 fold change (x-axis) indicates the direction of effect, with positive values representing species enriched in the device group (maroon) and negative values representing species depleted in the device group (pink)..... 86

Figure 4.2 Differentially abundant species identified using ADAPT, comparing participants who did not have a device (reference group) to participants with each device type (A) respiratory device or non-surgical tubing (B) surgical drain or vascular access device, adjusting for age and *Candida albicans* colonization. Significance was determined based on an adjusted p-value < 0.05 correcting for multiple hypothesis testing using Benjamini-Hochberg correction; however, species are displayed by their -log10(p-value) for easier interpretability, with non-significant species shown in gray. Labeled points reflect species with p-values < 0.001 or the top three most significant species if none were <0.001. Log10 fold change (x-axis) indicates the direction of effect, with positive values representing species enriched in the device group (maroon) and negative values representing species depleted in the device group (pink). 87

Figure 4.3 Differentially abundant species identified using ADAPT, comparing participants who did not use antibiotics (reference group) to participants who used antibiotics at any time point (collapsed categories), adjusting for age and Candida albicans colonization. Significance was determined based on an adjusted p-value < 0.05 correcting for multiple hypothesis testing using Benjamini-Hochberg correction; however, species are displayed by their -log10(p-value) for easier interpretability, with non-significant species shown in gray. Labeled points reflect species with p-values < 0.001. Log10 fold change (x-axis) indicates the direction of effect, with positive values representing species enriched in the device group (maroon) and negative values representing species depleted in the device group (pink). 88

Figure 4.4 Differentially abundant species identified using ADAPT, comparing participants who did not use antibiotics (reference group) to participants with each antibiotic usage type (A) antibiotics prior to the study only (B) any antibiotics during the study, adjusting for age and *Candida albicans* colonization. Significance was determined based on an adjusted p-value < 0.05 correcting for multiple hypothesis testing using Benjamini-Hochberg correction; however, species are displayed by their -log10(p-value) for easier interpretability, with non-significant species shown in gray. Labeled points reflect species with p-values < 0.001. Log10 fold change (x-axis) indicates the direction of effect, with positive values representing species enriched in the device group (maroon) and negative values representing species depleted in the device group (pink). 89

Supplemental Figure 4.1 Chao1 alpha diversity by study visit and (A) frailty status, (B) device type, and (C) antibiotic usage. Higher Chao1 values indicate greater microbial diversity. Kruskal-Wallis tests were performed to assess differences in Chao1 diversity across subgroups within each health characteristic and study visit independently. No significant differences were observed by frailty status. For device type and antibiotic usage, all subgroup comparisons were significant at each study visit. A single bracket is shown at visit 1 to illustrate significance (*) across device type subgroups and antibiotic subgroups, but similar significant differences were observed at all five study visits. 90

Supplemental Figure 4.2 Shannon alpha diversity by study visit and (A) frailty status (B) device type (C) antibiotic usage. Higher Shannon values indicate greater microbial diversity. Kruskal-Wallis tests were performed to assess differences in Shannon diversity across subgroups within each health characteristic and study visit independently. No significant differences were observed by frailty status. For device type and antibiotic usage, all subgroup

comparisons were significant at each study visit. A single bracket is shown at visit 1 to illustrate significance (*) across device type subgroups and antibiotic subgroups, but similar significant differences were observed at all five study visits. 91

Supplemental Figure 4.3 Bray-Curtis dissimilarity distance by participant comparing each study visit to baseline (study visit 1) by (A) frailty status (B) device type (C) antibiotic usage. Summary line was generated using LOESS regression to capture the central trend across all participants. 93

Supplemental Figure 4.4 Bray-Curtis dissimilarity distance by participant comparing each study visit to the consecutive visit by (A) frailty status (B) device type (C) antibiotic usage. Summary line was generated using LOESS regression to capture the central trend across all participants. 94

Supplemental Figure 4.5 Coefficient of variation evaluating species stability within each participant colored by genus. 95

Supplemental Figure 4.6 Coefficient of variation evaluating species stability across all participants colored by genus..... 96

Supplemental Figure 4.7 Microbial functional pathways inferred using PICRUSt2 and identified as enriched using ADAPT differential abundance based on timing of antibiotic usage and adjusted for age and *Candida albicans* colonization. Significance is based on adjusted p-value < 0.05 correcting for multiple hypothesis testing using Benjamini-Hochberg correction. Log10 fold change informs if enriched or depleted. 107

Supplemental Figure 4.8 Microbial functional pathways inferred using PICRUSt2 and identified as depleted using ADAPT differential abundance based on timing of antibiotic usage and adjusted for age and *Candida albicans* colonization. Significance is based on adjusted p-value < 0.05 correcting for multiple hypothesis testing using Benjamini-Hochberg correction. Log10 fold change informs if enriched or depleted. 108

Supplemental Figure 4.9 Microbial functional pathways inferred using PICRUSt2 and identified as enriched using ADAPT differential abundance based on device type and adjusted for age and *Candida albicans* colonization. Significance is based on adjusted p-value < 0.05 correcting for multiple hypothesis testing using Benjamini-Hochberg correction. Log10 fold change informs if enriched or depleted. 109

Supplemental Figure 4.10 Microbial functional pathways inferred using PICRUSt2 and identified as depleted using ADAPT differential abundance based on device type and adjusted for age and Candida albicans colonization. Significance is based on adjusted p-value < 0.05 correcting for multiple hypothesis testing using Benjamini-Hochberg correction. Log10 fold change informs if enriched or depleted. 110

Abstract

As the population ages and Medicare policies evolve to reduce healthcare costs, the demand for short-term, subacute care, particularly skilled nursing facilities (SNFs), continues to grow. The SNF population is uniquely vulnerable, with high prevalence of frailty, new functional disabilities, frequent antibiotic use, and need for post-hospitalization care. This dissertation spans multiple dimensions of health among older adults recovering at a single SNF and highlights opportunities to improve care planning, reduce hospital readmissions, and enhance health outcomes.

Chapters 2-4 consist of three unique manuscripts. In Chapter 2, I examine population characteristics and identify predictors of length of stay (LOS) among 313 SNF residents in the Tracking Acute Respiratory Illness in Older Adults (TARI), a prospective cohort study. Upon SNF entry, 23% reported one or more respiratory symptoms, but prevalence declined over time. Although most residents had visitors, movement within the facility was nearly limited to the rehabilitation center. Dependence in activities of daily living (ADL) was associated with a 63% increase in predicted LOS, corresponding to an 8.6-day longer median stay. The presence of a surgical drain or vascular access device was associated with a 37% increase in predicted LOS, or 5.2-day longer median stay. In contrast, older age (80+) was associated with a 22% decrease in predicted LOS, corresponding to a 3.0-day shorter median stay, while having heart disease was associated with a 28% decrease or 3.8-day shorter median stay. These findings introduce a new hypothesis into the literature, a potential “healthy-elder” bias where older adults who utilize SNF care may represent a healthier subset with higher baseline health, potentially influencing how we understand recovery trajectories.

In Chapter 3, I examine the association between self-assessed oral health (OH) and frailty in the TARI cohort and assess how previously identified risk factors modify this association. After adjusting for age, chronic respiratory disease, ADL status, and antidepressant usage, the odds of frailty were 2.45 times greater among those with poor OH compared to those with good OH (95% CI: 1.42–4.29). Further, after adjusting for poor OH and other risk factors, being less than 70 years old, having chronic respiratory disease, ADL dependent, or using antidepressants

were each independently associated with frailty. Given limited access to dental care in SNFs, this simple, non-invasive tool for measuring oral health could be integrated into routine care and inform individualized care plans.

In Chapter 4, I use 16S rRNA amplicon sequencing of bacterial DNA to evaluate the short-term dynamics of the nose/throat microbiome among the TARI cohort and identify differences in microbiome composition and function by common health characteristics: frailty, devices, and antibiotic use. Microbiome composition and diversity was relatively stable but participants with a device or taking antibiotics had distinct microbial profiles that remained stable within these subsets. I identified biochemical pathways associated with these health characteristics and found depletion of the denitrification, polyamine synthesis, and sulfate-cysteine biosynthesis pathways – important in biofilm formation, immune response, cardiovascular health, oxidative stress regulation, and metabolism. These findings highlight the importance of microbiome resilience in recovery.

In the final chapter, I discuss the public health significance of my findings and future research directions. Together, my findings inform strategies to improve quality of care, reduce hospital readmissions, and lower costs associated with post-acute care. The dissertation concludes with a personal reflection on lessons learned throughout my doctoral degree.

Chapter 1 Introduction

1.1 Dissertation Goal

The goal of this dissertation is to describe the population characteristics of older adults recovering in a skilled nursing facility (SNF) and examine how these factors relate to length of stay and frailty and influence the composition and stability of the nose/throat microbiome. SNF residents represent a distinct and understudied population, different from both community-dwelling (independent living) older adults and those in long-term care facilities, where long-term residence rather than short term care, is the primary objective. Despite SNF populations heightened vulnerability, research on viral transmission, oral health, and microbiome dynamics among SNF populations is limited. This dissertation addresses three key gaps: (1) descriptions of SNF residents' characteristics and how these characteristics impact length of stay; (2) assessments of the relationship between oral health and frailty in settings where standardized dental evaluations are rare, and prevalence of frailty is high; and (3) short-term nose/throat microbiome dynamics. By addressing these gaps, this dissertation aims to inform discharge planning, support targeted interventions to improve recovery, and ultimately enhance the quality of care in SNFs. Specifically, this dissertation identifies predictors of length of stay, evaluates the association between self-assessed oral health, and assesses how common health characteristics influence the short-term stability and composition of the nose/throat microbiome. Further, this work provided a vehicle for me to develop foundational skills in epidemiologic methods, laboratory techniques, and microbiome analysis within an aging cohort. This introductory chapter presents the rationale for studying SNF populations, outlines dissertation aims, and provides an overview of the subsequent chapters.

1.2 Aging and the need for care facilities

The American population is aging rapidly: the number of Americans aged 65 and older surpassed 52 million in 2018 and is projected to reach 73 million by 2030, 21% of the total population.[1] As individuals age, they are more prone to illness and injury, often requiring

additional resources to support recovery. Care facilities provide both short-term and long-term solutions to meet those needs. While traditional nursing homes offer chronic disease management and medication oversight for individuals who can no longer live independently, they are less suited for patients who only temporarily need support while they recover from surgery or illness. This widespread realization has contributed to the rise of skilled nursing facilities (SNF) as a short-term, subacute care alternative focused on rehabilitation and regaining independence. Unlike nursing homes, SNFs provide specialized medical care and rehabilitation services designed to prepare patients for discharge home. Medicare Part A covers up to 100 days in a SNF, making these facilities an accessible option for individuals unable to immediately transition to outpatient rehabilitation due to logistic challenges in transportation, living alone, or insufficient support to manage their care.[1,2] With the increasing demand for post-acute recovery support, SNFs have become a critical component of the healthcare system: SNF associated Medicare expenditures reached $60 billion in 2015.

1.3 Length of stay and managing care

SNFs play a critical role in helping patients regain independence following surgery, illness, or infection by providing specialized rehabilitation services. Designed to shorten hospital stays, most residents remain in a SNF for only a few days to weeks with their length of stay influenced by factors such as Medicare Part A coverage, age, overall health status, rehabilitation needs, and availability of home care.[3] While shorter stays align with recovery goals, prolonged stays in SNFs can increase exposure to pathogens, thereby increasing the risk of healthcare-associated infections (HAIs).[4] Shared spaces and limited visitor restrictions in SNFs further amplify the potential for respiratory infections making efficient discharge planning essential to mitigate the risks and ensure smooth care transitions. Understanding the determinants of SNF length of stay can help families and healthcare providers to make informed decisions about care transitions and post- discharge planning and support. Chapter two of this dissertation examines population characteristics of SNF residents and the determinants of their length of stay to improve patient outcomes and care.

1.4 Frailty and oral health

Frailty, a condition marked by aging-related declines in strength and physical function, occurs frequently among those with poor oral health. Frailty and poor oral health are

independently and jointly associated with adverse health outcomes such as cardiovascular disease.[5] Frailty prevalence in care facilities often exceeds 50%, and poor oral health is widespread among older adults: 68% have periodontal disease, nearly one in five have lost all their teeth, 96% have experienced cavities, and 20% have untreated tooth decay.[6,7] Poor oral health significantly impacts nutritional intake, chronic disease management, and overall well-being.

Frailty is detected via a validated set of questions, but oral health has been deemed to require a dental examination, measuring factors such as tooth decay, denture use, or periodontal status. Given the importance of poor oral health and its association with frailty, we wondered if questions on self-assessed poor oral health might be used as a screening tool in settings where dental examinations are impractical or where access to routine dental examinations is limited. A previous study in community-dwelling older adults found self-assessed oral health had strong predictive value for frailty.[8] However, its utility within SNFs, where frailty levels are higher and dental care access is more restricted, remains unexamined.

Understanding the association between frailty and poor oral health and identifying factors that modify this association in high-frailty populations is critical for developing targeted interventions to improve oral health and enhancing overall health outcomes. As the population aged 65 and older is projected to double by 2030, addressing these issues is a public health priority.[9] Chapter three of this dissertation evaluates the utility of this self-assessed oral health as a measure and examines factors that modify its association with frailty within a single SNF.

1.5 Nose and throat microbiome

The microbiome is a dynamic community made up of microorganisms that interact both independently and collaboratively to perform functions necessary to sustain their individual and collective lives. Microbiome dynamics, defined as the fluctuation of microbial composition and structure over time, may indicate robustness to external perturbations such as antibiotic usage or respiratory infections. Fluctuations in microbiome composition and structure may create environments that are hospitable or hostile for certain pathogens, and changes in the abundance of even a single species have been associated with increased risk of several chronic and acute conditions.[10] The nose and throat are the portal of entry for respiratory infections making it likely that pathogen resistance by the nose/throat microbiome plays an important role in host defense against respiratory infections.

Most microbiome studies are cross-sectional or - if longitudinal - samples are collected relatively infrequently. Few studies of the nose/throat microbiome or any microbiome have been conducted among older individuals. The most comprehensive study to date was by Zhou *et al*. These investigators followed 86 individuals quarterly for six years and assessed microbiome stability in four body sites: stool, skin, tongue/oral cavity, and nasal over the course of the study. Overall, the microbiomes were stable, but skin and nasal microbiomes were less stable than the stool and oral cavity, likely due to direct environmental exposures.[11] To address the limitations of existing microbiome studies, in chapter four of this dissertation I assess the short-term dynamics, approximately every three days, of the nose/throat microbiome among older adults recovering in a SNF. Additionally, I characterize the composition of the nose/throat microbiome by frailty, device use, and antibiotic exposure. This work provides a foundation for future research aimed at preventing microbiome dysbiosis and improving health outcomes among older adults.

1.6 Dissertation Chapters

In the following chapters, I explore distinct aspects of the SNF population to address critical gaps in understanding their care. In Chapter 2, I examine the population characteristics of SNF residents and determinants of length of stay. I introduce the "healthy elder effect" hypothesis, suggesting that the observed association between older age and shorter SNF stays reflects a selection bias, where older residents may be a healthier subgroup at baseline. In Chapter 3, I examine the association between self-assessed oral health and frailty in SNF residents and assess how prevalent risk factors modify this relationship. In Chapter 4, I use 16S rRNA amplicon sequencing of bacterial DNA to evaluate the stability of the nose/throat microbiome and characterize its composition based on frailty status, device type, and antibiotic usage, all common health characteristics among SNF residents.

All analyses were conducted using data from a prospective cohort study, Tracking Acute Respiratory Illness in Older Adults (TARI), that enrolled residents from April 5, 2021, to May 22, 2023, at a single SNF in Southeast Michigan. Participants were enrolled shortly after admission to the SNF. Over a 12 to 15-day period, patients underwent up to five total study visits approximately every 3 days, during which respiratory symptoms, facility movement, and nose/throat samples were collected. Demographics, health behaviors, and health history were collected after study discharge through detailed medical record review and a review of the

Minimum Dataset (MDS), a standardized tool used to evaluate the clinical status and functional capability of residents in care facilities.

In the final chapter, I synthesize my findings to highlight their public health significance and potential to inform SNF case management and intervention strategies. By identifying modifiable risk factors and potential intervention opportunities, this research aims to enhance case management and improve care and discharge planning for older adults residing in SNFs and their families. I close with some personal reflections on my graduate experience.

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Chapter 2 Skilled Nursing Facility Population Characteristics and Length of Stay: A Prospective Cohort Study

This manuscript is intended for publication and currently is under review.

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2.1 Abstract

Background: Skilled nursing facilities (SNFs) offer short-term, subacute care following injury or hospitalization, with Medicare fully covering stays ≤ 20 days. As the U.S. population ages, SNF demand is increasing, yet this population remains poorly characterized. Prolonged stays increase pathogen exposure, with shared spaces and visitors potentially elevating viral transmission. We estimated the prevalence of self-reported respiratory symptoms, shared space use, and visitor frequency among SNF residents and their associations with length of stay (LOS).

Methods: We conducted a prospective cohort study of 313 SNF residents enrolled in Tracking Acute Respiratory Illness in Older Adults (TARI) (April 2021 – May 2023). Participants were enrolled after SNF admission and followed every three days for up to five visits. Demographics, health behaviors, and medical history were collected, with respiratory symptoms and facility use assessed at each visit. We fitted lognormal survival models to evaluate associations between patient characteristics and LOS.

Results: Participants averaged 77 years of age; 63.3% were women. 23.2% reported respiratory symptoms at entry, but prevalence declined over time. Other than rehabilitation services, few

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used shared spaces; 84% had visitors. Average LOS was 21.82 days. Dependence for activities of daily living (HR: 1.63, 95% CI: 1.42 – 1.86) and having a vascular access and/or surgical drain (HR: 1.37, 95% CI: 1.09 – 1.73) were associated with significantly longer LOS, while older age and heart disease predicted shorter stays.
Conclusion: We identify LOS predictors to aid in discharge planning and introduce the "healthy elder effect" hypothesis into literature.

2.2 Introduction

In 2020, approximately one in six people in the United States were aged 65 and older, and by 2030, an estimated 20% will be 65 and older.[1] As we age, we are more prone to illness, injury, and interventions that require additional resources to facilitate recovery.[2,3] About one in four Medicare beneficiaries were discharged from a hospital to a skilled nursing facility (SNF) for short-term subacute care to aid recovery between 2000 to 2015.[4]

SNFs prioritize regaining independence post-surgery or infection by providing easy access to skilled nursing rehabilitation services before discharge home, aiming to reduce future hospital stays. SNF stays typically last only days or weeks.[5] Length of stay (LOS) is often determined by ability to cover the copay: Medicare Part A covers a 100-day stay in a SNF, with full coverage for days 0–20, and coverage with a copayment on days 21–100.[6,7] In 2015, Medicare spent $60 billion on subacute SNF care.[7] Other determinants of LOS include age, overall health status, rehabilitation needs, and home care availability.[5]

Prolonged stays in care facilities result in greater exposure to pathogens, which increases the risk of healthcare-associated infections (HAI).[8] Older patients often have decreased cough reflex, immunosenescence, frailty, decreased mobility, and comorbidities that increase susceptibility.[9] Shared spaces and limited visitor restrictions in SNFs may increase respiratory infection exposure. Although we found no data specific to SNFs, a 2017 Centers for Disease Control point prevalence survey of 161 nursing homes found that 1 in 43 residents had a HAI on any given day, most commonly affecting skin (32%), respiratory (29%) and urinary tract (20%).[10]

Here, we use data from a prospective cohort study of SNF residents in southeast Michigan to identify factors associated with increased LOS. We report detailed demographics, health behaviors, comorbidities, device and medication usage, and infection history among residents. Due to the paucity of data on viral transmission in SNFs, we additionally estimate the

prevalence of self-reported respiratory symptoms, the use of shared spaces, and visitor frequency, as these may contribute to viral transmission.

2.3 Methods

2.3.1 Study Population

The Tracking Acute Respiratory Illness in Older Adults (TARI) study is a longitudinal, prospective cohort study that enrolled residents recovering at a single SNF in southeast Michigan. Eligible participants were 18 years or older, English-speaking, and not scheduled for immediate discharge. Enrollment for this analysis occurred from April 5, 2021 to May 22, 2023 for a total enrollment of 313 participants. Residents were ineligible for the study if they had devices blocking access to their nose and mouth, were not oriented and alert, or were unable to self-consent (Figure 2.1). The study protocol was approved by the Institutional Review Board at the University of Michigan.

2.3.2 Data Collection & Storage

Participants were recruited shortly after admittance to the SNF. At enrollment, informed consent was obtained for each participant and initial demographics (age, gender, race, height, weight), health history (frailty, activities of daily living [ADL], comorbid conditions) and health-related behaviors (oral health, dietary habits, smoking history) were collected. After enrollment, participants were visited approximately every three days for up to five study visits over a 12 to 15-day period (Supplemental Figure 2.1). During every study visit, two nasal and two throat swabs were collected along with self-reported respiratory symptoms, room type, movement throughout the facility, visitor frequency, and any medical devices. One of each swab type was combined into one vial of UTM and one eSWAB tube. The swabs were transported to the lab and processed within 3 hours. The UTM was aliquoted into four vials of sample: one, 1mL with the swabs (A), two, 500ul of media (B-C), and one for the remainder of the media (D); extracted nucleic acids from these samples were used for viral detection of both DNA and RNA targets. The eSWAB media was aliquoted into four samples: two, 1 swab with 10% milk (A-B), one, 500ul with 20% milk and 500ul Amies media (C), and one for the remainder of the Amies media (D); these were used for bacterial growth studies. All aliquots were then frozen at -80 degrees Celsius. At study discharge, a detailed medical record review was completed for each participant

using the Minimum Data Set (MDS), capturing information on medication usage, infection history, and hospitalization history. Data was collected and stored using REDCap.

2.3.3 Participant Characteristics

Gender, race, smoking history, self-reported oral health, and dental care utilization were treated as categorical variables. Comorbid conditions, device use, medications (antipsychotics, anti-anxiety, or antidepressants), use of shared spaces, visitors, and self-reported respiratory symptoms were dichotomous (yes/no) variables.

Age was calculated using study enrollment date and date of birth and categorized as < 70, 70–79, and 80+ years old. Body mass index (BMI) was calculated and categorized as underweight (< 18.5) or normal (18.5–24.9), overweight (25–29.9), or obese (30+) based on participant's height and weight.[11] Smoking status was categorized as current, former, or never smoker based on a combination of smoking history and frequency questions. Self-reported oral health was collapsed into two categories: poor oral health (combining 'very poor', 'poor', and 'fair' responses) and good oral health (combining 'good' and 'very good' responses). We used a validated five question Frailty Short Interview to calculate a score between zero and one based on total questions answered and number of missing questions. Scores were dichotomized with < 0.6 considered 'non-frail' and ≥ 0.6 considered to be 'frail'.[12] Activities of Daily Living (ADL) categories included: bathing, dressing, toileting, transferring, continence, and feeding. Scores were summarized and classified into 'independent' (≥ 6), 'partially dependent' (> 2 - < 6), and 'dependent' (≤ 2); independent and partially dependent were collapsed into one category for this analysis.[13]

Antibiotic usage was classified as 'no antibiotic usage', 'antibiotics prior to the study only', and 'any antibiotics during the study'. Infections and pneumonia histories from the prior thirty days were classified as 'no history' versus 'any history' regardless of timing relative to the study. Devices were categorized into 'no devices', 'respiratory devices and/or non-surgical tubing', and 'any surgical drain and/or vascular access device' based on difficulty in managing the device at home. The devices utilized by TARI participants and how they were categorized are detailed in the supplementary information (Supplemental Figure 2.2).

LOS was calculated as the number of days between SNF date of admission and date of discharge. This variable was categorized into 0–2 weeks (0–14 days), 2–3 weeks (15–20 days),

and 3+ weeks (≥ 21 days) in the tables. Self-reported respiratory symptoms (cough, fever, chills, sore throat, nasal congestion, headache, and body aches) were analyzed individually. The total symptom count was also calculated for each resident. Movement throughout the facility was defined as use of any shared spaces (dining room, living room, and rehabilitation facilities).

2.3.4 Statistical Analysis

To determine whether differences in behaviors and medical conditions by patient characteristics were statistically significant, we used Pearson's Chi-squared test or Fisher's Exact Test for Count data (Fisher's when cell frequency is <5) to assess statistical significance ($p<0.05$) of associations between each covariate and LOS. To visualize LOS within SNF, we generated unadjusted Kaplan-Meier survival curves by age, ADL status, antibiotic usage, and medical device type. We fitted a lognormal survival model with an outcome of LOS at the SNF (in days until discharge) and covariates as named in Table 1 using the "survival" package in R (version 3.7.0) to estimate hazard ratios (HR).[14] The lognormal distribution of the outcome was selected as the proportional hazards assumption for Cox proportional hazards model was not met.

We calculated cumulative survival probabilities and plotted fit survival curves. For each model covariate, we plotted the survival curve for each covariate level, holding the other covariates constant. We used a simple substitution estimator to generate and compare the survival curve associated with all low-risk predictors (as defined by the model) to all high-risk predictors. Low-risk included being 80 years and older, having heart disease, no antibiotics, independent or partially dependent ADL, and having no devices; high-risk included less than 70 years of age, not having heart disease, having any antibiotics during the study, ADL dependent, and having a vascular access and/or surgical drain device. To isolate the absolute effect of each individual covariate on LOS, for each covariate of interest, we calculated the absolute differences in mean LOS by comparing the survival curves at the 25th, 50th, and 75th percentiles, holding all other variables constant at their reference level (Table 2.1). 95% confidence intervals were calculated using non-parametric bootstrapping with 1,000 iterations. All analyses were conducted using RStudio Version 4.3.2.

2.4 Results

2.4.1 Study Population Characteristics

Participants averaged 77 years of age (min: 37.8, max: 100.8), 63.3% were women, 88.5% were White, and 93.2% were discharged home from the SNF. We did not observe differences in health behaviors and health history by gender, but did observe significant differences across age categories. As compared to younger age groups (less than 70 years old and 70–79 years old), those 80 years and older were more likely to be underweight or have a healthy BMI, have never smoked, have better self-reported oral health, have consistently visited a dentist for regular check-ups, and be classified as non-frail upon entry to the SNF (Supplemental Table 2.1). Nearly 66% of participants were ADL dependent with similar rates seen across age and gender categories. Prevalence of asthma, diabetes, and chronic obstructive pulmonary disorder (COPD) were lower among participants aged 80 years and older compared to the other age groups, but the proportion with heart disease or dementia were higher (Supplemental Table 2.1). Prior to SNF entry, participants 80 years and older were more likely to have taken antibiotics, have had an infection, and/or had pneumonia compared to other age groups. In addition, those 80 years and older had the lowest consumption of antipsychotic and anti-anxiety medications of any age category. Participants 70–79 years old had the highest proportion of devices, and those less than 70 years old had the highest consumption of antipsychotics and anti-anxiety medications of any age category.

2.4.2 Self-Reported Symptom Frequency

Self-reported respiratory symptoms were common, especially at SNF entry. A total of 72 participants (23.0%) had one or more self-reported respiratory symptoms at enrollment (Figure 2.2). No participants had a fever. Among those with symptoms at enrollment, 52.8% had only one symptom, 29.2% had two symptoms, and 18.1% had three or more symptoms. The proportion of patients reporting respiratory symptoms decreased by almost half (from 23.0% to 13.5%) by the second study visit and remained consistently low across all subsequent visits (<15%). Facility staff confirmed SARS-CoV-2 circulated within the facility during the study period, but we did not test participant nose/throat swabs for this. We did not detect circulation of other respiratory viruses such as influenza and rhinovirus through viral testing of nose/throat swabs. From 2020 until Fall 2022, masking was required for all staff, patients, and their visitors.

2.4.3 Visitors & Movement Behaviors

Most SNF residents had visitors, with men having on average, more visitors than women (Figure 2.3). Among women, those 80 years and older were more likely than younger women to have visitors at study visits 2–4. Men 80 years and older were also more likely to have visits as compared to younger men (> 75% of men 80 and older had at least one visitor at each study visit compared to < 50% of men less than 70 years, consistent across all five study visits). A total of 94 (30.0%) participants had visitors for all their study visits and 53 (16.9%) had no visitors for their entire SNF stay.

We hypothesized that use of shared spaces would be common among residents. However, very little movement was reported by residents other than to the rehabilitation center. At the time of their enrollment visit, 71.4% participants did not utilize the rehabilitation center and either had rehabilitation in their room or not at all, which is common for an initial evaluation; for study visits 2–5, the majority (visit 2: 56.0%; visit 3: 58.6%; visit 4: 54.6%; visit 5: 54.1%) of residents used the rehabilitation center - likely related to improvements in ADL. Less than 5% of residents used the dining room or living room across all five visits (Figure 2.3; Supplemental Table 2.2); there were no significant differences observed by gender (Supplemental Table 2.3) or age category (Supplemental Table 2.4).

2.4.4 Length of Stay

TARI participants stayed an average of 21.82 days (range 1–168 days) in the SNF; the overall distribution is slightly right-skewed with some participants remaining at the SNF for greater than 50 days. Approximately 40% of the 313 participants resided for 0–14 days; 25% for 15–20 days; and 35% for 21 days or greater. There were no significant differences between those staying 0–2 weeks, 2–3 weeks, or 3+ weeks by age, but there were by gender, with men having shorter stays compared to women (Table 2.1). Faster discharges, indicating shorter LOS, were observed more frequently among those 80 years and older; those aged 70 years or less had the longest LOS. Those with ADL independent or partially dependent had significantly shorter LOS compared to those ADL dependent. Over 45% of residents who entered the SNF as ADL dependent had LOS greater than three weeks, whereas 86% of those who were independent or partially dependent stayed less than three weeks.

Participants who did not take antibiotics at any point and did not have a device had the shortest LOS. Among the 64 participants with a device, 21.8% had a LOS of 2–3 weeks and 50% a stay greater than three weeks. Over 80% of the participants that had a device at enrollment

retained that device for all their study visits and 18.8% of participants had more than one device (Supplemental Figure 2.3).

We visualized LOS (time to SNF discharge) using unadjusted Kaplan-Meier survival curves (Supplemental Figure 2.4). We fit a multivariable lognormal survival model to estimate cumulative survival probabilities based on age, heart disease, ADL status, type of medical device, and antibiotic usage, with an interaction between age and heart disease. The interaction term was significant, indicating that the effect of age on LOS depends on the presence of heart disease among the older adults, as 68.9% of those 80 years and older had heart disease. ADL dependence ADL was the strongest predictor of a longer LOS (HR: 1.63, 95% CI: 1.42 – 1.86) (as compared to independent), followed by having a vascular access and/or surgical drain device (HR: 1.37, 95% CI: 1.09 –1.73) (as compared to having no devices).

Individuals with all high-risk characteristics (as defined in Methods) had a mean LOS that was 34.9 days longer at the 75th percentile; 23.5 days longer at the 50th percentile; and 15.8 days longer at the 25th percentile (compared to individuals who had all low-risk characteristics) (Figure 2.4A). Absolute differences in mean LOS were calculated for each level of the model covariates, holding all other variables constant to the reference, to isolate the effect of each individual variable on LOS. Those ADL dependent had a mean LOS that was 5.8 days longer at the 75th percentile; 8.6 days longer at the 50th percentile; and 12.7 days longer at the 25th percentile compared to those ADL independent or partially dependent (Figure 2.4B). Mean differences in the LOS by age category, devices, heart disease, and antibiotic usage are included in Supplemental Figure 2.5 and Supplemental Figure 2.6.

2.5 Discussion

In this prospective cohort study of 313 SNF residents, we identified ADL dependence and having a vascular access device and/or surgical drain as independently associated with increased LOS. Our study adds to the sparse literature on SNF populations by describing demographics, health history, prevalence of self-reported respiratory symptoms, and shared space use.

Typically persons enter SNFs for subacute care following hospitalization because they require specialized care beyond what can be provided at home; travel to rehabilitation is complicated; they live alone; or their partner/caregiver is not capable of arranging travel or managing care.[6] This aligns with our findings that the strongest LOS predictors, even after

controlling for age, antibiotic usage, and heart disease status in lognormal survival models, were ADL dependence and having a vascular access device and/or surgical drain.

The demographic characteristics of our participants, prevalence of frailty and ADL-classified dependence, presence of infections, and antibiotic usage before SNF entry are consistent with the profiles of typical SNF admissions.[6,15] Participants were disproportionately women and White, consistent with nursing home residents.[16] However, we found older participants were less likely to be frail and had shorter LOS than younger participants, which is not consistent with the literature. Frailty is an aging-associated decline and multiple cross-sectional and longitudinal studies show that frailty increases with age.[17-19] This observed difference may reflect a specific selection bias for health status among persons who seek short-term care. If a person who is 80 years and older and recovering from a major surgery or illness only requires short-term care, it is possible they are in better overall health than their counterparts who already reside in long-term care. We hypothesize that there may be a 'healthy elder' bias in which older SNF adults who use subacute care have higher baseline health. This form of selection bias is akin to the healthy worker survivor bias observed and documented in occupational studies.[20] Additional studies across multiple SNFs are required to understand how SNF residents differ from community-dwelling older adults and long-term care populations.

Self-reported respiratory symptoms, particularly cough and body aches, were common at SNF entry, but declined rapidly. Despite respiratory-like symptoms, viral testing of combined nose/throat swab specimens detected no circulating respiratory illness, nor were participants diagnosed with viral infections. SARS-CoV-2 was circulating amongst the general population, but community influenza rates were low.[21] These symptoms were non-specific and could be consistent with recent hospitalization discharge, suggesting that hospital stays can be traumatic and result in respiratory symptoms.[22] Current infection control protocols require screening and isolation for respiratory symptoms at entry, but our findings highlight that such symptoms may be expected post-hospitalization and may not necessitate immediate clinical action.[23] In order to better understand the presence of symptoms, data collection would need to be repeated at a time when seasonal respiratory illnesses are circulating in the community and care facilities.

SNFs advertise benefits like rehabilitation, recreation, and increased activity to promote physical and mental health while receiving skilled nursing.[24] While most participants had visitors, few left their rooms except for rehabilitation. It is unclear if this resulted from

pandemic-related social distancing or is typical for short-term, subacute care. It is possible that the added benefit of more movement within this specialized recovery facility is not being used as residents either are insufficiently independent or prioritize rehabilitation to expedite discharge.

Our study has some limitations. The SNF participating in this study is in a predominantly White community and is one of the few SNFs that remain non-profit.[25] Known health disparities exist within skilled nursing care: African American/Black residents are approximately four times more likely to be in lower-tier facilities with reduced quality of care compared to White residents.[26] Seventy-eight percent of SNFs are for-profit, which reduces access to Medicaid recipients, and segregates high quality and sufficiently staffed facilities to higher income communities.[27-28] Thus, while gender and age distributions observed in our study were representative of national long-term care trends, results may not be generalizable to all SNFs. Additionally, many of the health characteristics were collected only at enrollment, preventing us from accounting for improvements in frailty or ADL status over time; these improvements might modify LOS. However, as frailty is a long-term, multifaceted condition that develops gradually, it would be unusual for it to change drastically in the short term. Although we have detailed medical histories, reasons for prior hospitalization were recorded only as general diagnostic categories and therefore, were not included in survival models. Additionally, trends in movement and visitors were significantly compromised based on implemented social distancing requirements and masking behaviors during the SARS-CoV-2 pandemic.

Our study has several strengths, including detailed collection of socio-demographics, medical history from MDS, and a validated measure of frailty using the Frailty Short Interview. We had minimal missing data regarding participants' demographics, health behaviors, and histories. Among the 313 residents enrolled, over 74% completed at least three study visits. Finally, this study fills an existing knowledge gap regarding the residents of short-term, subacute care and details of their stay.

2.6 Conclusion

With the rise of short-term, subacute care, understanding how SNFs are used and identifying factors that predict LOS is increasingly important to assist SNF residents and their families in effective discharge planning. In our SNF, ADL dependence and having a vascular access device and/or surgical drain were the most significant predictors of a longer LOS, but older age was associated with a shorter LOS, potentially indicating that older adults who attend

short-term care facilities have higher baseline health. These findings allow us to introduce a new hypothesis into the literature of the "healthy elder effect" among SNF residents.

2.7 Acknowledgements

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2.8 Tables and Figures

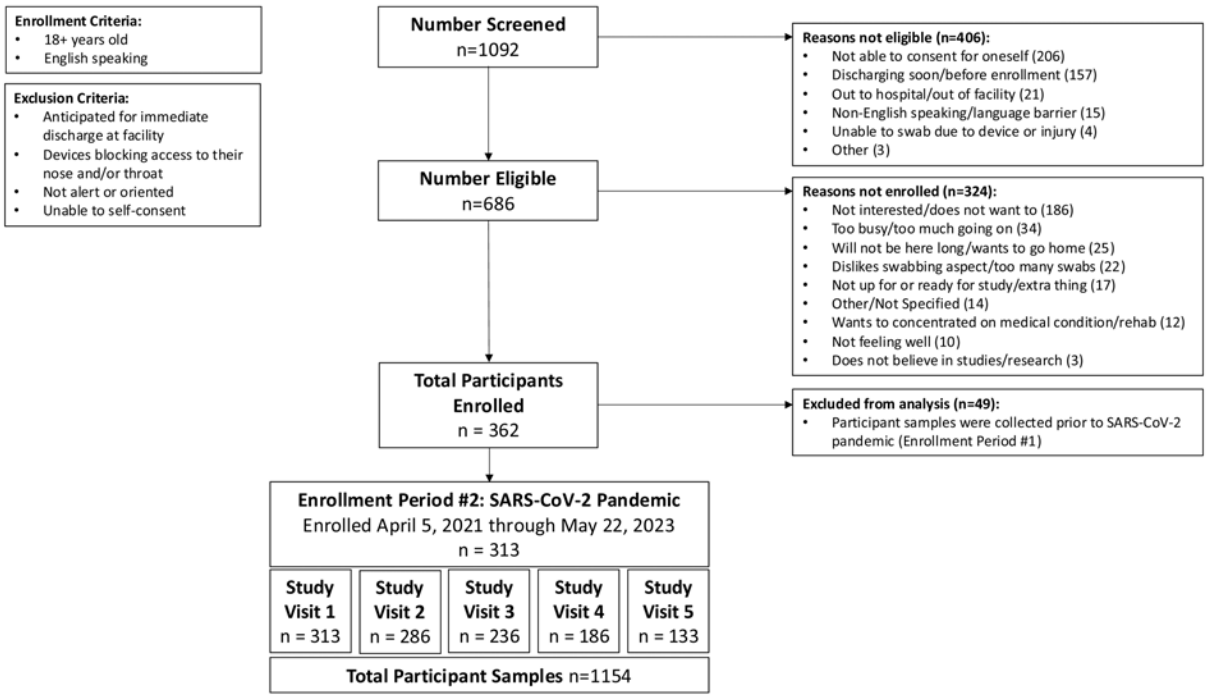


Figure 2.1 Consort diagram of 362 total participants in Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study (December 17, 2019 through May 22, 2023). Includes enrollment and exclusion criteria, detailing the two enrollment periods and the frequency of participants at each study visit.

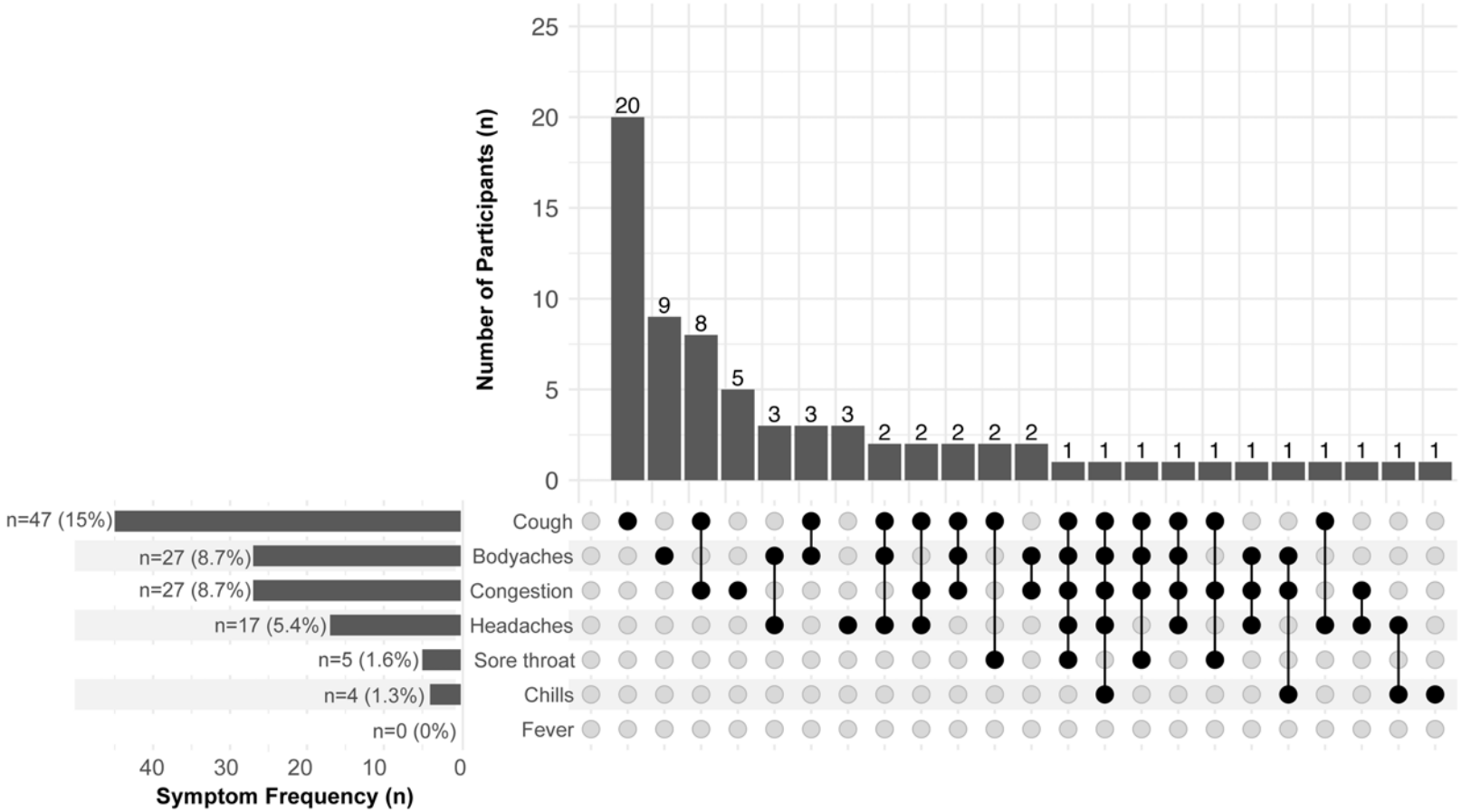


Figure 2.2 Upset plot reporting the frequency of different self-reported respiratory symptom combinations at enrollment collected from 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. Upset plot prepared using the "ComplexUpset" package in R (version 1.3.3).[29,30]

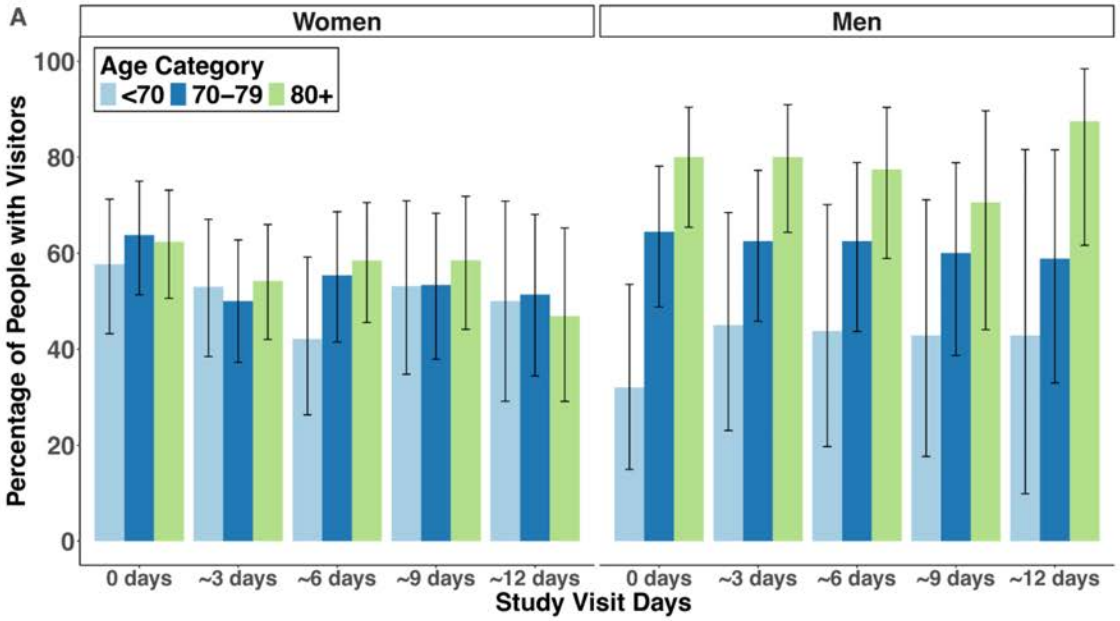
Table 2.1 Frequency [n (%)] of demographics, health behaviors, health history, external devices, and medication usage by length of stay at a single skilled nursing facility (SNF) in Michigan. 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023.

	0-2 weeks (n=125)	**2-3 weeks (n=88)**	**3+ weeks (n=100)**
DEMOGRAPHICS & HEALTH BEHAVIORS [n (%)]			
Age Category			
<70	33 (42.9%)	15 (19.5%)	29 (37.7%)
70-79	38 (33.3%)	32 (28.1%)	44 (38.6%)
80+	54 (44.3%)	32 (26.2%)	36 (29.5%)
Gender			
Female	**68 (34.3%)**	**59 (29.8%)**	**71 (35.9%)**
Male	**57 (49.6%)**	**20 (17.4%)**	**38 (33.0%)**
Race/Ethnicity			
American Indian or Alaskan			
Native	0 (NA%)	0 (NA%)	0 (NA%)
Asian	1 (16.7%)	3 (50.0%)	2 (33.3%)
Black	6 (24.0%)	8 (32.0%)	11 (44.0%)
Hispanic	0 (0%)	1 (100.0%)	0 (0%)
Mixed	1 (25.0%)	2 (50.0%)	1 (25.0%)
White	117 (42.2%)	65 (23.5%)	95 (34.3%)
Unknown	0 (NA%)	0 (NA%)	0 (NA%)
Body Mass Index (BMI)			
Underweight or healthy	41 (38.3%)	33 (30.8%)	33 (30.8%)
Overweight	40 (47.1%)	20 (23.5%)	25 (29.4%)
Obese	44 (36.4%)	26 (21.5%)	51 (42.1%)
Smoking Status			
Never	**77 (35.3%)**	**57 (26.1%)**	**84 (38.5%)**
Former	**41 (48.2%)**	**19 (22.4%)**	**25 (29.4%)**
Current	**7 (70.0%)**	**3 (30.0%)**	**0 (0%)**
Self-Reported Oral Health Status			
Good Oral Health	76 (37.8%)	50 (24.9%)	75 (37.3%)
Poor Oral Health	48 (43.6%)	28 (25.5%)	34 (30.9%)

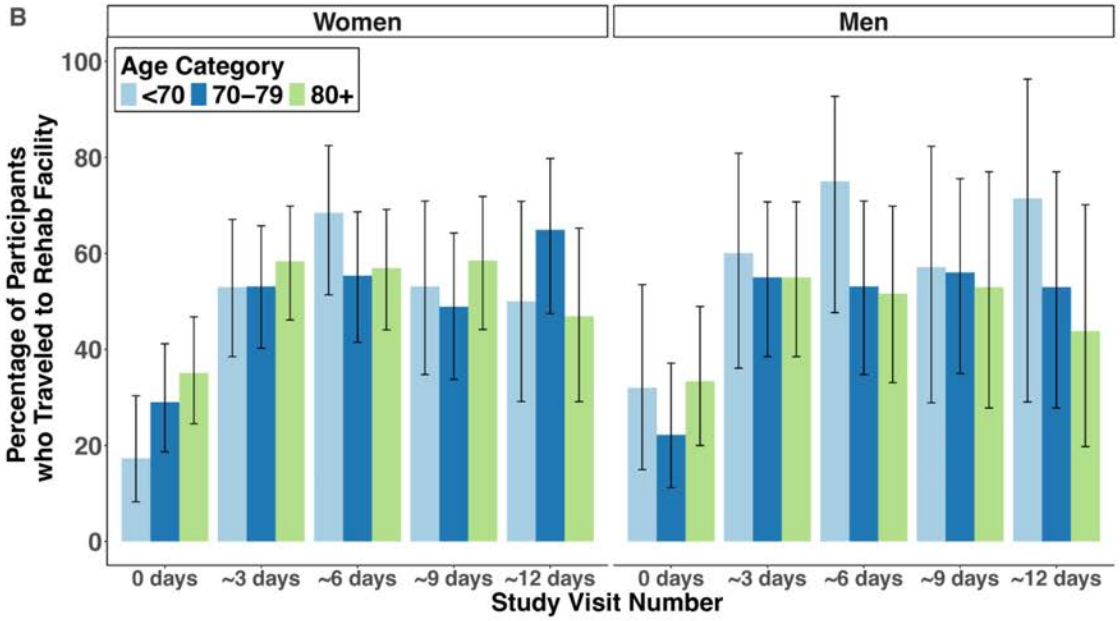
	0-2 weeks (n=125)	2-3 weeks (n=88)	3+ weeks (n=100)
Dental Visit Frequency			
Regular check-up	85 (38.6%)	58 (26.4%)	77 (35.0%)
Occasional check-up	16 (45.7%)	8 (22.9%)	11 (31.4%)
Only if problems	23 (41.8%)	12 (21.8%)	20 (36.4%)
Never been to the dentist	1 (50.0%)	0 (0%)	1 (50.0%)
HEALTH STATUS AT ENROLLMENT [n (%)]			
Total Self-Reported Respiratory Symptoms			
0 symptoms	100 (42.0%)	56 (23.5%)	82 (34.5%)
1-2 symptoms	18 (30.5%)	17 (28.8%)	24 (40.7%)
3+ symptoms	5 (38.5%)	5 (38.5%)	3 (23.1%)
Frailty Status			
Non-Frail	64 (42.1%)	42 (27.6%)	46 (30.3%)
Frail	61 (37.9%)	37 (23.0%)	63 (39.1%)
Activities of Daily Living (ADL)			
Independent or partially dependent	**69 (64.5%)**	**23 (21.5%)**	**15 (14.0%)**
Dependent	**56 (27.3%)**	**56 (27.3%)**	**93 (45.4%)**
Asthma			
No	**108 (41.7%)**	**56 (21.6%)**	**95 (36.7%)**
Yes	**17 (32.1%)**	**22 (41.5%)**	**14 (26.4%)**
Heart Disease			
No	47 (37.3%)	32 (25.4%)	47 (37.3%)
Yes	78 (41.7%)	47 (25.1%)	62 (33.2%)
Dementia			
No	118 (39.7%)	75 (25.3%)	104 (35.0%)
Yes	5 (35.7%)	4 (28.6%)	5 (35.7%)
Diabetes			
No	80 (40.2%)	53 (26.6%)	66 (33.2%)
Yes	45 (39.5%)	26 (22.8%)	43 (37.7%)
Chronic Obstructive Pulmonary Disorder (COPD)			
No	100 (39.2%)	63 (24.7%)	92 (36.1%)
Yes	25 (43.9%)	15 (26.3%)	17 (29.8%)

		0-2 weeks (n=125)	2-3 weeks (n=88)	3+ weeks (n=100)
ANTIBIOTICS, INFECTIONS, AND PNEUMONIA [n (%)]				
	Antibiotic Usage			
	No antibiotics at any time	**65 (40.9%)**	**49 (30.8%)**	**45 (28.3%)**
	Antibiotics prior to study only	**39 (48.1%)**	**12 (14.8%)**	**30 (37.0%)**
	Any antibiotics during study	**20 (28.6%)**	**18 (25.7%)**	**32 (45.7%)**
	Infections			
	No infection	**84 (43.5%)**	**52 (26.9%)**	**57 (29.5%)**
	Any infection (prior or during study)	**41 (34.2%)**	**27 (22.5%)**	**52 (43.3%)**
	Pneumonia			
	No pneumonia	115 (40.5%)	74 (26.1%)	95 (33.5%)
	Any pneumonia (prior or during study)	10 (34.5%)	5 (17.2%)	14 (48.3%)
DEVICES(S) & MEDICATION USAGE [n (%)]				
	External Devices			
	No devices	**107 (43.0%)**	**65 (26.1%)**	**77 (30.9%)**
	Respiratory and/or non-surgical tubing devices only	**9 (26.5%)**	**11 (32.4%)**	**14 (41.2%)**
	Minimum of surgical drain and/or vascular access devices	**9 (30.0%)**	**3 (10.0%)**	**18 (60.0%)**
	Anti-Psychotic Medications			
	No	121 (41.0%)	73 (24.7%)	101 (34.2%)
	Yes	4 (22.2%)	6 (33.3%)	8 (44.4%)
	Anti-Anxiety Medications			
	No	121 (41.0%)	73 (24.7%)	101 (34.2%)
	Yes	4 (22.2%)	6 (33.3%)	8 (44.4%)
	Antidepressant Medications			
	No	64 (40.3%)	45 (28.3%)	50 (31.4%)
	Yes	61 (39.6%)	34 (22.1%)	59 (38.3%)

Notes: Length of stay of 0-2 weeks: 0-14 days, 2-3 weeks: 15-20 days, and 3+ weeks: 21+ days. One person was still at the SNF at study completion; even though the exact length of stay is unknown, this participant was categorized into 3+ weeks for table reporting. Not all columns sum to total due to missing values (Detailed missingness: asthma n=1; dementia n=2; COPD n=1; antibiotic usage n=3). P-values determined through Pearson's Chi-squared test or Fisher's Exact Test for Count data. Variables with bolded numbers had statistically significant ($p<0.05$) differences across categories.

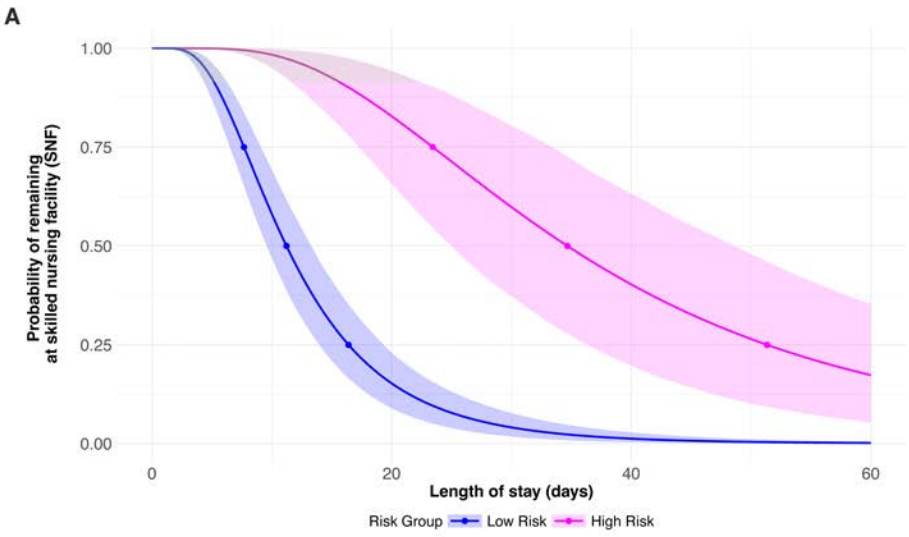


AGE CATEGORY	WOMEN					MEN				
	Study Visit 1 (0 days)	Study Visit 2 (~3 days)	Study Visit 3 (~6 days)	Study Visit 4 (~9 days)	Study Visit 5 (~12 days)	Study Visit 1 (0 days)	Study Visit 2 (~3 days)	Study Visit 3 (~6 days)	Study Visit 4 (~9 days)	Study Visit 5 (~12 days)
<70	30 (57.7%)	27 (52.9%)	16 (42.1%)	17 (53.1%)	12 (50.0%)	8 (32.0%)	9 (45.0%)	7 (43.8%)	6 (42.9%)	3 (42.9%)
70-79	44 (63.8%)	32 (50.0%)	31 (55.4%)	24 (53.3%)	19 (51.4%)	29 (64.4%)	25 (62.5%)	20 (62.5%)	15 (60.0%)	10 (58.8%)
80+	48 (62.3%)	39 (54.2%)	38 (58.5%)	31 (58.5%)	15 (46.9%)	36 (80.0%)	32 (80.0%)	24 (77.4%)	12 (70.6%)	14 (87.5%)

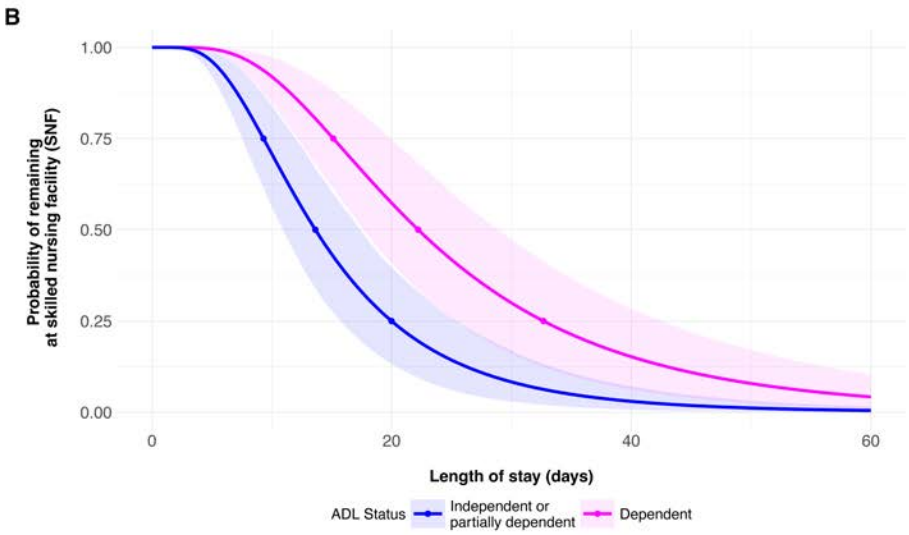


AGE CATEGORY	WOMEN					MEN				
	Study Visit 1 (0 days)	Study Visit 2 (~3 days)	Study Visit 3 (~6 days)	Study Visit 4 (~9 days)	Study Visit 5 (~12 days)	Study Visit 1 (0 days)	Study Visit 2 (~3 days)	Study Visit 3 (~6 days)	Study Visit 4 (~9 days)	Study Visit 5 (~12 days)
<70	9 (17.3%)	27 (52.9%)	26 (68.4%)	17 (53.1%)	12 (50.0%)	8 (32.0%)	12 (60.0%)	12 (75.0%)	8 (57.1%)	5 (71.4%)
70-79	20 (29.0%)	34 (53.1%)	31 (55.4%)	22 (48.9%)	24 (64.9%)	10 (22.2%)	22 (55.0%)	17 (53.1%)	14 (56.0%)	9 (52.9%)
80+	27 (35.1%)	42 (58.3%)	37 (56.9%)	31 (58.5%)	15 (46.9%)	15 (33.3%)	22 (55.0%)	16 (51.6%)	9 (52.9%)	7 (43.8%)

Figure 2.3 Visitors and movement to in-facility rehabilitation center by age and gender (A) Percentage of 313 participants that had visitors at each study visit by gender (women and men) and age category (<70, 70–79, and 80+ years) (B) Percentage of participants that traveled to the in-facility rehabilitation center during each study visit by gender (women and men) and age category (<70, 70–79, and 80+ years).

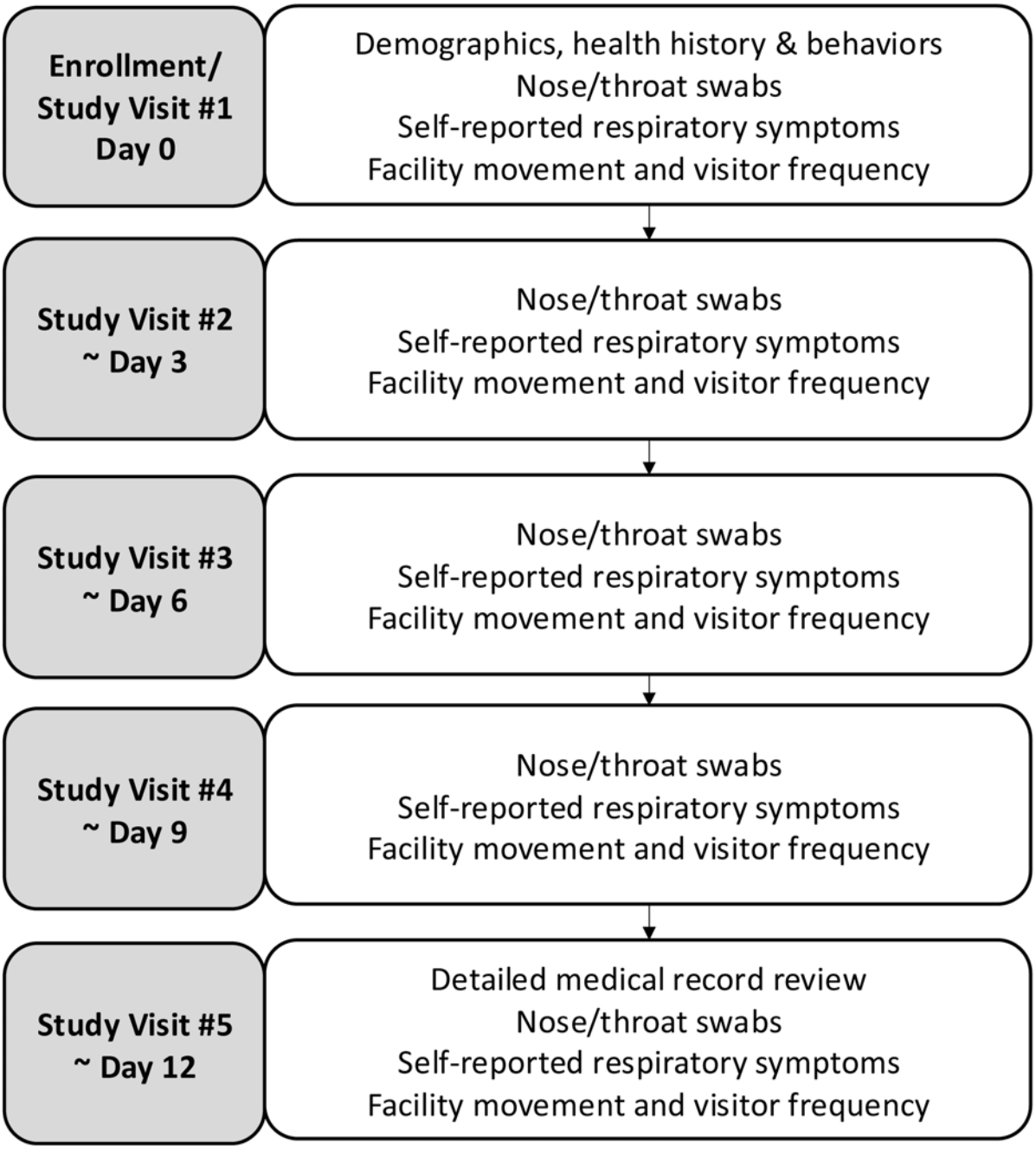


Comparison	Absolute Difference at 25th percentile	Absolute Difference at 50th percentile	Absolute Difference at 75th percentile
High Risk versus Low Risk	34.9	23.5	15.8

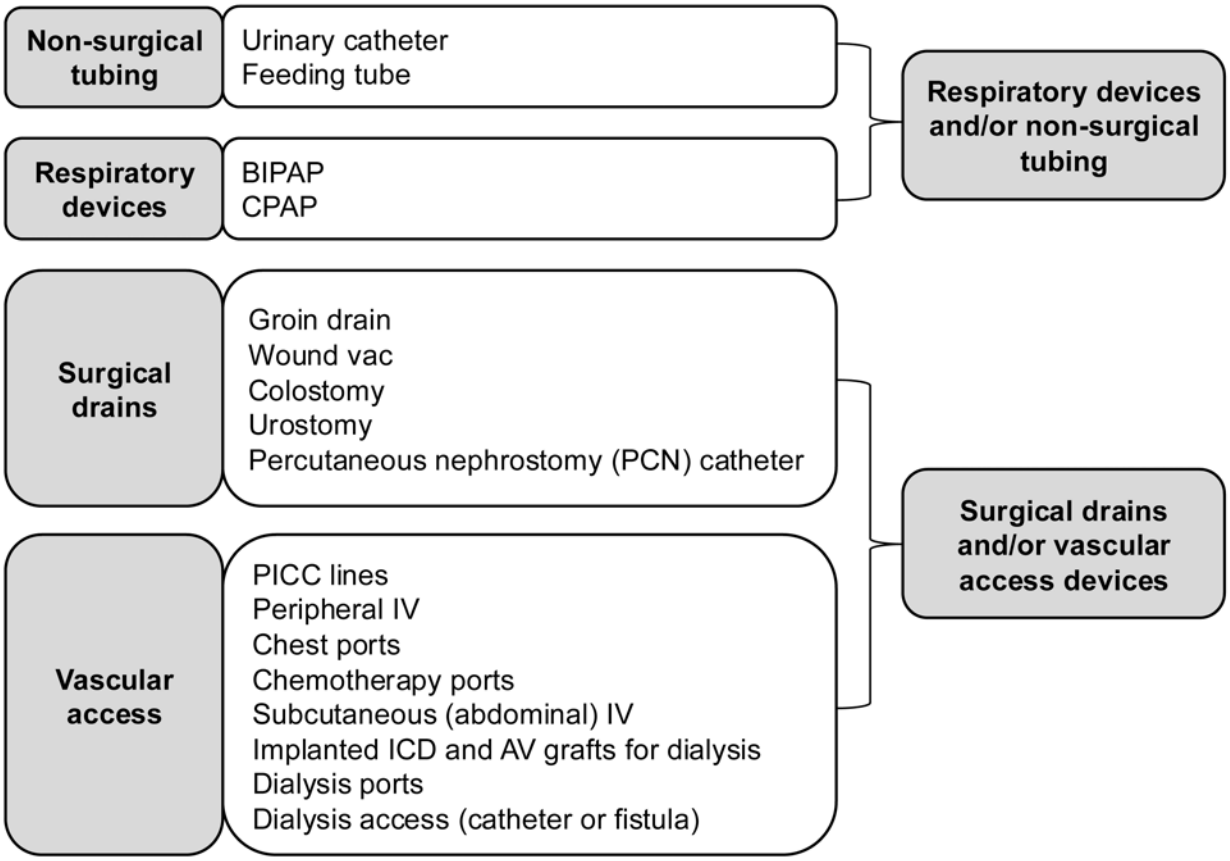


Comparison	Absolute Difference at 25th percentile	Absolute Difference at 50th percentile	Absolute Difference at 75th percentile
Dependent versus Independent or partially dependent	12.7	8.6	5.8

Figure 2.4 Cumulative survival curves estimating mean length of stay at skilled nursing facility (SNF) from 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. Tables calculate absolute difference in mean length of stay at 75th, 50th, and 25th percentiles (shown with dots) by (A) risk group and (B) ADL status from the lognormal survival model. For the risk survival curve, low-risk included being 80 years and older, having heart disease, no antibiotics, independent or partially dependent for ADL, and having no devices while high-risk included being less than 70 years of age, not having heart disease, having any antibiotics during the study, being dependent for ADL, and having a vascular access and/or surgical drain device. For the ADL survival curve, all other variables are held constant in the model at the reference: less than 70 years of age, not having heart disease, not taking any antibiotics, and having no devices. Confidence intervals were calculated using non-parametric bootstrapping with 1,000 iterations.



Supplemental Figure 2.1 Study protocol outlining study visit frequency, biological samples, and information collected from 362 participants in the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study from December 17, 2019 through May 22, 2023.



Supplemental Figure 2.2 List of the different types of devices present at entry to the Skilled Nursing Facility (SNF) and how each device was categorized for modeling. 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study.

Supplemental Table 2.1 Frequency of demographics, health behaviors, health history, external devices, and medication usage at enrollment by gender (women and men) and age category (<70, 70–79, and 80+ years). 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023.

	WOMEN (n=198)			**MEN (n=115)**			**OVERALL (n=313)**		
	<70	**70-79**	**80+**	**<70**	**70-79**	**80+**	**<70**	**70-79**	**80+**
Race/Ethnicity									
American Indian or Alaskan Native	**0 (NA%)**	**0 (NA%)**	**0 (NA%)**	0 (NA%)	0 (NA%)	0 (NA%)	0 (NA%)	0 (NA%)	0 (NA%)
Asian	**0 (0%)**	**1 (25%)**	**3 (75%)**	0 (0%)	1 (50%)	1 (50%)	0 (0%)	2 (33%)	4 (67%)
Black	**11 (55%)**	**6 (30%)**	**3 (15%)**	2 (40%)	2 (40%)	1 (20%)	13 (52%)	8 (32%)	4 (16%)
Hispanic	**0 (0%)**	**0 (0%)**	**1 (100%)**	0 (NA%)	0 (NA%)	0 (NA%)	0 (0%)	0 (0%)	1 (100%)
Mixed	**0 (0%)**	**1 (100%)**	**0 (0%)**	0 (0%)	2 (67%)	1 (33%)	0 (0%)	3 (75%)	1 (25%)
White	**41 (24%)**	**61 (35%)**	**70 (41%)**	23 (22%)	40 (38%)	42 (40%)	64 (23%)	101 (36%)	112 (40%)
Body Mass Index (BMI)									
Underweight or healthy	**11 (16%)**	**22 (33%)**	**34 (51%)**	5 (12%)	20 (50%)	15 (38%)	**16 (15%)**	**42 (39%)**	**49 (46%)**
Overweight	**12 (21%)**	**17 (30%)**	**27 (48%)**	7 (24%)	7 (24%)	15 (52%)	**19 (22%)**	**24 (28%)**	**42 (49%)**
Obese	**29 (39%)**	**30 (40%)**	**16 (21%)**	13 (28%)	18 (39%)	15 (33%)	**42 (35%)**	**48 (40%)**	**31 (26%)**
Smoking Status									
Never	36 (24%)	56 (37%)	59 (39%)	15 (22%)	27 (40%)	25 (37%)	51 (23%)	83 (38%)	84 (39%)
Former	13 (32%)	11 (27%)	17 (41%)	9 (20%)	16 (36%)	19 (43%)	22 (26%)	27 (32%)	36 (42%)
Current	3 (50%)	2 (33%)	1 (17%)	1 (25%)	2 (50%)	1 (25%)	4 (40%)	4 (40%)	2 (20%)
Self-Report Oral Health Status									
Good Oral Health	**24 (18%)**	**48 (37%)**	**58 (45%)**	**10 (14%)**	**29 (41%)**	**32 (45%)**	**34 (17%)**	**77 (38%)**	**90 (45%)**
Poor Oral Health	**28 (42%)**	**21 (31%)**	**18 (27%)**	**15 (35%)**	**15 (35%)**	**13 (30%)**	**43 (39%)**	**36 (33%)**	**31 (28%)**

	WOMEN (n=198)			MEN (n=115)			OVERALL (n=313)		
	<70	**70-79**	**80+**	**<70**	**70-79**	**80+**	**<70**	**70-79**	**80+**
Dental Visit Frequency									
Regular check-up	**34 (23%)**	**49 (33%)**	**64 (44%)**	**8 (11%)**	**30 (41%)**	**35 (48%)**	**42 (19%)**	**79 (36%)**	**99 (45%)**
Occasional check-up	**10 (53%)**	**5 (26%)**	**4 (21%)**	**9 (56%)**	**2 (12%)**	**5 (31%)**	**19 (54%)**	**7 (20%)**	**9 (26%)**
Only if problems	**8 (26%)**	**15 (48%)**	**8 (26%)**	**7 (29%)**	**12 (50%)**	**5 (21%)**	**15 (27%)**	**27 (49%)**	**13 (24%)**
Never been to the dentist	**0 (0%)**	**0 (0%)**	**1 (100%)**	**1 (100%)**	**0 (0%)**	**0 (0%)**	**1 (50%)**	**0 (0%)**	**1 (50%)**
Total Self-Reported Respiratory Symptoms									
0 symptoms	41 (28%)	46 (32%)	59 (40%)	18 (20%)	36 (39%)	38 (41%)	**59 (25%)**	**82 (36%)**	**97 (41%)**
1-2 symptoms	6 (15%)	17 (44%)	16 (41%)	4 (20%)	9 (45%)	7 (35%)	**10 (17%)**	**26 (44%)**	**23 (39%)**
3+ symptoms	5 (46%)	5 (46%)	1 (9%)	2 (100%)	0 (0%)	0 (0%)	**7 (54%)**	**5 (39%)**	**1 (8%)**
Frailty Status at Study Enrollment									
Non-Frail	15 (17%)	26 (30%)	47 (53%)	**11 (17%)**	**21 (33%)**	**32 (50%)**	**26 (17%)**	**47 (31%)**	**79 (52%)**
Frail	37 (34%)	43 (39%)	30 (27%)	**14 (27%)**	**24 (47%)**	**13 (25%)**	**51 (32%)**	**67 (42%)**	**43 (27%)**
Activities of Daily Living (ADL) at Enrollment									
Independent or partially dependent	22 (33%)	18 (27%)	27 (40%)	10 (25%)	19 (48%)	11 (28%)	32 (30%)	37 (35%)	38 (36%)
Dependent	30 (23%)	51 (39%)	50 (38%)	14 (19%)	26 (35%)	34 (46%)	44 (21%)	77 (38%)	84 (41%)
Length of Stay at SNF									
0-2 weeks	21 (31%)	16 (24%)	31 (46%)	12 (21%)	22 (39%)	23 (40%)	33 (26%)	38 (30%)	54 (43%)
2-3 weeks	11 (19%)	23 (39%)	25 (42%)	4 (20%)	9 (45%)	7 (35%)	15 (19%)	32 (41%)	32 (41%)
3+ weeks	20 (28%)	30 (42%)	21 (30%)	9 (24%)	14 (38%)	14 (38%)	29 (27%)	44 (41%)	35 (32%)
Asthma									
No	**40 (25%)**	**49 (31%)**	**70 (44%)**	20 (20%)	40 (40%)	40 (40%)	**60 (23%)**	**89 (34%)**	**110 (42%)**
Yes	**12 (31%)**	**20 (51%)**	**7 (18%)**	5 (36%)	5 (36%)	4 (29%)	**17 (32%)**	**25 (47%)**	**11 (21%)**
Heart Disease									
No	26 (29%)	33 (36%)	32 (35%)	**13 (37%)**	**16 (46%)**	**6 (17%)**	**39 (31%)**	**49 (39%)**	**38 (30%)**
Yes	26 (24%)	36 (34%)	45 (42%)	**12 (15%)**	**29 (36%)**	**39 (49%)**	**38 (20%)**	**65 (35%)**	**84 (45%)**

	WOMEN (n=198)			MEN (n=115)			OVERALL (n=313)		
	<70	**70-79**	**80+**	**<70**	**70-79**	**80+**	**<70**	**70-79**	**80+**
Dementia									
No	52 (28%)	65 (34%)	72 (38%)	23 (21%)	43 (40%)	42 (39%)	75 (25%)	108 (36%)	114 (38%)
Yes	0 (0%)	3 (38%)	5 (62%)	2 (33%)	2 (33%)	2 (33%)	2 (14%)	5 (36%)	7 (50%)
Diabetes									
No	36 (27%)	42 (32%)	55 (41%)	**9 (14%)**	**27 (41%)**	**30 (45%)**	45 (23%)	69 (35%)	85 (43%)
Yes	16 (25%)	27 (42%)	22 (34%)	**16 (33%)**	**18 (37%)**	**15 (31%)**	32 (28%)	45 (39%)	37 (32%)
Chronic Obstructive Pulmonary Disorder (COPD)									
No	45 (27%)	56 (34%)	64 (39%)	19 (21%)	34 (38%)	37 (41%)	64 (25%)	90 (35%)	101 (40%)
Yes	7 (22%)	13 (41%)	12 (38%)	6 (24%)	11 (44%)	8 (32%)	13 (23%)	24 (42%)	20 (35%)
Antibiotic Usage									
No antibiotics at any time	26 (25%)	38 (36%)	41 (39%)	11 (20%)	23 (43%)	20 (37%)	37 (23%)	61 (38%)	61 (38%)
Antibiotics prior to study only	12 (23%)	13 (25%)	27 (52%)	5 (17%)	13 (45%)	11 (38%)	17 (21%)	26 (32%)	38 (47%)
Any antibiotics during study	12 (32%)	18 (47%)	8 (21%)	9 (28%)	9 (28%)	14 (44%)	21 (30%)	27 (39%)	22 (31%)
Infections									
No infection	30 (23%)	43 (33%)	56 (43%)	12 (19%)	29 (45%)	23 (36%)	42 (22%)	72 (37%)	79 (41%)
Any infection (prior or during study)	22 (32%)	26 (38%)	21 (30%)	13 (25%)	16 (31%)	22 (43%)	35 (29%)	42 (35%)	43 (36%)
Pneumonia									
No pneumonia	48 (26%)	66 (35%)	72 (39%)	23 (23%)	40 (41%)	35 (36%)	71 (25%)	106 (37%)	107 (38%)
Any pneumonia (prior or during study)	4 (33%)	3 (25%)	5 (42%)	2 (12%)	5 (29%)	10 (59%)	6 (21%)	8 (28%)	15 (52%)

	WOMEN (n=198)			MEN (n=115)			OVERALL (n=313)		
	<70	**70-79**	**80+**	**<70**	**70-79**	**80+**	**<70**	**70-79**	**80+**
External Devices									
No devices	41 (25%)	55 (33%)	71 (43%)	18 (22%)	33 (40%)	31 (38%)	59 (24%)	88 (35%)	102 (41%)
Respiratory and/or non-surgical tubing devices only	4 (27%)	7 (47%)	4 (27%)	3 (16%)	7 (37%)	9 (47%)	7 (21%)	14 (41%)	13 (38%)
Minimum of surgical drain and/or vascular access devices	7 (44%)	7 (44%)	2 (12%)	4 (29%)	5 (36%)	5 (36%)	11 (37%)	12 (40%)	7 (23%)
Anti-Psychotic Medications									
No	44 (24%)	64 (35%)	74 (41%)	25 (22%)	43 (38%)	45 (40%)	69 (23%)	107 (36%)	119 (40%)
Yes	8 (50%)	5 (31%)	3 (19%)	0 (0%)	2 (100%)	0 (0%)	8 (44%)	7 (39%)	3 (17%)
Anti-Anxiety Medications									
No	44 (24%)	64 (35%)	74 (41%)	25 (22%)	43 (38%)	45 (40%)	69 (23%)	107 (36%)	119 (40%)
Yes	8 (50%)	5 (31%)	3 (19%)	0 (0%)	2 (100%)	0 (0%)	8 (44%)	7 (39%)	3 (17%)
Antidepressant Medications									
No	**12 (13%)**	**34 (38%)**	**43 (48%)**	17 (24%)	26 (37%)	27 (39%)	**29 (18%)**	**60 (38%)**	**70 (44%)**
Yes	**40 (37%)**	**35 (32%)**	**34 (31%)**	8 (18%)	19 (42%)	18 (40%)	**48 (31%)**	**54 (35%)**	**52 (34%)**

Note: Note all columns sum to total due to missing values (Detailed missingness: self-report oral health status n=1 for 70-79 and n=1 for 80+; dental visit frequency n=1 for 70-79; ADL n=1 for <70; asthma n=1 for 80+; dementia n=1 for 70-79 and n=1 for 80+; COPD n=1 for 80+; Respiratory failure n=1 for 80+; ILD n=1 for 80+; Other lung diseases n=1 for 80+; antibiotic usage n=2 for <70 and n=1 for 80+) (2) P-value determined through Pearson's Chi-squared test or Fisher's Exact Test for Count data. Bold denotes significant at $p<0.05$

Supplemental Table 2.2 Frequency (n[%]) of visitors and facility movement across five study visits overall. 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023.

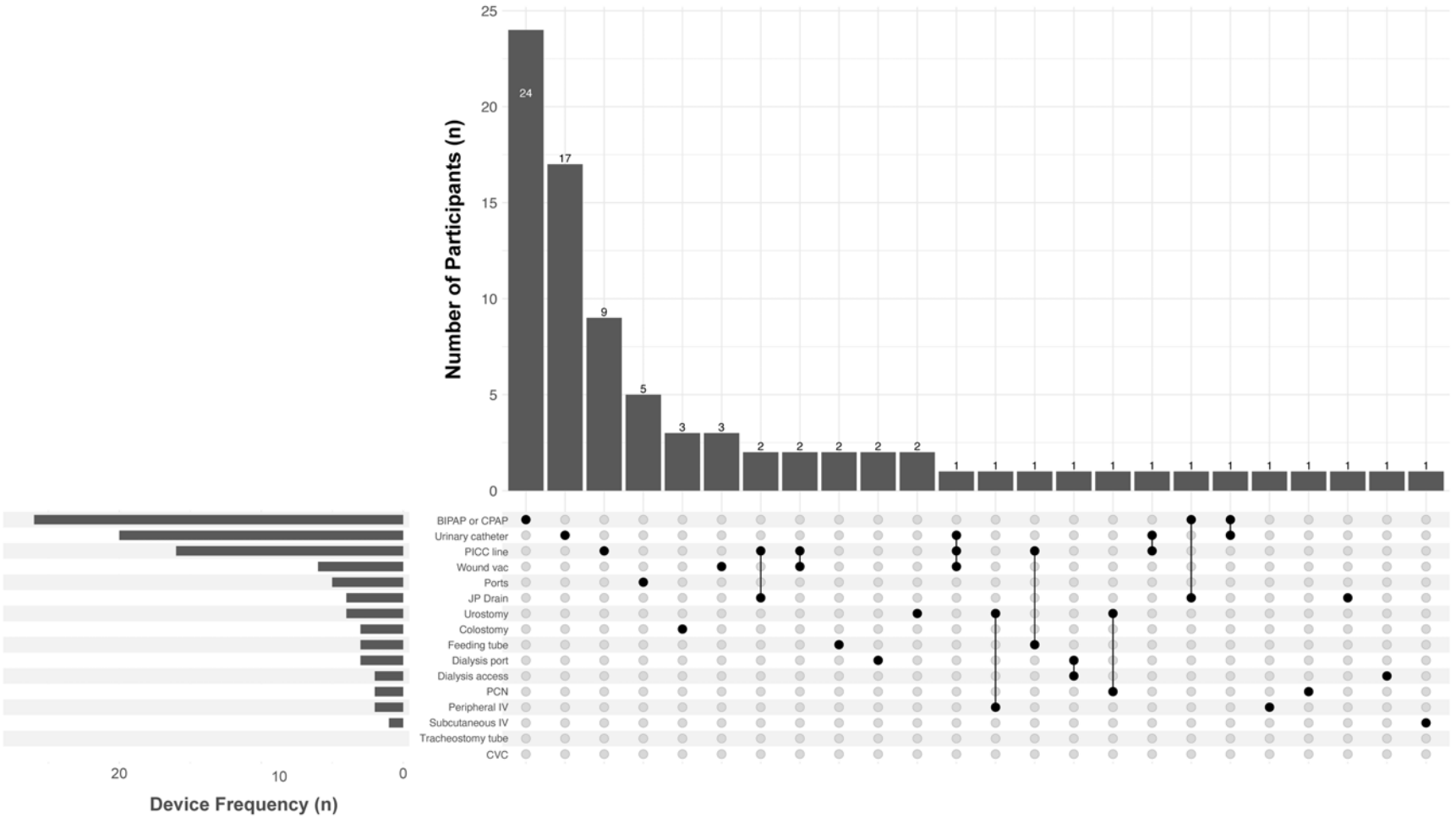
	Study Visit 1	**Study Visit 2**	**Study Visit 3**	**Study Visit 4**	**Study Visit 5**
HAD VISITORS (p = 0.54)					
No	118 (38%)	122 (43%)	100 (42%)	81 (44%)	60 (45%)
Yes	195 (62%)	164 (57%)	136 (58%)	105 (56%)	73 (55%)
UTILIZED DINING ROOM (p = 0.89)					
No	303 (98%)	281 (98%)	232 (98%)	182 (98%)	132 (99%)
Yes	7 (2%)	5 (2%)	4 (2%)	4 (2%)	1 (1%)
UTILIZED LIVING ROOM (p = 0.65)					
No	307 (98%)	276 (97%)	232 (98%)	180 (97%)	131 (98%)
Yes	6 (2%)	10 (3%)	5 (2%)	6 (3%)	2 (2%)
UTILIZED REHAB CENTER (p <0.0001)					
No	222 (71%)	125 (44%)	98 (41%)	84 (45%)	61 (46%)
Yes	89 (29%)	159 (56%)	139 (59%)	101 (55%)	72 (54%)
HAD REHAB IN ROOM (p = 0.12)					
No	156 (70%)	100 (80%)	80 (82%)	66 (80%)	46 (75%)
Yes	66 (30%)	25 (20%)	18 (18%)	17 (20%)	15 (25%)

Supplemental Table 2.3 Frequency [n (%)] of visitors and facility movement across five study visits by gender (women and men). 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023.

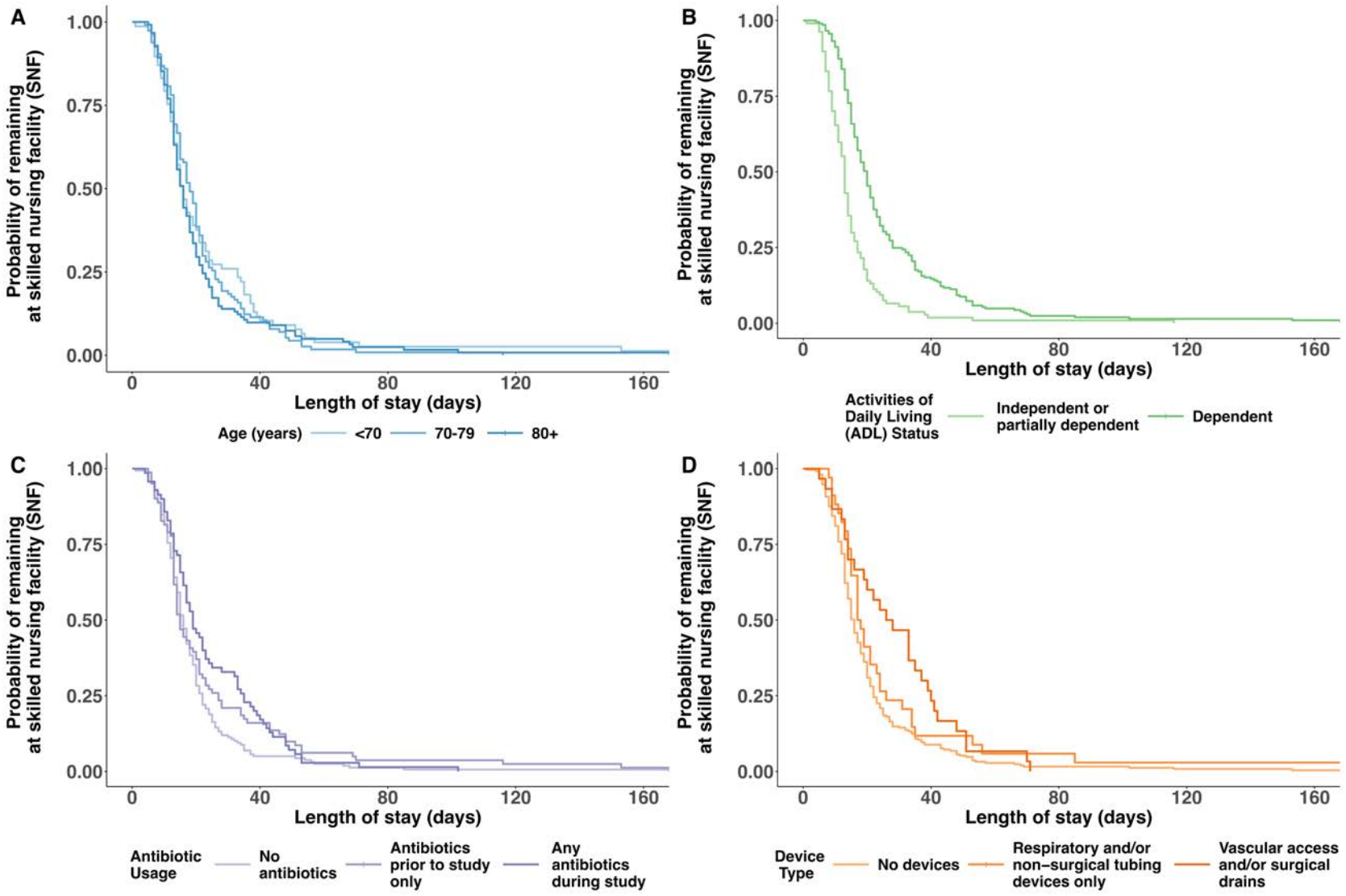
	Study Visit 1		**Study Visit 2**		**Study Visit 3**		**Study Visit 4**		**Study Visit 5**	
	Female	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**
HAD VISITORS (p =0.11)										
No	76 (38%)	42 (37%)	88 (47%)	34 (34%)	72 (46%)	28 (35%)	58 (45%)	23 (41%)	47 (51%)	13 (33%)
Yes	122 (62%)	73 (63%)	98 (53%)	66 (66%)	85 (54%)	51 (65%)	72 (55%)	33 (59%)	46 (49%)	27 (67%)
UTILIZED DINING ROOM (p = 0.91)										
No	192 (98%)	111 (97%)	184 (99%)	97 (97%)	154 (98%)	78 (99%)	127 (98%)	55 (98%)	92 (99%)	40 (100%)
Yes	3 (2%)	4 (3%)	2 (1%)	3 (3%)	3 (2%)	1 (1%)	3 (2%)	1 (2%)	1 (1%)	0 (0%)
UTILIZED LIVING ROOM (p = 0.60)										
No	196 (99%)	111 (97%)	179 (96%)	97 (97%)	154 (97%)	78 (99%)	127 (98%)	53 (95%)	92 (99%)	39 (97%)
Yes	2 (1%)	4 (3%)	7 (4%)	3 (3%)	4 (3%)	1 (1%)	3 (2%)	3 (5%)	1 (1%)	1 (3%)
UTILIZED REHAB CENTER (p <0.0001)										
No	140 (71%)	82 (71%)	82 (44%)	43 (43%)	64 (41%)	34 (43%)	60 (46%)	24 (44%)	42 (45%)	19 (48%)
Yes	56 (29%)	33 (29%)	103 (56%)	56 (55%)	94 (59%)	45 (57%)	70 (54%)	31 (56%)	51 (55%)	21 (52%)
HAD REHAB IN ROOM (p = 0.40)										
No	96 (69%)	60 (73%)	68 (83%)	32 (74%)	51 (80%)	29 (85%)	47 (78%)	19 (83%)	32 (76%)	14 (74%)
Yes	44 (31%)	22 (27%)	14 (17%)	11 (26%)	13 (20%)	5 (15%)	13 (22%)	4 (17%)	10 (24%)	5 (26%)

Supplemental Table 2.4 Frequency [n (%)] of visitors and facility movement across five study visits by age category (<70, 70–79, and 80+ years). 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023

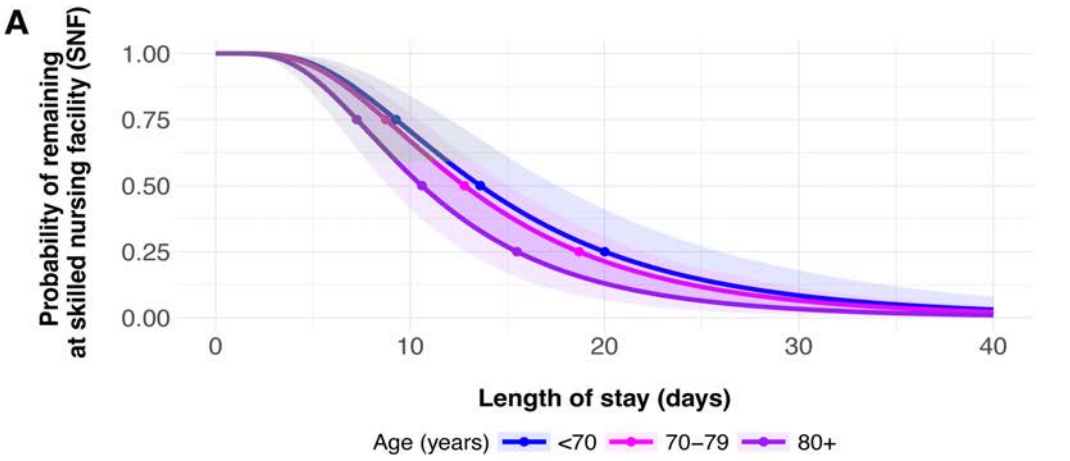
	Study Visit 1			Study Visit 2			Study Visit 3			Study Visit 4			Study Visit 5		
	<70	**70-79**	**80+**	**<70**	**70-79**	**80+**	**<70**	**70-79**	**80+**	**<70**	**70-79**	**80+**	**<70**	**70-79**	**80+**
HAD VISITORS (p = 0.062)															
No	39 (51%)	41 (36%)	38 (31%)	35 (49%)	46 (45%)	41 (37%)	30 (57%)	36 (41%)	34 (35%)	23 (50%)	31 (44%)	27 (39%)	16 (52%)	25 (46%)	19 (40%)
Yes	38 (49%)	73 (64%)	84 (69%)	36 (51%)	57 (55%)	71 (63%)	23 (43%)	51 (59%)	62 (67%)	23 (50%)	39 (56%)	43 (61%)	15 (48%)	29 (54%)	29 (60%)
UTILIZED DINING ROOM (p = 0.85)															
No	73 (97%)	112 (98%)	118 (98%)	68 (96%)	103 (100%)	110 (98%)	52 (98%)	86 (99%)	94 (98%)	45 (98%)	69 (99%)	68 (97%)	30 (97%)	54 (100%)	48 (100%)
Yes	2 (3%)	2 (2%)	3 (3%)	3 (4%)	0 (0%)	2 (2%)	1 (2%)	1 (1%)	2 (2%)	1 (2%)	1 (1%)	2 (3%)	1 (3%)	0 (0%)	0 (0%)
UTILIZED LIVING ROOM (p = 0.61)															
No	74 (96%)	112 (98%)	121 (99%)	70 (99%)	99 (96%)	107 (96%)	51 (96%)	86 (99%)	95 (99%)	43 (94%)	69 (99%)	68 (97%)	31 (100%)	52 (96%)	48 (100%)
Yes	3 (4%)	2 (2%)	1 (1%)	1 (1%)	4 (4%)	5 (5%)	2 (4%)	2 (2%)	1 (1%)	3 (7%)	1 (1%)	2 (3%)	0 (0%)	2 (4%)	0 (0%)
UTILIZED REHAB CENTER (p < 0.0001)															
No	60 (78%)	83 (74%)	79 (65%)	32 (45%)	46 (45%)	47 (42%)	15 (28%)	40 (46%)	43 (45%)	21 (46%)	33 (48%)	30 (43%)	14 (45%)	21 (39%)	26 (54%)
Yes	17 (22%)	30 (27%)	42 (35%)	39 (55%)	56 (55%)	64 (58%)	38 (72%)	48 (55%)	53 (55%)	25 (54%)	36 (52%)	40 (57%)	17 (55%)	33 (61%)	22 (46%)
HAD REHAB IN ROOM (p = 0.71)															
No	38 (63%)	60 (72%)	58 (73%)	27 (84%)	36 (78%)	37 (79%)	12 (80%)	34 (85%)	34 (79%)	17 (81%)	26 (81%)	23 (77%)	10 (71%)	15 (71%)	21 (81%)
Yes	22 (37%)	23 (28%)	21 (27%)	5 (16%)	10 (22%)	10 (21%)	3 (20%)	6 (15%)	9 (21%)	4 (19%)	6 (19%)	7 (23%)	4 (29%)	6 (29%)	5 (19%)



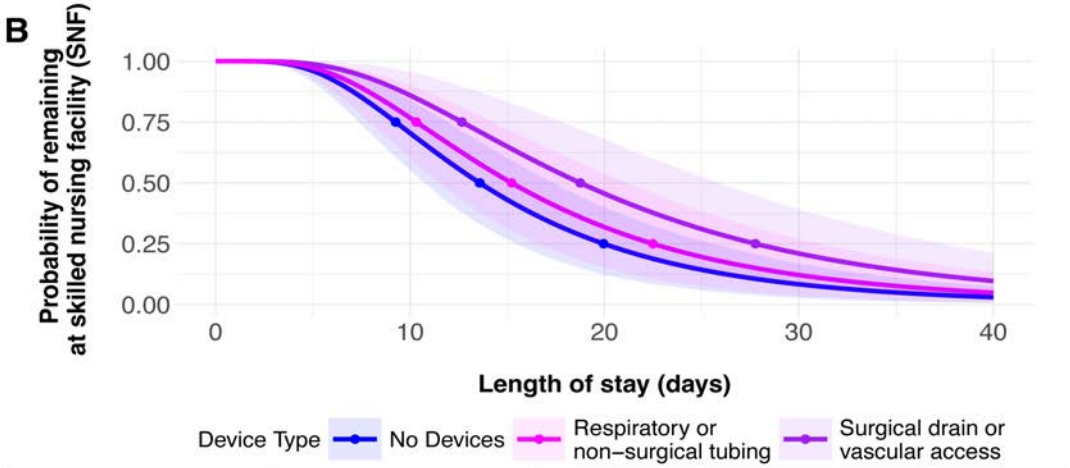
Supplemental Figure 2.3 Upset plot reporting the frequency of different device combinations at enrollment collected from 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. Upset plot prepared using the "ComplexUpset" package in R (version 1.3.3)[29,30].



Supplemental Figure 2.4 Kaplan Meier curves reporting length of stay at skilled nursing facility by covariates (A) age, (B), activities of daily living [ADL], (C) antibiotic usage, (D), device type, from 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023.

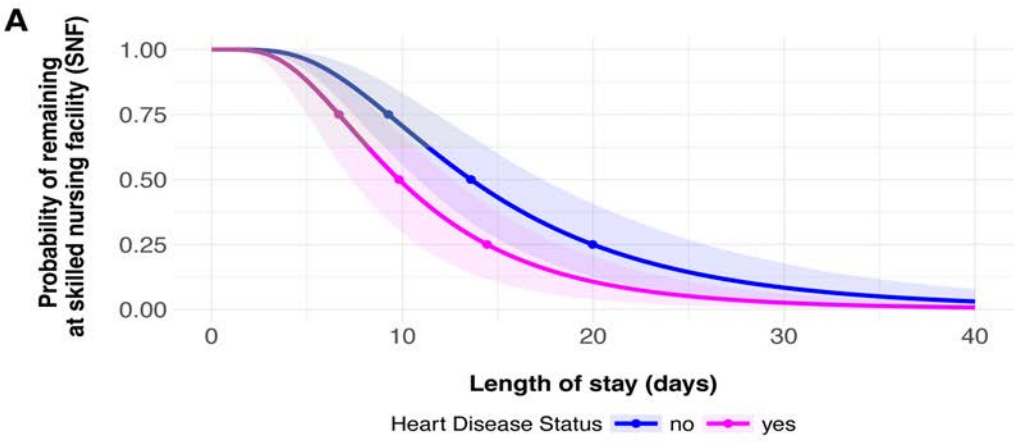


Comparison	Absolute Difference at 25th percentile	Absolute Difference at 50th percentile	Absolute Difference at 75th percentile
70–79 versus 80+	3.2	2.2	1.5
70–79 versus <70	1.3	0.8	0.5
80+ versus <70	4.5	3.0	2.0

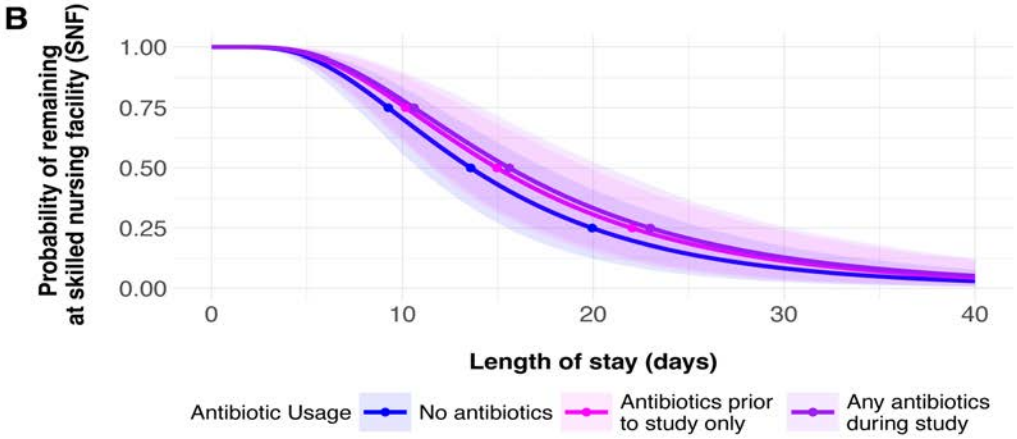


Comparison	Absolute Difference at 25th percentile	Absolute Difference at 50th percentile	Absolute Difference at 75th percentile
Surgical drain or vascular versus No devices	7.8	5.2	3.4
Surgical drain or vascular access versus Respiratory or non–surgical tubing	5.3	3.5	2.3
No devices versus Respiratory or non-surgical tubing	2.5	1.6	1.1

Supplemental Figure 2.5 Cumulative survival curves estimating mean length of stay at skilled nursing facility (SNF) from 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. Tables calculate absolute difference in mean length of stay at 75th, 50th, and 25th percentiles (shown with dots) by covariates [(A) age category, (B) device type] included in the lognormal survival model. For each covariate survival curve, all other variables are held constant in the model at the reference: less than 70 years of age, having no devices, not having heart disease, not taking any antibiotics, and being independent or partially dependent for ADL. Confidence intervals were calculated using non-parametric bootstrapping with 1,000 iterations.



Comparison	Absolute Difference at 25th percentile	Absolute Difference at 50th percentile	Absolute Difference at 75th percentile
No versus Yes	5.5	3.8	2.6



Comparison	Absolute Difference at 25th percentile	Absolute Difference at 50th percentile	Absolute Difference at 75th percentile
No antibiotics versus Prior antibiotics only	2.1	1.4	0.9
No antibiotics versus Any antibiotics during study	3.0	2.0	1.3
Prior antibiotics only versus Any antibiotics during study	1.0	0.6	0.4

Supplemental Figure 2.6 Cumulative survival curves estimating mean length of stay at skilled nursing facility (SNF) from 313 participants from the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. Tables calculate absolute difference in mean length of stay at 75th, 50th, and 25th percentiles (shown with dots) by covariates [(A) heart disease, (B) antibiotics usage] included in the lognormal survival model. For each covariate survival curve, all other variables are held constant in the model at the reference: less than 70 years of age, having no devices, not having heart disease, not taking any antibiotics, and being independent or partially dependent for ADL. Confidence intervals were calculated using non-parametric bootstrapping with 1,000 iterations.

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Chapter 3 Oral Health and Frailty Among Oder Adults: A Prospective Cohort Study of Skilled Nursing Facility Residents

This manuscript is intended for publication and currently is under review.

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3.1 Abstract

Objectives: Frailty and poor oral health (assessed via dental examination) are independently and jointly associated with each other and adverse health outcomes. Whether this association is detectable when using patient self-assessment of oral health - a more feasible method where access to dentists is limited - is unclear. We describe associations between self-assessed oral health and frailty among skilled nursing facility (SNF) residents and identify factors that modify this association.

Methods: We analyzed data from 309 SNF residents enrolled in the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study between April 5, 2021 and May 22, 2023. Participants self-assessed oral health and frailty (using validated Frailty Short Interview); demographics and health history were collected via medical record review. We fit multivariable logistic regression models to describe associations between self-assessed oral health and frailty, and modifications by age, chronic respiratory disease, activities of daily living (ADL), and antidepressant usage.

Results: Individuals with poor self-assessed oral health had 2.45 times the odds of frailty compared to those with good oral health (CI 95% CI: 1.42 – 4.29); being less than 70 years old,

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having chronic respiratory disease, being ADL dependent, or using antidepressants were also independently associated with increased odds of frailty. Age, chronic respiratory disease, ADL status, and antidepressant usage were not significant modifiers of the association between oral health and frailty.

Conclusion: Self-assessed oral health is strongly associated with frailty among older adults. Routine self-assessed oral health within care facilities could serve as a simple, non-invasive tool for identifying individuals at risk for dental disease, frailty, and other poor health outcomes, potentially improving the quality of life for older adults in care facilities.

3.2 Introduction

Frailty, defined as aging-related declines in strength and physical function, is strongly associated with poor oral health. Individually and jointly, frailty and poor oral health are associated with adverse health outcomes, including cardiovascular disease and increased risk of infection.[1] While 4 to 16% of older adults who live independently (i.e., are community dwelling) are estimated to be frail, over 50% of residents of care facilities are frail.[2] Frailty significantly increases the risk of morbidity and mortality.[2] Similarly, poor oral health is widespread among older adults: 96% of adults aged 65 and older have experienced cavities, 68% have periodontal disease, 20% have untreated tooth decay, and nearly one in five have lost all their teeth.[3] In addition to adversely affecting quality of life,[4] poor oral health is associated with chronic conditions including cardiovascular disease and diabetes, and is exacerbated by side effects of medications, such as dry mouth and reduced salivary flow.[3] As the population aged 65 and older is projected to grow from 35 million to 73 million by 2030, addressing frailty and poor oral health is a critical public health priority.[5]

Most studies of the association between frailty and oral health assess oral health via dental examination: measurements of number of decayed, missing and filled teeth, denture usage, or periodontal status. Hakeem *et al*. described the relationship between periodontitis and frailty among 2,368 community-dwelling adults aged 60 and older from the 2011–2014 cycles of National Health and Nutrition Examination Survey (NHANES).[6] In this population-based sample, people with moderate-to-severe periodontitis had an 8% higher relative risk (RR: 1.08, 95% CI: 1.02 – 1.14) of frailty compared to those who had no periodontitis, after adjusting for age, gender, and poor nutritional intake.[6] Having good dental health decreased frailty risk in a cross-sectional study of 365 nursing home residents aged 65 years and older participating in the

Chinese Longitudinal Healthy Longevity Survey (CLHLS): each additional tooth present was associated with a reduction in the odds of frailty by 6% (OR: 0.94, 95% CI: 0.91 – 0.98), and regular tooth brushing was associated with a 63% lower odds of frailty (OR: 0.37, 95% CI: 0.13 – 0.99) compared to those who never brush their teeth, after adjusting for sex, age group, years of education, drinking, false teeth, visual impairment, hearing loss, cognitive impairments, sufficient financial support, and exercise.[7]

Conducting dental examinations can be challenging and overly invasive for ill or recovering individuals and often are unfeasible to conduct in short-term care facilities. Therefore, a non-invasive method is desirable. A study of 3,630 community-dwelling adults from NHANES cycles 2011–2014 found that the self-rated oral health question, "Rate the health of your teeth and gums," was a good predictor of frailty after adjusting for age, gender, ethnicity, poverty-income ratio, education, poor nutritional intake, and smoking.[8] There was a dose response relationship: the relative rate of frailty increased as self-reported oral health decreased from very good, good, fair to poor (compared to excellent) (RRs: 1.03, 95% CI: 0.95 – 1.13; 1.15, 95% CI: 1.05 – 1.25; 1.30, 95% CI: 1.17 – 1.45; 1.44, 95% CI: 1.28 – 1.54, respectively).[8] However, no studies to date have explored self-reported oral health and frailty in either short- or long-term care facilities, leaving a critical gap in understanding this relationship in populations with higher frailty levels.

Our study addresses these gaps by examining the association between self-assessed oral health and frailty and identifying potential modifiers of this association among 309 residents from a single skilled nursing facility (SNF) in southeast Michigan. Identifying a reliable, easy-to-collect oral health measure that staff can obtain at facility entry could inform targeted interventions to improve oral health, potentially mitigating risk of frailty, and enhancing overall health outcomes in vulnerable populations.

3.3 Methods

3.3.1 Study population

We analyzed data from 309 participants in the Tracking Acute Respiratory Illness in Older Adults (TARI) study between April 5, 2021, through May 22, 2023. TARI is a longitudinal, prospective cohort study that enrolled residents recovering a single SNF in southeast Michigan. Participants were recruited shortly after admittance to the SNF. After

enrollment, participants were visited approximately every three days for up to five study visits over a 12 to15-day period. Residents were eligible for inclusion if they were 18 years or older, English-speaking, and not scheduled for immediate discharge. Residents were ineligible if they had devices blocking access to their nose and mouth, were not oriented and alert, or were not able to self-consent. Participants who did not have complete data on self-reported oral health, frailty, or any other covariate included in the final model were excluded from analysis (n = 4), resulting in a final sample of 309 participants. The study protocol was approved by the Institutional Review Board at the University of Michigan.

3.3.2 Data collection

3.3.2.1 Self-assessed oral health and frailty measures

At enrollment, after informed consent was obtained, research personnel administered a questionnaire to participants that included measures of respiratory symptoms, oral health, and frailty status. Participants self-assessed oral health as "very poor", "poor", "fair", "good", or "very good" in response to the question, "How is your current dental health (that is of your mouth, teeth and/or dentures)?". For our primary analysis, we collapsed responses into two categories: poor oral health (combining "very poor", "poor", and "fair" responses) and good oral health (combining "good" and "very good" responses). We also examined a dose response by evaluating each of the five responses separately. Frailty was assessed using a validated Frailty Short Interview that consists of five questions related to weight loss, exhaustion, weakness, difficulty walking, and physical activity. The average score, ranging from zero to one, was calculated based on the number of questions answered, excluding any missing responses. For analysis, scores were dichotomized with < 0.6 considered "non-frail" and ≥ 0.6 considered to be "frail", based on prior guidelines.[9]

3.3.2.2 Other oral health measures collected at enrollment

We also captured information on oral health according to alternative metrics. We evaluated self-reported tooth/gum pain ("During the past 3 months, how much pain or distress have your teeth or gums caused you?"), tooth/gum worry ("During the past 3 months, how much have your teeth or gums worried or concerned you?"), and concerns regarding the appearance of their teeth ("During the past 3 months, how much of the time have problems with the way your teeth look caused you to avoid conversations with people?").[10] Potential responses to these

questions included “a lot of the time”, “some of the time”, “a little of the time”, or “none of the time”. For analysis, we collapsed these responses into a binary metric for “any of the time” or “none of the time”.

3.3.2.3 Covariate measures

We conducted a detailed medical record review and used the latest Minimum Data Set (MDS; a standardized assessment tool that collects information about a nursing home resident's health) to collect participant demographics and health behaviors, at study discharge. We abstracted information on comorbidities, tobacco usage, infection history, and medication history (e.g., antidepressant use). Age was calculated using study enrollment date and date of birth and categorized as < 70, 70–79, and 80+ years old. Activities of Daily Living (ADL), bathing, dressing, toileting, transferring, continence, and feeding, were measured using the Katz Index based on patient assessments, progress notes, and MDS. Scores were summed across the six categories and the index classified into “independent” ($\geq$ 6), “partially dependent” (> 2 - < 6), and “dependent” ($\leq$ 2).[11] We collected information on asthma and chronic obstructive pulmonary disease (COPD) from the medical record review. As medications for controlling asthma and managing symptoms of COPD are often similar and contribute to dry mouth, we anticipated the impact to oral health would be consistent; therefore, we collapsed both conditions into a single chronic respiratory disease category for analysis.[12]

3.3.3 Statistical analysis

We used a Cochran-Armitage test for trend to evaluate if frailty varied linearly across the five self-assessed oral health categories overall and when stratified by gender. We described associations between each covariate and frailty or oral health status and used Pearson’s Chi-squared test or Fisher’s Exact Test for Count data (Fisher’s when cell frequency is <5) to assess statistical significance ($p<0.05$). To estimate odds ratios (OR) and 95% confidence intervals (CIs), we used logistic regression, regressing our binary indicator for frailty against our primary, binary indicator for self-reported oral health, as well as other covariates. We chose covariates that may potentially confound the relationship between oral health and frailty based on prior reports of association with both oral health and frailty or observation of statistically significant univariate associations in our study population. For example, inhaled medications commonly used to manage asthma or COPD can reduce salivary flow and alter salivary pH, increasing the

risk of dental caries, erosion, periodontitis, and candidiasis, and those with asthma have two times higher risk of becoming frail compared to those without asthma.[13,14] We assessed possible interactions for gender, age, and smoking status with comorbid conditions; none of these interactions were statistically significant and were dropped from the model. Each covariate included in the model was also evaluated as a potential effect modifier in the relationship between oral health and frailty by including an interaction term between the covariate and self-assessed oral health.

To examine how the relationship between self-assessed oral health and frailty varies by each level of a covariate, we created predicted probability plots, which show how the predicted probability of frailty changes with self-assessed oral health across levels of a single covariate, while keeping all other variables constant at their reference value. This approach allowed us to visually isolate and understand the modifying effect of each covariate on the association between self-assessed oral health and frailty. 95% confidence intervals were calculated using non-parametric bootstrapping with 1,000 iterations. All analyses were conducted using RStudio Version 4.4.2.

3.4 Results

3.4.1 Study population characteristics

Participants averaged 77 years of age (min: 37.8, max: 100.8), 63.8% were women, and 88.7% were White. Poor oral health was reported by 35% of participants and 51.8% were classified as frail. There was a significant increase in the percent frail as oral health declined: very good (35.3% frail); good (44.9%); fair (65.9%); poor (73.7%); very poor (100% frail) (Cochran-Armitage test for trend $p<0.001$); the same trend was observed when stratified by gender (women: $p<0.001$; men: $p=0.036$). Those with poor oral health (mean: 74 years, SD: 9.8 years) were younger than their counterparts with good oral health (mean: 78.6 years, SD: 9.9 years; $p=0.0001$) (Table 3.1). Prevalence of poor oral health was significantly higher among participants who currently smoke ($p=0.01$), who never went to the dentist or only went to a dentist if there was a problem ($p<0.0001$), or who had chronic respiratory disease ($p=0.001$) or diabetes ($p=0.01$) (Table 1). Those who were frail (mean: 74.9 years, SD: 9.7 years) were younger than their counterparts who were non-frail (mean: 79.2 years, SD: 10.0 years; $p<0.0001$). Prevalence of frailty was significantly higher among participants with chronic

respiratory disease (p=0.001), other lung diseases (p=0.01), and those taking antidepressants (p<0.0001). Prevalence of poor oral health was significantly greater among those with any tooth/gum pain (p<0.0001) and tooth/gum worry (p<0.0001), but not significantly associated with tooth appearance/conversational avoidance.

3.4.2 Association between oral health and frailty

The odds of frailty among those with poor self-assessed oral health were 2.45 times the odds of frailty among those with good self-assessed oral health (95% CI: 1.42 – 4.29) after adjusting for age, chronic respiratory disease, activities of daily living, and antidepressant usage. The covariates in the model were also significantly associated with increased the odds of frailty after adjustment for each other and poor oral health (Table 3.2). In another analysis using all categories (rather than the dichotomized measure) of self-assessed oral health, we observed a dose-response relationship: as oral health declined, the odds of frailty increased (Good Oral Health OR: 1.73, 95% CI: 0.74 – 4.21; Fair Oral Health OR: 3.63, 95% CI: 1.44 – 9.51; Poor/Very Poor Oral Health OR: 5.74, 95% CI: 1.51 – 24.93) after adjusting for age, chronic respiratory disease, activities of daily living, and antidepressant usage (Table 3.3). The odds of frailty remained significant for tooth/gum pain (OR: 3.68, 95% CI: 1.69 – 8.63) and tooth/gum worry (OR: 3.95, 95% CI: 1.82 – 9.27) after adjusting for the same covariates. These findings suggest that poor oral health is a significant risk factor for frailty, highlighting the importance of oral health in overall well-being.

3.4.3 Effect modification of the relationship between oral health and frailty

There were no statistically significant variables that modified the association between self-assessed oral health and frailty. However, the association between poor oral health and frailty was of higher magnitude among younger age groups (<70 years and 70-79 years) compared to older age groups (Figure 3.1A), even though poor oral health was consistently associated with higher odds of frailty among all age groups. The association between poor oral health and frailty was also of greater magnitude among participants with chronic respiratory disease (Figure 3.1B). Oral health was not associated with frailty among those who were independent in ADL (Figure 3.1C) but was associated with frailty among participants with some degree of dependence in ADL. While antidepressant usage was associated with higher predicted

probability of frailty as a main effect, it did not alter the association between oral health and frailty (Figure 3.1D).

3.5 Discussion

In this analysis of 309 residents of a single SNF, the odds of frailty among those with poor self-assessed oral health was 2.45 times the odds of frailty among those with good self-assessed oral health (95% CI: 1.42 – 4.29) after adjusting for age, chronic respiratory disease, activities of daily living, and antidepressant usage. Additionally, being less than 70 years old, having chronic respiratory disease, being ADL dependent, or using antidepressants also increased the odds of frailty after adjusting for poor oral health. Further, the odds of frailty increased as self-assessed oral health declined. While an association between oral health and frailty is well known, to our knowledge, our study is the first to evaluate the association using self-assessed oral health among SNF residents. Thus, our study addresses an important gap in understanding the association between oral health and frailty in populations where frailty levels are high and dental examinations are challenging. The associations we found using self-assessed oral health metrics mirrored those found using metrics collected by a dentist. Additionally, overall self-assessed oral health was correlated with other self-reported measures for tooth or gum pain, worry, and appearance or conversational avoidance. However, it is important to note that very few (n=5) participants reported any concerns regarding appearance or conversational avoidance. Our additional analysis revealed tooth/gum pain and tooth/gum worry predicted a similar magnitude of odds of frailty compared to the primary analysis, suggesting that overall self-assessed oral health is a perception-based measure.

The consistent findings between self-assessed measures and dental assessments aligns with results from an analysis of 2368 adult participants (aged 18 to 64) in the RAND Health Insurance Experiment. In the RAND study, pain was reported by 24% of individuals with 0-12 decayed missing or filled teeth compared to 31% of those with 23-28 to decayed, missing or filled teeth and pain and worry were reported more frequently than concerns about appearance or conversational avoidance, consistent with our findings.[10] These findings suggest that even if a dentist rates a person’s oral health as poor based on objective measures, an individual with filled cavities, intact teeth, and minimal pain may still perceive their oral health as fair or good. This highlights the value of using a self-assessed oral health measure as a complement to clinical evaluation, in that it provides insight into an individual's perception of their own oral health.

Incorporating a single self-assessed oral health question into questionnaires offers a simple, non-invasive tool to better understand SNF residents' wellbeing and overall health. This is particularly valuable in care facilities where access to routine dental care and evaluations may be limited.[15] By leveraging self-assessment measures, either overall self-assessed oral health, tooth/gum pain, or tooth/gum worry, healthcare providers may gain an additional perspective on a resident's health status to consider in case management.

Our focus on SNFs fills an important gap in the literature, as most studies evaluating the association between oral health and frailty have been conducted among community-dwelling adults.[6,8] SNF residents are recovering from surgery, injury or illness in addition to having a higher prevalence of underlying comorbid conditions, use of medications that influence oral health, and frailty than community-dwelling adults.[12,16] We identified age, asthma or COPD, and antidepressant use as potential modifiers of the oral health - frailty relationship, although not at a statistically significant level. Nevertheless, as medications used to treat asthma, COPD and depression can cause dry mouth that is associated with increased risk of oral disease we believe that oral health care among ill individuals with these factors is especially important.[12,17]

Our study has several limitations. TARI participants were enrolled from a single, non-profit SNF located in Southeast Michigan whose residents are predominately White. While the study participant distribution by gender and age is consistent with nationwide long-term care facilities, our findings may not be generalizable to all SNF populations.[18] Despite these limitations, our study has several strengths. Frailty was assessed using the validated Frailty Short Interview[9] and detailed covariate information was collected, including sociodemographics, health behaviors, self-assessed oral health measures, and health history using MDS. Importantly, this study fills a critical gap in knowledge by evaluating the association between oral health and frailty among a population of care facility residents with multiple other risk factors for frailty and demonstrates consistent associations between oral health and frailty even when using self-assessed measures of oral health.

3.6 Conclusion

This first report on the association between self-assessed oral and frailty among SNF residents highlights the potential utility of a simple, easily obtainable measure in case management within these facilities, and by the dentists working with short- and long-term care populations. Incorporating self-assessed oral health into patient intake forms could provide

valuable opportunities to identify early signs of frailty risk and implement interventions to potentially slow functional decline. Prospective studies are needed to establish the temporal understanding of whether oral health precedes frailty or vice versa as plausible mechanisms support both directions of association. As the mouth serves as a window to overall health, improving oral health among those with poor self-assessed oral health could play a significant role in enhancing care and improving the quality of life for our aging population.

3.7 Acknowledgements

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3.8 Tables and Figures

Table 3.1 Frequency (n[%]) of demographics, health behaviors, oral history, health history, and medication usage by self-assessed oral health and frailty status among 309 residents at a single skilled nursing facility (SNF) participating in the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023.

CHARACTERISTICS*	**Good Oral Health n (%)**	**Poor Oral Health n (%)**	***P*****	**Non-Frail n (%)**	**Frail n (%)**	***P*****
SEX						
Female	130 (66.0%)	67 (34.0%)	0.6	88 (44.7%)	109 (55.3%)	0.1
Male	71 (63.4%)	41 (36.6%)		61 (54.5%)	51 (45.5%)	
AGE CATEGORY						
<70	34 (44.7%)	42 (55.3%)		25 (32.9%)	51 (67.1%)	
70-79	77 (68.1%)	36 (31.9%)	**0.0001**	46 (40.7%)	67 (59.3%)	**<0.0001**
80+	90 (75.0%)	30 (25.0%)		78 (65.0%)	42 (35.0%)	
RACE/ETHNICITY						
American Indian/ Alaskan Native	0 (NA)	0 (NA)		0 (NA)	0 (NA)	
Asian	3 (50.0%)	3 (50.0%)		4 (66.7%)	2 (33.3%)	
Black	12 (50.0%)	12 (50.0%)	0.2	8 (33.3%)	16 (66.7%)	0.4
Hispanic	1 (100.0%)	0 (0%)		1 (100.0%)	0 (0%)	
Mixed	4 (100.0%)	0 (0%)		2 (50.0%)	2 (50.0%)	
White	181 (66.1%)	93 (33.9%)		134 (48.9%)	140 (51.1%)	
BODY MASS INDEX (BMI)						
Underweight or healthy	69 (64.5%)	38 (35.5%)		59 (55.1%)	48 (44.9%)	
Overweight	58 (68.2%)	27 (31.8%)	0.8	40 (47.1%)	45 (52.9%)	0.2
Obese	74 (63.2%)	43 (36.8%)		50 (42.7%)	67 (57.3%)	
SMOKING STATUS						
Never	149 (68.7%)	68 (31.3%)		106 (48.8%)	111 (51.2%)	
Former	50 (60.2%)	33 (39.8%)	**0.01**	40 (48.2%)	43 (51.8%)	0.7
Current	2 (22.2%)	7 (77.8%)		3 (33.3%)	6 (66.7%)	

CHARACTERISTICS*	Good Oral Health n (%)	Poor Oral Health n (%)	*P***	Non-Frail n (%)	Frail n (%)	*P***
DENTAL VISIT FREQUENCY						
Regular check-up	165 (75.3%)	54 (24.7%)		114 (52.1%)	105 (47.9%)	
Occasional check-up	15 (44.1%)	19 (55.9%)	**<0.0001**	15 (44.1%)	19 (55.9%)	0.1
Only if problems	20 (37.0%)	34 (63.0%)		20 (37.0%)	34 (63.0%)	
Never been to the dentist	0 (0%)	1 (100.0%)		0 (0%)	1 (100.0%)	
TOOTH PAIN						
Any of the time	17 (36.2%)	30 (63.8%)	**<0.0001**	10 (21.3%)	37 (78.7%)	**0.0001**
None of the time	183 (70.1%)	78 (29.9%)		138 (52.9%)	123 (47.1%)	
TOOTH WORRY						
Any of the time	18 (36.0%)	32 (64.0%)	**<0.0001**	10 (20.0%)	40 (80.0%)	**<0.0001**
None of the time	183 (70.7%)	76 (29.3%)		139 (53.7%)	120 (46.3%)	
TOOTH APPEARANCE						
Any of the time	1 (20.0%)	4 (80.0%)	0.1	1 (20.0%)	4 (80.0%)	0.4
None of the time	200 (65.8%)	104 (34.2%)		148 (48.7%)	156 (51.3%)	
SELF-ASSESSED ORAL HEALTH						
Good	·	·	·	114 (56.7%)	87 (43.3%)	**<0.0001**
Poor	·	·		35 (32.4%)	73 (67.6%)	
FRAILTY AT BASELINE						
Non-Frail	114 (76.5%)	35 (23.5%)	**<0.0001**	·	·	·
Frail	87 (54.4%)	73 (45.6%)		·	·	
ACTIVITIES OF DAILY LIVING (ADL)						
Independent	11 (57.9%)	8 (42.1%)		14 (73.7%)	5 (26.3%)	
Partially dependent	54 (61.4%)	34 (38.6%)	0.5	44 (50.0%)	44 (50.0%)	0.1
Dependent	136 (67.3%)	66 (32.7%)		91 (45.0%)	111 (55.0%)	
CHRONIC RESPIRATORY DISEASE						
No	156 (70.9%)	64 (29.1%)	**0.001**	119 (54.1%)	101 (45.9%)	**0.001**
Yes	45 (50.6%)	44 (49.4%)		30 (33.7%)	59 (66.3%)	
HEART DISEASE						
No	85 (67.5%)	41 (32.5%)	0.5	60 (47.6%)	66 (52.4%)	0.9
Yes	116 (63.4%)	67 (36.6%)		89 (48.6%)	94 (51.4%)	

CHARACTERISTICS*	Good Oral Health n (%)	Poor Oral Health n (%)	*P***	Non-Frail n (%)	Frail n (%)	*P***
DEMENTIA						
No	192 (64.9%)	104 (35.1%)	0.3	143 (48.3%)	153 (51.7%)	0.7
Yes	9 (81.8%)	2 (18.2%)		6 (54.5%)	5 (45.5%)	
DIABETES						
No	139 (70.2%)	59 (29.8%)	**0.01**	101 (51.0%)	97 (49.0%)	0.2
Yes	62 (55.9%)	49 (44.1%)		48 (43.2%)	63 (56.8%)	
MEDICAL DEVICES						
No devices	160 (65.0%)	86 (35.0%)		115 (46.7%)	131 (53.3%)	
Respiratory and/or non-surgical tubing only	22 (66.7%)	11 (33.3%)	1.0	18 (54.5%)	15 (45.5%)	0.6
Vascular access device and/or surgical drain	19 (63.3%)	11 (36.7%)		16 (53.3%)	14 (46.7%)	
ANTIPSYCHOTIC MEDICATIONS						
No	186 (63.9%)	105 (36.1%)	0.1	143 (49.1%)	148 (50.9%)	0.2
Yes	15 (83.3%)	3 (16.7%)		6 (33.3%)	12 (66.7%)	
ANTI-ANXIETY MEDICATIONS						
No	186 (63.9%)	105 (36.1%)	0.1	143 (49.1%)	148 (50.9%)	0.2
Yes	15 (83.3%)	3 (16.7%)		6 (33.3%)	12 (66.7%)	
ANTIDEPRESSANT MEDICATIONS						
No	103 (65.2%)	55 (34.8%)	1.0	97 (61.4%)	61 (38.6%)	**<0.0001**
Yes	98 (64.9%)	53 (35.1%)		52 (34.4%)	99 (65.6%)	

*Not all characteristics sum to total (n=309) due to missing values (Detailed missingness: dental visits n=1; tooth pain n=1; dementia n=2) .

**P-value determined through Pearson's Chi-squared test or Fisher's Exact Test for Count data. Bold p-value denotes significance at $p<0.05$.

Table 3.2 Results of multivariable logistic regression model showing odds ratios between self-assessed oral health and frailty status obtained through Frailty Short Interview among 309 residents at a single skilled nursing facility (SNF) participating in the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023.

MODEL COMPONENTS	OR	95% CI*	*P**
SELF-ASSESSED ORAL HEALTH			
Good	REF		
Poor	**2.45**	**(1.42, 4.29)**	**0.001**
AGE			
<70 years old	REF		
70-79 years old	0.86	(0.43, 1.7)	0.7
80+ years old	**0.33**	**(0.17, 0.66)**	**0.002**
CHRONIC RESPIRATORY DISEASE			
No	REF		
Yes	**2.02**	**(1.14, 3.64)**	**0.02**
ACTIVITIES OF DAILY LIVING (ADL)			
Independent	REF		
Partially dependent	3.29	(0.97, 12.85)	0.07
Dependent	**4.78**	**(1.47, 18.04)**	**0.01**
ANTIDEPRESSANT USAGE			
No	REF		
Yes	**2.79**	**(1.68, 4.69)**	**<0.001**

*Bold odds ratio (OR), 95% confidence interval (CI) and p-value denotes significance at $p<0.05$.

Table 3.3 Additional analyses using univariate and multivariable logistic regression model showing odds ratios between three independent oral health measures (non-collapsed self-assessed oral health, tooth/gum pain, and tooth/gum worry) and frailty status obtained through Frailty Short Interview among 309 residents at a single skilled nursing facility (SNF) participating in the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023.

	Univariate Model			**Adjusted Model**[***]		
MODEL COMPONENTS	**OR**	**95% CI**[**]	***P***[**]	**OR**	**95% CI**[**]	***P***[**]
Primary Model[*]: Poor	**2.73**	**(1.68, 4.50)**	**<0.001**	**2.45**	**(1.42, 4.29)**	**0.001**
SELF-ASSESSED ORAL HEALTH						
Very Good	REF					
Good	1.49	(0.70, 3.30)	0.3	1.73	(0.74, 4.21)	0.2
Fair	**3.54**	**(1.57, 8.34)**	**0.003**	**3.63**	**(1.44, 9.51)**	**0.007**
Poor - Very Poor	**5.50**	**(1.69, 20.51)**	**0.007**	**5.74**	**(1.51, 24.93)**	**0.014**
TOOTH/GUM PAIN						
None of the time	REF					
Any of the time	**4.15**	**(2.05, 9.14)**	**<0.001**	**3.68**	**(1.69, 8.63)**	**0.002**
TOOTH/GUM WORRY						
None of the time	REF					
Any of the time	**4.63**	**(2.30, 10.16)**	**<0.001**	**3.95**	**(1.82, 9.27)**	**<0.001**

[*]Primary Model is from Table 2 with collapsed self-assessed oral health (Good vs. Poor).
[**]Bold odds ratio (OR), 95% confidence interval (CI) and p-value denotes significance at p<0.05.
[***]Multivariable model adjusted for age category, chronic respiratory disease, activities of daily living (ADL), and antidepressant usage.

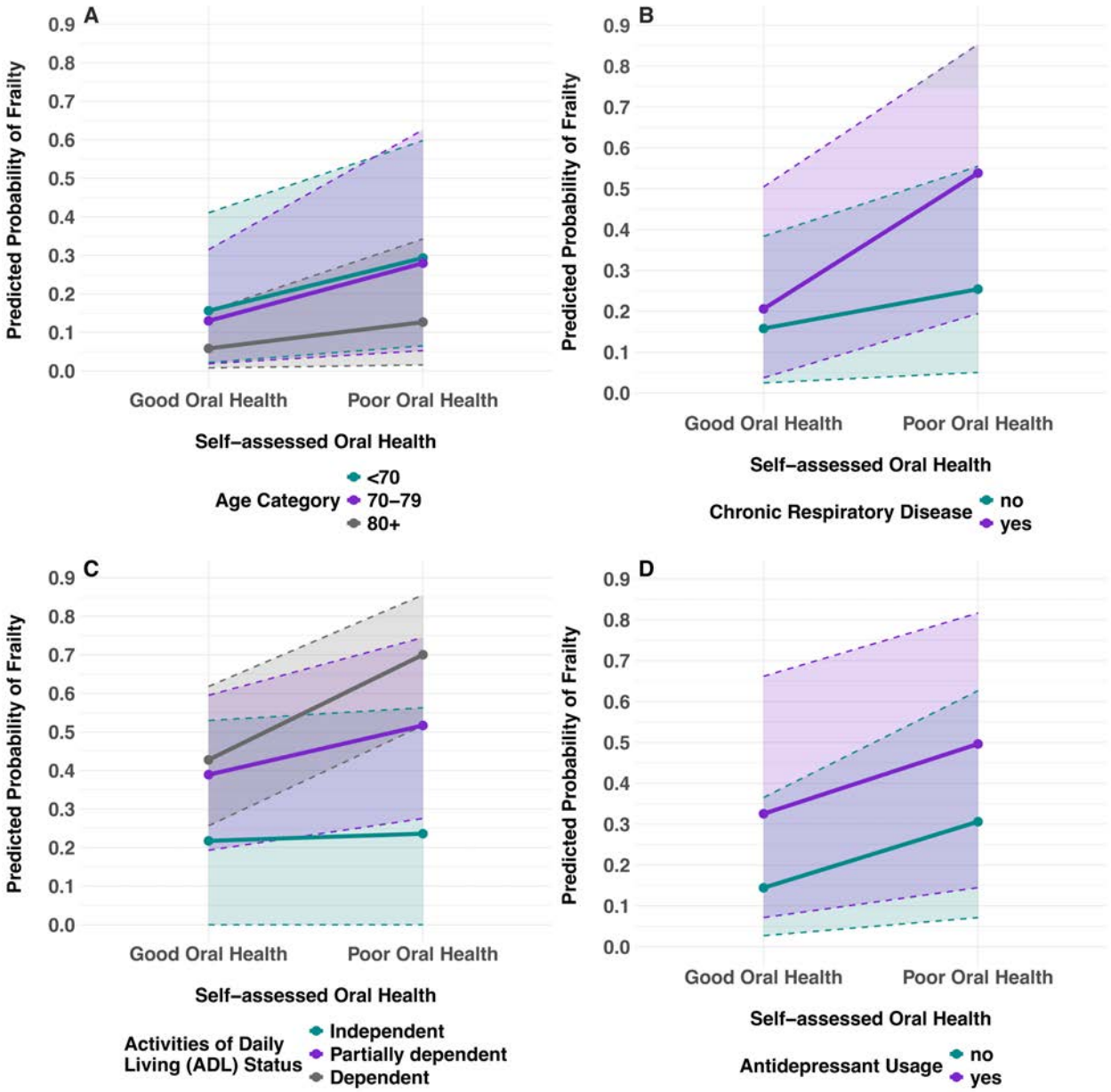


Figure 3.1 Plots visualizing how the predicted probability of frailty changes with self-assessed oral health across each level of a single covariate, (A) age category (ref <70), (B) chronic respiratory disease (ref no), (C) activities of daily living [ADL] (ref independent), (D) antidepressant usage (ref no), while keeping all other variables constant at their reference value. Confidence intervals were calculated using non-parametric bootstrapping with 1,000 iterations.

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Chapter 4 Associations of Microbiome Structure and Composition by Health Characteristics Among Older Adults in a Skilled Nursing Facility

This manuscript is intended for publication.

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4.1 Abstract

*Introduction***:** The nose/throat microbiome plays a crucial role in host defense, particularly among older adults recovering from surgery or illness – characteristic of residents of skilled nursing facilities (SNFs) – who are more susceptible to respiratory infections. However, longitudinal studies on microbiome stability and response to health factors in SNF residents are few. Our study described the short-term dynamics of the nose/throat microbiome in older adults residing in SNFs and documented associations between microbiome composition and function and frailty, medical devices, and antibiotic use.

*Methods***:** We analyzed the nose/throat microbiome of 301 SNF residents enrolled in prospective cohort study, Tracking Acute Respiratory Infection Among Older Adults (TARI). Participants were followed every three days for up to five study visits. At each visit, two nasal and two throat swabs were collected and combined for microbiome analysis. Samples were amplicon sequenced using the V4 region of the 16S rRNA gene, processed using the DADA2 pipeline, and taxonomy assigned using the Human Oral Microbiome Database (HOMD); microbial function was inferred

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using PICRUSt2. We assessed alpha and beta diversity, and coefficient of variation (CV) to assess the stability of the nose/throat microbiome. We conducted differential abundance analyses to identify microbiome composition and functional changes associated with frailty, device type, and antibiotic use adjusting for age and *Candida albican*s colonization.
Results: Over half of participants were frail (51.2%), 48.5% took antibiotics at any time point, and 20.3% had a device at entry. The nose/throat microbiome remained stable over short intervals, with *Veillonella dispar* (median CV within: 0.59; across: 1.14), *Streptococcus ASV1* (0.74; 1.31), and *Streptococcus. parasanguinis clade 411* (0.68; 1.53) showing the greatest median stability within and across participants. However, diversity and composition varied significantly by device presence and timing of antibiotic use ($p<0.001$). Participants with medical devices exhibited distinct microbial profiles and depleted biofilm-related functions, while current antibiotic use reduced microbial diversity and disrupted functional pathways related to immune response, oxidative stress, and metabolism.
Conclusion: This study provides novel insights into the short-term dynamics of the nose/throat microbiome among older adults recovering in a SNF. Our findings highlight the influence of medical devices and antibiotic use on microbiome composition and function, underscoring the need for strategies to maintain microbiome resilience and support positive recovery outcomes in this vulnerable population.

4.2 Introduction

The microbiome is a dynamic community made up of microorganisms that interact both independently and collaboratively to perform functions necessary to sustain their individual and collective lives. Microbiome dynamics refers to the fluctuation of microbial composition and structure over time within a community. Stability, defined as the ability of the microbiome to maintain or quickly recover its structure after disturbance, may indicate robustness to external perturbations such as exposure to respiratory pathogens or antibiotic usage. These fluctuations influence the microbial community's ability to resist or facilitate the invasion of potential pathogens and can modulate host immune responses, either upregulating or down regulating immune activity.[1,2] Changes in microbial composition, particularly shifts in the abundance of specific taxa, can be associated with increased susceptibility to chronic and acute conditions.[3] The nose and throat serve as primary entry ports for respiratory infections, suggesting that the

nose/throat microbiome plays a crucial role in host defense. Much of the existing research on microbial community dynamics has focused on the gut microbiome.

Studies of the nose/throat microbiome remain limited, and there is a notable absence of prospective studies and studies describing nose/throat microbiome dynamics over short intervals, such as every few days. A systematic review by Zeng & Liang identified 24 studies on nasal microbiome composition and its associations with respiratory diseases, revealing strong links between nasal microbiome dysbiosis and asthma, particularly in early life.[4] However, most studies identified by the review were cross-sectional or –if longitudinal– sampled infrequently (e.g., biweekly or monthly). Furthermore, sample sizes were often small: 16 of 24 studies had fewer than 250 participants, and only two included adults over 20 years old, both of which were cross-sectional and focused on the microbiome of those with asthma.[4] A longitudinal study by Zhou *et al*., sampled 86 participants from multiple body sites (stool, skin, tongue/oral cavity, and nasal) quarterly over six years, but did not specifically focus on the combined nose/throat microbiome.[5] The sample included some older adults (participant mean: 55 years; range: 29 – 75 years). In this sample, microbiome stability varied by body site and was heavily influenced by the host. The skin and nasal microbiomes, which are continuously exposed to the environment, exhibited greater fluctuations over time compared to the stool and oral microbiomes, indicating lower baseline stability. However, despite these fluctuations, individual microbiomes within each body site demonstrated a consistent capacity to recover after perturbations, suggesting that overall microbiome stability was robust to external disturbances such as viral infections.

Among the microorganisms inhabiting the nose and throat, fungal and bacterial species can play important roles in shaping microbiome stability and host outcomes. *Candida albicans* (*C. albicans)* is a commensal fungus common in the nose and throat; however, it is associated with poor recovery outcomes in patients with pneumonia and lower respiratory tract infections.[6] Results from *in vitro* and mouse models suggest possible mechanisms for this phenomenon. *In vitro*, *C. albicans* disrupts the pH, depleting resources for beneficial commensals and favoring more potentially pathogenic species such as *Staphylococcus aureus* (*S. aureus*) and inducing mucosal dysbiosis.[7,8] Further, *C. albicans* increases *S. aureus* proliferation and biofilm formation; in a peritonitis mouse model co-infection reduced survival over independent infections with each species.[9] While bacterial dysbiosis is well-documented, fungal-bacterial interactions in the human nose/throat remains poorly characterized.[10,11]

We address the lack of prospective studies of short-term nose/throat microbiome dynamics, and bacterial-fungal interactions using samples collected every three days for up to 15 days from 301 older adults residing in a single skilled nursing facility (SNF) in southeast Michigan. We assess the stability of the microbiome using alpha diversity, beta diversity, and coefficient of variation measures. SNF residents are an especially relevant population as the nose/throat microbiome may play a particularly influential role in host defense against respiratory infections to which older adults and individuals recovering from surgery or illness are more susceptible.[12] Within SNFs, the risk of exposure to respiratory and other pathogens is heightened due to shared living spaces, decreased mobility, a high level of medical interventions and interactions with healthcare workers. SNFs provide short-term acute care following surgery, illness or injury; residents generally have multiple comorbidities, commonly experience frailty, use medical devices, and have frequent antibiotic exposure. We assess for differences in microbial composition and function by these SNF health characteristics using differential abundance analysis. Antibiotic use can suppress bacterial competitors creating an environment for *C. albicans* to thrive and amplify.[8] Together these factors can disrupt the microbiome, and as SNF residents tend to be older these disruptions may be compounded by age-related declines in microbial diversity which can indicate declining overall health.[13] Our work provides a foundation for future research aimed at preventing microbiome dysbiosis and improving health outcomes among vulnerable older adults.

4.3 Methods

4.3.1 Study population

We analyzed data from 301 participants in the Tracking Acute Respiratory Illness in Older Adults (TARI) study, a prospective cohort study. Participants were enrolled shortly after admittance to a single SNF in southwest Michigan between April 5, 2021, through May 22, 2023. After enrollment, participants were visited approximately every three days for up to five study visits over a 12 to15-day period. Residents were eligible for inclusion if they were 18 years or older, English-speaking, and not scheduled for immediate discharge. Residents were ineligible if they had devices blocking access to their nose and mouth, were not oriented and alert, or were not able to self-consent. Participants who had insufficient sequencing reads, whose samples were contaminated during transport or testing or insufficient sample volume for complete *C. albicans*

and *S. aureus* testing, and who did not have complete covariate information for frailty, device type, and antibiotic usage were excluded from this analysis (n = 12), resulting in a final sample of 301 participants. The study protocol was approved by the Institutional Review Board at the University of Michigan.

4.3.2 Nose/throat sample collection & storage

During each study visit, two nasal and two throat swabs were collected. One of each swab type was combined into a single vial of UTM and into one eSWAB tube with Amies Media (BD, 220220, 220245 respectively). Swabs were transported to the Foxman laboratory and processed within 3 hours. Each UTM vial was vortexed for a minute at full speed then aliquoted into four vials: one with 1mL of media and the swabs (A), two vials with 500ul of media (B-C); and any remaining media was stored in a separate vial (D). Extracted nucleic acids from UTM and the swabs (aliquot A) were used for viral detection of DNA and RNA targets, and 16S community screening. The eSWAB media was aliquoted into four samples: two vials containing a swab placed in 10% milk (A-B), one vial with 500ul of 20% milk and 500ul Amies media (C), and any remaining media was stored in a separate vial (D); these were used for bacterial growth studies. All aliquots were then frozen at -80 degrees Celsius.

4.3.3 DNA extraction and sequencing

Bacterial DNA was extracted from the UTM and swabs from combined nose/throat samples. Library preparation and amplicon sequencing of the 16S rRNA V4 region was performed by the University of Michigan Medical School Microbiome Core.[14] DNA extraction was performed using the Eppendorf EpMotion 5075 liquid handling system using the Qiagen MagAttract PowerMicrobiome kit (Cat: 27500-4-EP) protocol. Sequencing was performed on the Illumina MiSeq platform, using MiSeq Reagent Kit V2 500 cycles (Illumina cat# MS-102-2003). Each plate of samples included a positive control, a DNA extraction kit control, and a negative water control. The 16S rRNA sequencing data from the TARI study is publicly available at the National Center for Biotechnology Information (NCBI) repository under PRJNA1160331.

4.3.4 Bioinformatics

Reads were processed to amplicon sequence variants (ASVs) using DADA2 (version 1.30.1 within R version 4.3.1) and assigned taxonomy using the Human Oral Microbiome

Database (HOMD).[15,16] We filtered out nonbacterial amplified sequences, samples with fewer than 1,000 reads, and species that were rare (<0.05%), but common across all participants. ASVs that could not be resolved to the species level were named using the assigned ASV number from the DADA2 package. After quality filtering, there were 25,497,376 total reads with an average read count of 22,604.

4.3.5 Staphylococcus aureus and Candida albicans testing

Colonization status was determined based on testing using Fast Track Diagnostics (FTD) kits (Siemens Healthineers ref. 10921703) and quantitative polymerase chain reactions (qPCR) for each combined nose/throat swab. FTD kits test for an array of respiratory pathogens, but not fungal species. Preliminary testing revealed a lack of meaningful positives for targets other than *Staphylococcus aureu*s; therefore, *S. aureus* testing was initially performed using the FTD kit and then qPCR after depletion of all kits whereas *Candida albicans* testing was performed using qPCR only. Participants were classified as either not colonized, single colonized (*S. aureus* only or *C. albicans* only), or co-colonized (both *S. aureus* and *C. albicans*) at enrollment.

The PCR assay for *S. aureus* was performed with forward (5′-GCG ATT GAT GGT GAT ACG GTT-3′) and reverse primers (5′-AGC CAA GCC TTG ACG AAC TAA AGC-3′) that targeted the nuc gene of *S. aureus* marker.[17] Amplification was performed in 20uL total volume with 5uL of DNA and 10uL Evagreen Sofast Supermix and 2.5uL of each primer (2uM). Cycle parameters included fluorescence FAM with initial denaturation at 95 degrees Celsius with 10min hold, melt at 95 degrees Celsius with 1s hold, annealing at 55 degrees Celsius with 5s hold, and extension at 72 degrees Celsius with 8s hold, followed by creation of the melt curve.[17] Melt temperatures for *S. aureus* were between 77.5-79.5 degrees Celsius.

The PCR assay for *C. albicans* was performed with Khan forward (5′-TTT ATC AAC TTG TCA CAC CAG A-3′) and reverse primers (5′-GGT CAA AGT TTG AAG ATA TAC GT-3′) that targeted the ITS gene of *C. albicans* marker.[18] Amplification was performed in 20uL total volume with 5uL of DNA and 10uL Evagreen Sofast Supermix and 2.5uL of each primer (5pmol). Cycle parameters included fluorescence FAM with initial denaturation at 95 degrees Celsius with 2min hold, melt at 95 degrees Celsius with 10s hold, annealing at 58 degrees Celsius with 10s hold, and extension at 72 degrees Celsius with 15s hold, followed by creation of the melt curve.[18] Melt temperatures for *C. albicans* were between 83.5-84.5 degrees Celsius.

For both *C. albicans* and *S. aureus* qPCR cycle threshold (Ct) values and melt temperatures were reviewed for each sample tested. Ct values <35 and consistent with expected melt temperatures for the target were determined positive for colonization. Ct values from 35-<38 were classified as high positives. For this analysis high positives were considered positive for colonization. Retesting was performed for 10% positives, 10% high positives, and 10% of negatives to confirm results.

A total of 257 of visit one samples were tested for the *S. aureus* target using the FTD kit.[19] The remaining 44 samples were tested only using qPCR. To assure methods were comparable, ~170 samples previously tested by FTD were tested with qPCR method for comparison. After considering expected DNA degradation and potentially more sensitive qPCR assays, the methods were concluded to be comparable.

4.3.6 Demographic collection

Demographics, health behaviors, and comorbidities were collected using a detailed medical record review and review of the most recent Minimum Data Set (MDS), a standardized assessment tool that collects information about a nursing home resident's health. Age was calculated using study enrollment date and date of birth and categorized as < 70, 70–79, and 80+ years old for analysis.

4.3.7 Health characteristics

Frailty, devices, and antibiotic use occur frequently among SNF residents. Frailty, an age-related decline in strength and physical function, is common among older adults; among SNF residents the prevalence can exceed 50%.[20] Frailty was assessed using a validated Frailty Short Interview that consisted of five questions related to weight loss, exhaustion, weakness, difficulty walking, and physical activity. The average score, ranging from zero to one, was calculated based on the number of questions answered, excluding any missing responses. For analysis, scores were dichotomized with < 0.6 considered ‘non-frail’ and ≥ 0.6 considered to be ‘frail’, based on prior guidelines.[21]

A patient is often admitted to a skilled nursing facility (SNF) when a partner or caregiver can no longer manage their care needs, often due to new functional disabilities, antibiotic use, wound care requirements, or the need for post-hospitalization observation and support.[22] Often a device is present that requires help from a trained professional to manage. For this analysis,

devices were categorized into 'no devices', 'respiratory devices and/or non-surgical tubing', and 'any surgical drain and/or vascular access device' based on difficulty in managing the device at home. The devices found among TARI participants and how they were categorized are detailed in the supplementary information (Supplemental Figure 2.2). Nearly two-thirds of hospitalized patients entering nursing homes are currently taking antibiotics.[23] Further, especially if a device is present, antibiotics may be continued or prescribed if symptoms occur while in the SNF. After participants exited the study, we reviewed their medical record and the Minimum Dataset (MDS) to assess whether antibiotics were prescribed prior to the SNF (up to 30 days prior to SNF entry) and/or while residing at the SNF. For analysis, antibiotic use was included as a single measurement coded as 'no antibiotic usage', 'antibiotics prior to the study only', and 'any antibiotics during the study'. 'Any antibiotics during the stay' would capture participants with both antibiotic use prior to the SNF and during their stay.

4.3.8 Short-term dynamics analysis

We used multiple approaches to assess the short-term dynamics of the nose/throat microbiome, described in detail, below.

4.3.8.1 Alpha diversity analysis

Alpha diversity refers to within-sample diversity and provides information about the distribution of microbes.[24] Measurements may capture richness –defined as– the number of different microbes present, or evenness, which describes how uniformly those microbes are distributed.[24] Alpha diversity metrics were calculated using the "estimate_richness" function from the "phyloseq" R package (version 1.46.0). We evaluated Alpha diversity using the Shannon diversity index, which takes into account both evenness and richness, and Chao1, which is a measure of richness. Larger alpha diversity values indicate more diversity. We tested for significant differences in alpha diversity by each health characteristics subgroup across each study visit using the Kruskal-Wallis test.

4.3.8.2 Beta diversity analysis

Beta diversity is a measure of dissimilarity between microbial communities, either within the same participant or between different participants.[24] Beta diversity metrics were calculated using the "distance" function from the "phyloseq" R package (version 1.46.0). We evaluated Beta diversity using the Bray–Curtis dissimilarity that is based on abundance. Beta diversity

ranges from 0–1 with 0 indicating the samples are identical and 1 indicating they are completely different. We visualized Bray–Curtis dissimilarity distances across visits for each participant by health characteristics using spaghetti plots. We assessed how each person changed from baseline (study visit 1) and each consecutive visit. A summary line was generated using LOESS regression to capture the central trend in dissimilarity across all individuals by health characteristic.

4.3.8.3 Coefficient of variation

The coefficient of variation (CV) was used to quantify the relative variability in microbial abundance, providing a normalized measure for comparison across different taxa.[25] We calculated CV using relative abundance to assess the stability of each taxon. CV is calculated as the ratio of the standard deviation of relative abundance to the mean relative abundance for each taxon. A lower CV indicates higher stability, suggesting that a species is consistently present, while a higher CV suggests greater variability and potential sensitivity to perturbations, such as exposure to antibiotics. We calculated CV two ways: (1) within participants over time, grouping by ID and species, to identify species that were stable within each participant, and (2) across all participants at each visit, grouping by visit and species, to evaluate species that exhibit stability across all participants at each study visit.

4.3.8.4 Reference taxa across study visits based on colonization of Candida albicans or Staphylococcus aureus

Differential abundance (DA) analysis is a method commonly used in microbiome studies to identify taxa whose abundance significantly differs between two groups. To conduct DA analysis, we used ADAPT (Analysis of Microbiome Differential Abundance by Pooling Tobit Models)[26] that accounts for the compositional nature and the excessive number of zeros in metagenomic count tables. This allows ADAPT to achieve a good balance between false discovery rate and detection power. ADAPT identifies a subset of taxa that are not differentially abundant as reference taxa and uses the sum of these reference taxa abundances to normalize the abundances of all taxa. ADAPT treats zeros as left-censored observations and uses Tobit models to compare normalized taxa abundances between different conditions.[27] We adjusted the p-values for multiple hypothesis testing using Benjamini-Hochberg correction.[28] We used ADAPT to identify taxa that were differentially abundant between individuals who were colonized with at

least one of *Candida albicans* and *Staphylococcus aureus* and those who were not, based on preliminary evidence suggesting that colonization influenced stability in the microbiome.

To evaluate stability across each visit, we conducted DA analyses for samples from each of the five study visits separately. DA analysis requires the establishment of a stable reference taxa for normalization in order to identify the differentially abundant taxa in each visit. We identified a subset of stable taxa, which were prevalent among the study participants and consistently not differentially abundant between residents with and without colonization, according to our stability criteria (defined as consistently large unadjusted p-values and small estimated fold change). Any species that met this criterion was used to normalize data across all five study visits. ADAPT was then rerun to identify any differentially abundant taxa.

4.3.9 Microbiome differences associated with health characteristics

We used ADAPT to study the association between the abundance of each nose/throat species and commonly identified SNF health characteristics: frailty status, device type, and antibiotic usage.[26] We considered a microbiome taxon to be significantly associated if the adjusted p-value was smaller than 0.05.[28] In terms of frailty status, we performed one analysis; we identified differentially abundant taxa between individuals who were frail and non-frail. In terms of device usage, we performed three analyses. First, we identified differentially abundant taxa between individuals who used at least one device and those who did not. Second, we reported differentially abundant taxa between individuals who used a respiratory device and/or non-surgical tubing device and those who did not use any devices. Third, we revealed differentially abundant taxa between individuals who had a surgical drain or vascular access device and those who did not use any devices. We applied similar analysis schemes to antibiotic usage, distinguishing individuals who did not use antibiotics during or in the 30 days prior to the study period, who used antibiotics before entering the SNF (up to 30 days prior to SNF entry) and those who used antibiotics during the study period while residing in the SNF. For each individual, we calculated the average relative abundance of each taxon across all five study visits to represent their microbiome abundance profile.

4.3.10 Functional pathway analysis

We inferred the functional potential of the nose/throat microbiota at the ASV level using picrust2_pipeline.py with default parameters implemented in PICRUSt2 (version 2.5.3).[29] We

calculated the relative abundance of each pathway and averaged the relative abundance across all five study visits. We investigated differentially abundant pathways using the same approach described for the species DA analyses described above by frailty, device type, and antibiotic use. Unless otherwise stated, all analyses were conducted using RStudio Version 4.4.2.

4.4 Results

4.4.1 Study population characteristics

The 301 included TARI participants averaged 77 years of age (min: 37.8, max: 100.8), 63.8% were women, and 88.7% were White. Over half were frail (51.2%), 20.3% had a device at entry with over 80% retaining their device across all study visits, 48.5% took antibiotics either prior to and/or during the study, 29.2% were colonized with *Candida albicans* at enrollment, and 44.9% were colonized with *Staph aureus* at enrollment. Prevalence of frailty was greater among women as compared to men ($p=0.0458$), among participants less than 70 years old as compared to greater than 70 ($p<0.001$), and among those with asthma ($p=0.001$), and chronic obstructive pulmonary disease (COPD; $p<0.001$) as compared to those without. Devices were more common among men compared to women ($p=0.007$) and those with COPD ($p<0.001$). Demographics and comorbidities were not statistically different by antibiotics use and *Candida albicans* colonization (Table 4.1).

4.4.2 Short-term stability of the nose/throat microbiome

We visualized Chao1 (Supplemental Figure 4.1) and Shannon (Supplemental Figure 4.2) alpha diversity measures by frailty status, device type, and antibiotic usage across study visits. Within each health characteristic subgroup, all five study visits were relatively stable. For frailty status, Chao1 and Shannon diversity were not significantly different between those with and without frailty by visit. By device type, Chao1 and Shannon diversity of study participants with devices were significantly less rich and diverse than among those with no devices. The microbiomes of study participants who did not take antibiotics at any time point were significantly richer and more diverse, and microbiomes of the study participants who took antibiotics during the study period were significantly less rich and diverse (Supplemental Table 4.1).

We visualized changes in Bray–Curtis dissimilarity distances by frailty status, device type, and antibiotic usage using spaghetti plots. For each participant, dissimilarity distances were

plotted comparing each study visit to baseline (Supplemental Figure 4.3) and comparing each consecutive study visit (Supplemental Figure 4.4). LOESS regression captured the central trend in dissimilarity across all individuals by health characteristic. While variability was evident at the individual level across all three health characteristics, the overall central trend reflected a flat line at approximately 0.5 dissimilarity across subgroups, suggesting no substantial changes in microbial composition over time. This indicated that the microbial communities were maintaining a relatively stable composition over time.

Coefficient of variation was calculated within participants (Supplemental Figure 4.5) and across participants (Supplemental Figure 4.6) to evaluate stability of individual species. Species CV within participants across study visits identified *Veillonella dispar, Streptococcus parasanguinis clade 411, Veillonella parvula, Granulicatella adiacens, Streptococcus ASV1* were the most stable species and *Moraxella catarrhalis, Escherichia coli, Lactobacillus casei, Corynebacterium propinquum, Pseudomonas aeruginosa* were the least stable species (Supplemental Figure 4.5). Species CV across participants identified similar species as the most stable, although the order differed. These species included *Veillonella dispar, Streptococcus ASV1, Streptococcus parasanguinis clade 411, Veillonella parvula,* and *Granulicatella adiacens.* The least stable species were, *Staphylococcus pettenkoferi, Corynebacterium ASV36, Corynebacterium propinquum, Pseudomonas aeruginosa,* and *Escherichia coli* (Supplemental Figure 4.6).

4.4.2.1 Microbiome differences associated with colonization of C. albicans and S. aureus

We applied ADAPT to samples from each of the five visits separately and investigated differentially abundant taxa between individuals who were colonized with *C. albicans* or *S. aureus* colonization and those who were not. The selected reference taxa by ADAPT differed from visit to visit (Supplemental Table 4.2). We considered the prevalence, fold change estimates and the p-values to select *Veillonella parvula* and *Veillonella dispar* as a refined reference taxa set for all the DA analyses related to colonization. DA analysis with the reference taxa revealed no differentially abundant taxa identified by *C. albicans* or *S. aureus* colonization (no colonization (REF) vs. any colonization, no *C. albicans* colonization (REF) vs. any *C. albicans* colonization, and no *S. aureus* colonization (REF) vs. any *S. aureus* colonization) suggesting that the initially observed changes surrounding colonization were artifacts of the analysis rather than biologically meaningful changes.

4.4.3 Microbiome differences associated with health characteristics

Based on the evidence described above, we inferred that the nose/throat microbiome is stable over short time periods. Therefore, we repeated our DA analyses averaging the relative abundance of each species across each participant's five study visits. We used ADAPT to identify species that were significantly different between levels of frailty, device type (Supplemental Table 4.3), and antibiotic usage (Supplemental Table 4.4) adjusting for age and *C. albicans* colonization. We adjusted for age and *C. albicans* colonization, as prior work shows that the microbiome changes as we age and *C. albicans* is a known disruptor of the bacterial community. There were no differentially abundant species by frailty status.

4.4.3.1 Device type

As device type is a three-level categorical variable, three separate analyses were performed as DA analyses are only capable of comparing two levels of a variable. We compared: (1) no device vs. at least one device, (2) no device vs. respiratory device and/or non-surgical tubing, and (3) no device vs. surgical tubing or vascular access device to identify microbiome differences between at least one device and specific types of devices. When comparing the microbiome between participants who did not use any device to the participants who used at least one device, 15 differentially abundant species were identified with five being enriched and ten being depleted among participants who used at least one device compared to no devices (Figure 4.1). Among the most significant species, identified by the smallest adjusted p-values (or the largest -log10(p-value) for visualization): *Corynebacterium matruchotii* (Log10 fold change: -1.79, adjusted $p<0.001$) and *Fusobacterium periodonticum* (Log10 fold change: -1.76, adjusted $p=0.002$) were depleted and *Lactobacillus casei* (Log10 fold change: 3.10, adjusted $p<0.001$) was enriched among participants who used at least one device compared to participants who did not use any device.

When comparing the microbiome between participants who did not use a device to the participants who specifically used a respiratory device or non-surgical tubing, seven differentially abundant species were identified with only one species being enriched and six being depleted among participants with respiratory devices or non-surgical tubing (Figure 4.2A). Among the most significant species, *Actinomyces sp. HMT180* (Log10 fold change: -0.66, adjusted $p=0.032$), *Fusobacterium periodonticum* (Log10 fold change: -1.99, adjusted $p=0.032$), and *Corynebacterium matruchotii* (Log10 fold change: -1.42, adjusted $p=0.032$) were depleted

among participants who used a respiratory device or non-surgical tubing compared to participants who did not use any device.

When comparing the microbiome between participants who did not use a device to the participants who used a surgical drain or vascular access device, 18 differentially abundant species were identified with three being enriched and 15 being depleted among participants with this a surgical drain or vascular access device (Figure 4.2B). Among the most significant species, *Streptococcus ASV1* (Log10 fold change: -0.56, adjusted p=0.009) was depleted and *Lactobacillus casei* (Log10 fold change: 3.04, adjusted p=0.002) and *Staphylococcus aureus* (Log10 fold change: 0.73, adjusted p=0.009) were enriched among participants who used a surgical drain or vascular access device compared to participants who did not use any device.

When comparing the findings of the three independent DA analyses for device type, there were some differences in the differentially abundant taxa (Supplemental Table 4.3). Four taxa that were identified as differentially abundant when comparing the microbiome of participants who did not use a device to the microbiome of participants who used at least one device were not differentially abundant in the analyses that accounted for a specific type of device: *Capnocytophaga leadbetteri, Aggregatibacter ASV70, Lactobacillus fermentum,* and *Staphylococcus pettenkoferi*. Additionally, the DA analyses that accounted for a specific device type identified differentially abundant species that were not differentially abundant when compared to the microbiome of participants who did not use any device. *Actinomyces sp. HMT 180* was depleted among participants who used a respiratory device or non-surgical tubing compared to participants who did not use any device. *Streptococcus ASV1, Oribacterium sinus, Selenomonas ASV101, Streptococcus ASV28, Leptotrichia sp. HMT 417, Atopobium parvulum, Streptococcus mutans, Parvimonas micra, Granulicatella adiacens, Fusobacterium nucleatum subsp. Vincentii* were depleted among participants who used a surgical drain or vascular access device compared to participants who did not use any device.

4.4.3.2 Antibiotic usage

As antibiotic usage is a three-level categorical variable, three separate analyses were also performed as DA analyses are only capable of comparing two levels of a variable. These were: (1) no antibiotics (either in the 30 days prior before SNF entry or during the study) vs. any antibiotics, (2) no antibiotics vs. antibiotics prior to the study only (up to 30 days prior to SNF entry), and (3) no antibiotics vs. any antibiotics during study (during SNF stay with or without

antibiotics in the 30 days prior) to identify if any differences between any antibiotic usage and timing of antibiotics. When comparing the microbiome between participants who did not use any antibiotics to the participants who used antibiotics, 32 differentially abundant species were identified with five being enriched and 27 being depleted among participants who used antibiotics (Figure 4.3). Among the most significant species, identified by the smallest adjusted p-values (or the largest -log10(p-value) for visualization): *Corynebacterium matruchotii* (Log10 fold change: -1.61, adjusted $p<0.001$) and *Gemella morbillorum* (Log10 fold change: -0.67, adjusted $p<0.001$) were depleted and *Lactobacillus casei* Log10 fold change: 2.39, adjusted $p<0.001$) was enriched among participants who used antibiotics compared to participants who did not use antibiotics.

When comparing the microbiome between participants who did not use antibiotics to the participants who only used antibiotics in the 30 days prior to SNF entry, 20 differentially abundant species were identified with seven being enriched and 13 depleted among participants with prior antibiotics use (Figure 4.4A). Among the most significant species, *Corynebacterium matruchotii* (Log10 fold change: -1.54, adjusted $p<0.001$) was depleted and *Lactobacillus casei* (Log10 fold change: 2.30, adjusted $p=0.001$) and *Scardovia wiggsiae* (Log10 fold change: 1.24, adjusted $p=0.002$) were enriched among participants who only used antibiotics prior to the study compared to participants who did not use antibiotics.

When comparing the microbiome between participants who did not use antibiotics to the participants who used antibiotics during the study period, nine differentially abundant species were identified with five being enriched and four being depleted among participants with antibiotic use during the study (Figure 4.4B). Among the most significant species, *Corynebacterium matruchotii* (Log10 fold change: -1.37, adjusted $p<0.001$) and *Fusobacterium nucleatum subsp. vincentii* (Log10 fold change: -1.17, adjusted $p<0.001$) were depleted and *Lactobacillus casei* (Log10 fold change: 2.81, adjusted $p<0.001$) was enriched among participants who used antibiotics during the study compared to participants who did not use antibiotics.

When comparing the findings of the three independent DA analyses for antibiotic usage, there were some differences in the differentially abundant taxa (Supplemental Table 4). Fifteen taxa that were identified as differentially abundant when comparing the microbiome of participants who did not use any antibiotics to the microbiome among participants who used

antibiotics at any time were not differentially abundant in the analyses that accounted for specific timing of antibiotic use. These were: *Alloprevotella sp. HMT 308, Fusobacterium periodonticum, Prevotella nigrescens, Leptotrichia sp. HMT 212, Prevotella nanceiensis, Corynebacterium durum, Leptotrichia sp. HMT 215, Prevotella pallens, Neisseria subflava, Campylobacter concisus, Dolosigranulum pigrum, Selenomonas ASV101, Prevotella oris, Rothia mucilaginosa*, and *Pseudomonas aeruginosa.* Additionally, the DA analyses that accounted for specific timing of antibiotic use identified differentially abundant species that were not differentially abundant when compared to the microbiome of participants who did not use any antibiotics. *Kingella sp. HMT 012* was depleted and *Veillonella dispar* and *Streptococcus parasanguinis clade 411* were enriched among participants who only used antibiotics prior to the study compared to participants who did not use antibiotics. There were no species that were differentially abundant when comparing participants who used antibiotics during the study to participants who did not use antibiotics. Notably, other known antibiotic targets, including *Haemophilus influenzae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, were also identified as differentially abundant in at least one of the analyses among participants who used antibiotics.

4.4.4 Impact of health factors on microbial function

We inferred microbial pathway abundance using the averaged relative abundance of each species across each participant's five study visits. We used ADAPT to identify functional pathways that significantly varied in abundance by frailty status, device type, and antibiotic usage adjusting for age and *C. albicans* colonization. Microbial pathway abundance did not vary by frailty status.

4.4.4.1 Device type

Consistent with the taxon-level analysis, three separate analyses were performed for functional pathways: (1) no device vs. at least one device, (2) no device vs. respiratory device and/or non-surgical tubing, and (3) no device vs. surgical tubing or vascular access device to identify if any differences between at least one device and specific types of devices. Multiple pathways were differentially varied in abundance by device type. We identified 65 pathways that were differentially abundant between participants who used at least one device and those who did not use any device. When comparing the findings of the three independent DA analyses for

device type, there were some differences in differentially abundant pathways; a heatmap is provided showing which pathways were enriched in Supplemental Figure 4.7 and which were depleted in Supplemental Figure 4.8 based on each analysis. There were no pathways that were differentially abundant in all three analyses by device type. Of the 65 pathways, three were enriched among participants who used a respiratory device or non-surgical tubing compared to participants who did not use any device. Fourteen pathways were depleted and 29 pathways were enriched among participants who used a surgical drain or vascular access device compared to participants who did not use any device. In addition, there were 23 pathways (20 depleted, 3 enriched) that were unique to this last analysis and not in the initially identified 65 pathways. To highlight a few pathways relevant to recovering older adults, the polyamine synthesis pathway and the arginine-polyamine synthesis pathway were both depleted among participants who used at least one device compared to participants who did not use any device.

4.4.4.2 Antibiotic usage

Consistent with the taxon-level analysis, three separate analyses were performed for functional pathways: (1) no antibiotics vs. any antibiotics, (2) no antibiotics vs. antibiotics prior to the study only (up to 30 days prior to SNF entry), and (3) no antibiotics vs. any antibiotics during study (during SNF stay) to identify if any differences between any antibiotic usage and timing of antibiotics. Multiple pathways were differentially varied in abundance by antibiotic usage. We identified 139 pathways that were differentially abundant between participants who used antibiotics 30 days prior to the SNF entry and/or during the study period and those who did not use any antibiotics. When comparing the findings of the three independent DA analyses for antibiotic usage, there were some differences in differentially abundant pathways; a heatmap is provided showing which pathways were enriched in Supplemental Figure 4.9 and which were depleted in Supplemental Figure 4.10 based on each analysis. There were 46 pathways (36 depleted, 10 enriched) that were differentially abundant in all three analyses by antibiotic usage. Of the 139 pathways, 12 pathways were also depleted and five pathways also enriched among participants who used antibiotics during the study compared to participants who did not use antibiotics. In addition, there were 12 pathways (7 depleted, 5 enriched) that were unique to this last analysis and not in the initially identified 139 pathways. Of the initially identified 139 pathways, 33 pathways were also depleted and 38 pathways also enriched among participants who used antibiotics during the study compared to participants who did not use antibiotics. In

addition, there were 22 pathways (13 depleted, 9 enriched) that were unique to this last analysis and not in the initially identified 139 pathways. To highlight a few pathways relevant to recovering older adults, the denitrification pathway and the sulfate-cysteine pathway were depleted among participants who used antibiotics (either 30 days prior to SNF entry or during SNF stay) compared to participants who did not use antibiotics.

4.5 Discussion

In this analysis of 301 residents of a single SNF followed approximately every 3 days for up to 15 days, the nose/throat microbiome remained stable based on the evaluations of alpha diversity across visits, beta diversity within participants, and the species identified to be the most stable and least stable using the coefficient of variation within and across participants. *Streptococcus*, *Veillonella*, and *Granulicatella* species were the most stable genera within and across participants. However, subgroups of health characteristics modified the Alpha diversity. There was significantly higher diversity – as measured by the Chao1 and Shannon alpha indices – at each study visit among participants who did not use a device, compared to those who used a respiratory device or non-surgical tubing, or a surgical drain or vascular access device. There was also significantly higher Chao1 and Shannon alpha diversity at each study visit among those who did not use antibiotics compared to those who used antibiotics in the 30 days prior to the study or during the study. Distinct microbial compositions were observed based on device type and antibiotic use timing, and these same factors also shaped the predicted microbial functional potential. While antibiotic usage and medical devices are known to influence the microbiome, to our knowledge, our study is the first study to examine differentially abundant species in the nose/throat microbiome based on both device type and timing of antibiotic use among older adults.

It is well established that medical devices influence microbial colonization, with biofilm formation playing a critical role in increasing infection risk.[30] While these findings are often site-specific, we hypothesized that the presence of devices, especially those requiring maintenance, insertion through mucosal surfaces, or associated with higher antibiotic use, could also impact the nose/throat microbiome composition due to differences in materials, surface properties, and usage conditions.[31] While common microbial species have been identified on certain devices, direct comparative analyses of microbiome compositions across device types remain limited. Common bacterial species found in catheter-associated biofilms include *Staphylococcus*

epidermidis, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Enterococcus faecalis*.[32] In our study, we observed depletion of many species among participants with respiratory and non-surgical tubing, with *Lactobacillus casei* being the only species enriched, which was not expected. *Lactobacillus* species, commonly used in probiotics, can reduce biofilm growth by up to 99.9%, according to a systematic review by Carvalho *et al*.[33] Surgical drain infections are often associated with members of the *Enterobacteriaceae* family (*Escherichia coli*, *Klebsiella pneumoniae*) and *Staphylococcus* species (*Staphylococcus aureus*), which aligns with our finding that *Staphylococcus aureus* was enriched in participants with surgical drains or vascular access devices.[34] While the mechanisms underlying the enrichment of specific species by device type remains unclear, our findings highlight the need for further investigation into these associations and the significant correlation between device use and antibiotic therapy.

Antibiotics are known to reduce microbiome diversity by targeting susceptible species, which aligns with our findings that more species were depleted than enriched in the microbiome of participants who did versus did not use antibiotics either 30 days prior to the SNF entry or during the study period.[35] *Haemophilus influenzae,* a common antibiotic target due to its role in respiratory infections, was depleted among antibiotic users, suggesting that it is still susceptible to antibiotic treatment.[36] The few species that were enriched among antibiotic users may reflect antibiotic resistance or biofilm formation, particularly among participants with medical devices who are also more likely to be taking antibiotics. *Pseudomonas aeruginosa* and *Staphylococcus aureu*s are both well documented antibiotic-resistant pathogens.[37,38] Indeed, we found *Pseudomonas aeruginosa* enriched in participants with any antibiotic use, regardless of whether use was in the 30 days prior to or during the study, while *Staphylococcus aureus* was enriched only in independent analyses on timing of antibiotic use, up to 30 days prior to SNF entry or during the study. Additionally, we identified enrichment of *Scardovia wiggsiae* among our antibiotic users, a known biofilm producer in the oral cavity and a key species in early childhood caries.[39]

Both independently and jointly, the presence of a medical device and antibiotic use influenced microbiome composition, but these health factors also result in functional changes relevant to biofilm formation, immune response, and metabolic pathways. The arginine-polyamine synthesis and polyamine synthesis pathways play essential roles in cell growth and

biofilm formation, and their depletion that we observed within our participants with medical devices may impair immune response and wound healing.[40] The denitrification pathway facilitates the reduction of nitrate (NO_3^-) to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O), and ultimately nitrogen (N_2) gas.[41] Nitric oxide production declines with age and has been linked to cardiovascular disease and the progression of Alzhemier's disease.[42] While antibiotics are known to inhibit nitric oxide production, most research on this effect has focused on soil environments. A study by Semedo *et al*. found that antibiotic exposure led to increased nitrous oxide production and decreased nitrogen gas production, indicating incomplete denitrification.[43] Consistent with this, we observed depletion of the denitrification pathway in older adults who had used antibiotics (either prior to or during the study) compared to participants who did use antibiotics. Additionally, the sulfate-cysteine biosynthesis pathway plays a critical role in sulfur metabolism, and its disruption can increase susceptibility to oxidative stress and antibiotic sensitivity.[44] Given the greater number of depleted species among antibiotic users in our study, the observed reduction in this pathway aligns with known mechanisms of antibiotic-induced stress. Long-term disruptions of sulfate-cysteine biosynthesis could exacerbate oxidative stress, accelerate aging, reduce glutathione synthesis, a key factor in frailty and age-related diseases, and weaken immune response, increasing infection risk.[45,46]

Our findings highlight key microbial shifts driven by antibiotic use and medical devices, as well as their functional consequences. However, several limitations should be considered when interpreting these results. Participants were enrolled from a single SNF in Southeast Michigan. While the gender and age distributions were consistent with nationwide long-term care facilities, this community lacked racial diversity, limiting the generalizability of our findings.[47] Additionally, 16S rRNA sequencing provides information on species presence and their relative abundance, but functional pathways are inferred using bioinformatics rather than directly assessed through metagenomics. This approach also precludes the detection of antibiotic resistance genes. Furthermore, the V4 region of 16S rRNA gene does not always allow for species-level resolution, particularly among *Streptococcus* species. Microbial function was inferred using PICRUSt2, which may introduce bias since some taxa in the nose/throat microbiome may be underrepresented or absent in reference databases. Additionally, PICRUSt2 does not account for functional redundancy, where multiple taxa may perform the same functions, potentially affecting prediction accuracy. For differential abundance analyses, we used

ADAPT, which is not specifically designed for longitudinal microbiome data. Given that we found the nose/throat microbiome to be stable over short intervals, we averaged species relative abundance across a participant's five study visits, though this may have resulted in some loss of information. While we controlled for age and *C. albicans* colonization, other unmeasured environmental and health factors may have influenced the microbiome and confounded our findings.

Despite these limitations, our study has several strengths. The longitudinal design allowed us to evaluate short-term microbiome dynamics and consider the influence of fungal species, *Candida albicans*, on the microbial community. We collected detailed data on socio-demographics, health behaviors, and medical history using MDS and frailty was assessed using a validated technique, the Frailty Short Interview.[21] Our sample size is also relatively large for a microbiome study. Importantly, our study fills a critical gap in knowledge by examining nose/throat microbiome stability at 3-day intervals in an older adult population recovering in a skilled nursing facility.

4.6 Conclusion

In this study of SNF residents, the nose/throat microbiome remained stable over short time intervals, with *Streptococcus*, *Veillonella*, and *Granulicatella* species exhibiting the greatest stability. However, the presence of a medical device such as BIPAP/CPAP device or urinary catheter, and antibiotic use independent of devices (in the 30 days prior to SNF entry and during the SNF stay), both common in SNF populations, had different microbiome compositions. The reasons for these differences are unclear, but we note that those with medical device tended to also be taking antibiotics. These findings have important clinical implications for infection prevention and microbiome resilience in this vulnerable population. The depletion of biofilm-related pathways among residents with medical devices may influence immune response and wound healing, while disruptions in denitrification and sulfate-cysteine biosynthesis pathways in antibiotic users may have broader effects on cardiovascular health, oxidative stress, and immune function. Understanding how medical interventions shape the microbiome can help inform strategies to minimize unintended microbial disruptions and improve recovery outcomes in older adults.

4.7 Acknowledgements

The authors thank the TARI participants. The authors also thank our colleagues in the Center for Molecular and Clinical Epidemiology (MAC-EPID) at the University of Michigan School of Public Health and Department of Internal Medicine, Geriatric & Palliative Medicine at the University of Michigan Medical School for their comments and guidance on this work. This research was supported by the University of Michigan Medical School Microbiome Core.

4.8 Tables and Figures

Table 4.1 Frequency (n[%]) of demographics, health behaviors, and comorbidities by frailty status, device type, antibiotics (AB) use, and Candida albicans colonization among 301 residents at a single skilled nursing facility (SNF) participating in the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023.

Characteristic[*]	**Non-Frail** n (%)	**Frail** n (%)	***p***[**]	**No Devices** n (%)	**Resp/ Non-Surg Tubing** n (%)	**Surgical Drain or Vascular Access** n (%)	***p***[**]	**No AB** n (%)	**Prior AB Only** n (%)	**Any AB During Study** n (%)	***p***[**]	***Candida* Neg**	***Candida* Pos**	***p***[**]
GENDER														
Women	83 (44%)	104 (56%)	***0.048***	159 (85%)	12 (6%)	16 (9%)	***0.007***	101 (54%)	50 (27%)	36 (19%)	*0.3*	137 (73%)	50 (27%)	*0.2*
Men	64 (56%)	50 (44%)		81 (71%)	19 (17%)	14 (12%)		54 (47%)	29 (26%)	31 (27%)		76 (67%)	38 (33%)	
AGE CATEGORY														
<70	25 (34%)	48 (66%)	***<0.001***	56 (77%)	6 (8%)	11 (15%)	*0.3*	36 (49%)	17 (23%)	20 (28%)	*0.4*	53 (73%)	20 (27%)	*0.6*
70-79	44 (41%)	63 (59%)		83 (78%)	12 (11%)	12 (11%)		58 (54%)	24 (23%)	25 (23%)		72 (67%)	35 (33%)	
80+	78 (64%)	43 (36%)		101 (83%)	13 (11%)	7 (6%)		61 (51%)	38 (31%)	22 (18%)		88 (73%)	33 (27%)	

Characteristic*	Non-Frail n (%)	Frail n (%)	*p***	No Devices n (%)	Resp/ Non-Surg Tubing n (%)	Surgical Drain or Vascular Access n (%)	*p***	No AB n (%)	Prior AB Only n (%)	Any AB During Study n (%)	*p***	Candida Neg	Candida Pos	*p***
RACE/ETHNICITY														
Asian	4 (67%)	2 (33%)		5 (83%)	0 (0%)	1 (17%)		1 (17%)	3 (50%)	2 (33%)		2 (33%)	4 (67%)	
Black	8 (32%)	17 (68%)		18 (72%)	3 (12%)	4 (16%)		16 (64%)	5 (20%)	4 (16%)		20 (80%)	5 (20%)	
Hispanic	1 (100%)	0 (0%)	*0.3*	0 (0%)	1 (100%)	0 (0%)	*0.2*	1 (100%)	0 (0%)	0 (0%)	*0.6*	1 (100%)	0 (0%)	*0.2*
Mixed	2 (50%)	2 (50%)		3 (75%)	0 (0%)	1 (25%)		2 (50%)	1 (25%)	1 (25%)		3 (75%)	1 (25%)	
White	132 (50%)	133 (50%)		214 (81%)	27 (10%)	24 (9%)		135 (51%)	70 (26%)	60 (23%)		187 (71%)	78 (29%)	
BODY MASS INDEX (BMI)														
Underweight or healthy	55 (54%)	46 (46%)		84 (83%)	7 (7%)	10 (10%)		52 (51%)	22 (22%)	27 (27%)		68 (67%)	33 (33%)	
Overweight	39 (47%)	44 (53%)	*0.4*	67 (81%)	7 (8%)	9 (11%)	*0.4*	38 (46%)	30 (36%)	15 (18%)	*0.1*	56 (67%)	27 (33%)	*0.3*
Obese	53 (45%)	64 (55%)		89 (76%)	17 (15%)	11 (9%)		65 (56%)	27 (23%)	25 (21%)		89 (76%)	28 (24%)	

Characteristic*	Non-Frail n (%)	Frail n (%)	*p***	No Devices n (%)	Resp/ Non-Surg Tubing n (%)	Surgical Drain or Vascular Access n (%)	*p***	No AB n (%)	Prior AB Only n (%)	Any AB During Study n (%)	*p***	Candida Neg	Candida Pos	*p***
SMOKING STATUS														
Never	102 (50%)	104 (50%)		168 (82%)	21 (10%)	17 (8%)		111 (54%)	50 (24%)	45 (22%)		150 (73%)	56 (27%)	
Former	42 (49%)	43 (51%)	*0.5*	65 (77%)	7 (8%)	13 (15%)	*0.1*	38 (45%)	27 (32%)	20 (23%)	*0.6*	59 (69%)	26 (31%)	*0.09*
Current	3 (30%)	7 (70%)		7 (70%)	3 (30%)	0 (0%)		6 (60%)	2 (20%)	2 (20%)		4 (40%)	6 (60%)	
ASTHMA														
No	132 (53%)	116 (47%)		194 (78%)	27 (11%)	27 (11%)		129 (52%)	64 (26%)	55 (22%)		179 (72%)	69 (28%)	
			<0.001				*0.4*				*0.9*			*0.2*
Yes	14 (27%)	38 (73%)		45 (86%)	4 (8%)	3 (6%)		25 (48%)	15 (29%)	12 (23%)		33 (63%)	19 (37%)	
HEART DISEASE														
No	59 (49%)	62 (51%)		102 (84%)	7 (6%)	12 (10%)		63 (52%)	29 (24%)	29 (24%)		86 (71%)	35 (29%)	
			0.9				*0.1*				*0.7*			*0.9*
Yes	88 (49%)	92 (51%)		138 (77%)	24 (13%)	18 (10%)		92 (51%)	50 (28%)	38 (21%)		127 (71%)	53 (29%)	
DEMENTIA														
No	139 (49%)	146 (51%)		227 (80%)	28 (10%)	30 (10%)		146 (51%)	73 (26%)	66 (23%)		201 (71%)	84 (29%)	
			0.6				*0.2*				*0.4*			*0.8*
Yes	8 (57%)	6 (43%)		11 (79%)	3 (21%)	0 (0%)		9 (64%)	4 (29%)	1 (7%)		11 (79%)	3 (21%)	

Characteristic*	Non-Frail n (%)	Frail n (%)	*p***	No Devices n (%)	Resp/ Non-Surg Tubing n (%)	Surgical Drain or Vascular Access n (%)	*p***	No AB n (%)	Prior AB Only n (%)	Any AB During Study n (%)	*p***	Candida Neg	Candida Pos	*p***
DIABETES														
No	98 (51%)	93 (49%)	*0.2*	154 (81%)	15 (8%)	22 (11%)	*0.1*	104 (55%)	48 (25%)	39 (20%)	*0.4*	136 (71%)	55 (29%)	*0.8*
Yes	49 (45%)	61 (55%)		86 (78%)	16 (15%)	8 (7%)		51 (46%)	31 (28%)	28 (26%)		77 (70%)	33 (30%)	
CHRONIC PULMONARY OBSTRUCTIVE DISORDER (COPD)														
No	131 (54%)	113 (46%)	***<0.001***	201 (82%)	17 (7%)	26 (11%)	***<0.001***	133 (55%)	59 (24%)	52 (21%)	*0.1*	178 (73%)	66 (27%)	*0.07*
Yes	16 (29%)	40 (71%)		38 (68%)	14 (25%)	4 (7%)		22 (39%)	19 (34%)	15 (27%)		34 (61%)	22 (39%)	

*Not all characteristics sum to total (n=301) due to missing values (Detailed missingness: asthma n=1; dementia n=2; COPD n=1) .
**P-value determined through Pearson's Chi-squared test or Fisher's Exact Test for Count

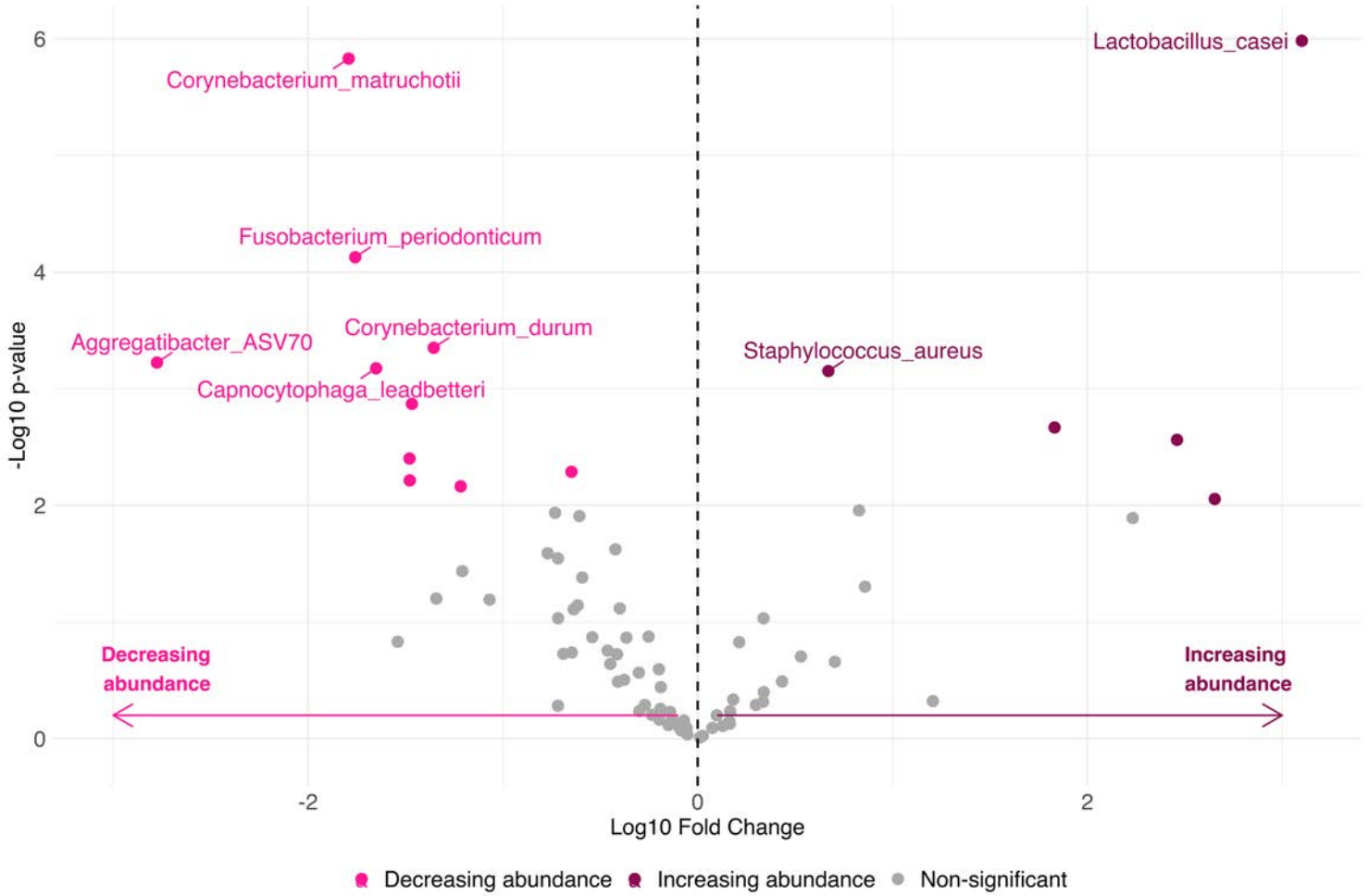


Figure 4.1 Differentially abundant species identified using ADAPT, comparing participants who did not have a device (reference group) to participants with at least one device (collapsed categories), adjusting for age and Candida albicans colonization. Significance was determined based on an adjusted p-value < 0.05 correcting for multiple hypothesis testing using Benjamini-Hochberg correction; however, species are displayed by their -log10(p-value) for easier interpretability, with non-significant species shown in gray. Labeled points reflect species with p-values < 0.001. Log10 fold change (x-axis) indicates the direction of effect, with positive values representing species enriched in the device group (maroon) and negative values representing species depleted in the device group (pink).

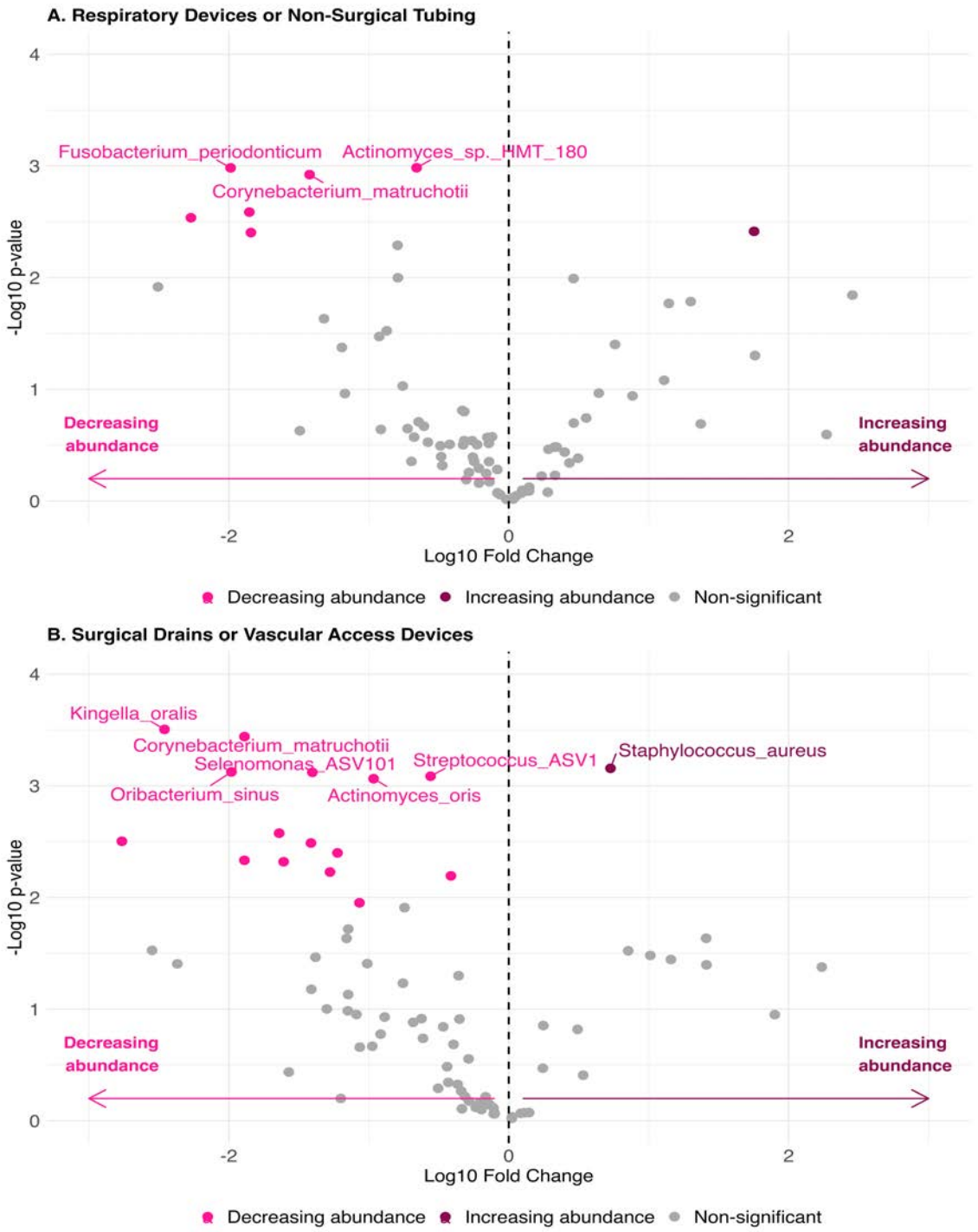


Figure 4.2 Differentially abundant species identified using ADAPT, comparing participants who did not have a device (reference group) to participants with each device type (A) respiratory device or non-surgical tubing (B) surgical drain or vascular access device, adjusting for age and *Candida albicans* colonization. Significance was determined based on an adjusted p-value < 0.05 correcting for multiple hypothesis testing using Benjamini-Hochberg correction; however, species are displayed by their -log10(p-value) for easier interpretability, with non-significant species shown in gray. Labeled points reflect species with p-values < 0.001 or the top three most significant species if none were <0.001. Log10 fold change (x-axis) indicates the direction of effect, with positive values representing species enriched in the device group (maroon) and negative values representing species depleted in the device group (pink)

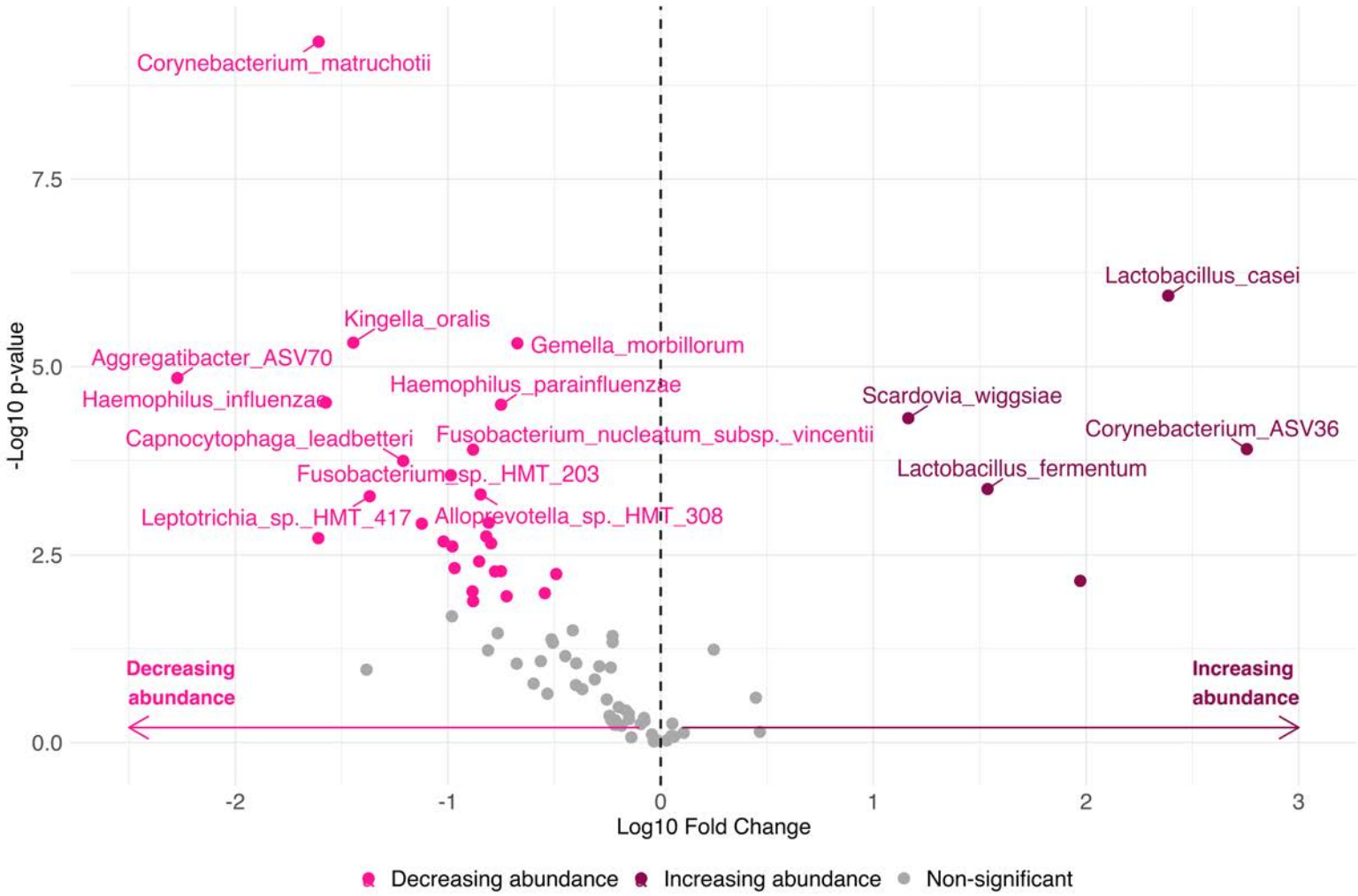


Figure 4.3 Differentially abundant species identified using ADAPT, comparing participants who did not use antibiotics (reference group) to participants who used antibiotics at any time point (collapsed categories), adjusting for age and Candida albicans colonization. Significance was determined based on an adjusted p-value < 0.05 correcting for multiple hypothesis testing using Benjamini-Hochberg correction; however, species are displayed by their -log10(p-value) for easier interpretability, with non-significant species shown in gray. Labeled points reflect species with p-values < 0.001. Log10 fold change (x-axis) indicates the direction of effect, with positive values representing species enriched in the device group (maroon) and negative values representing species depleted in the device group (pink).

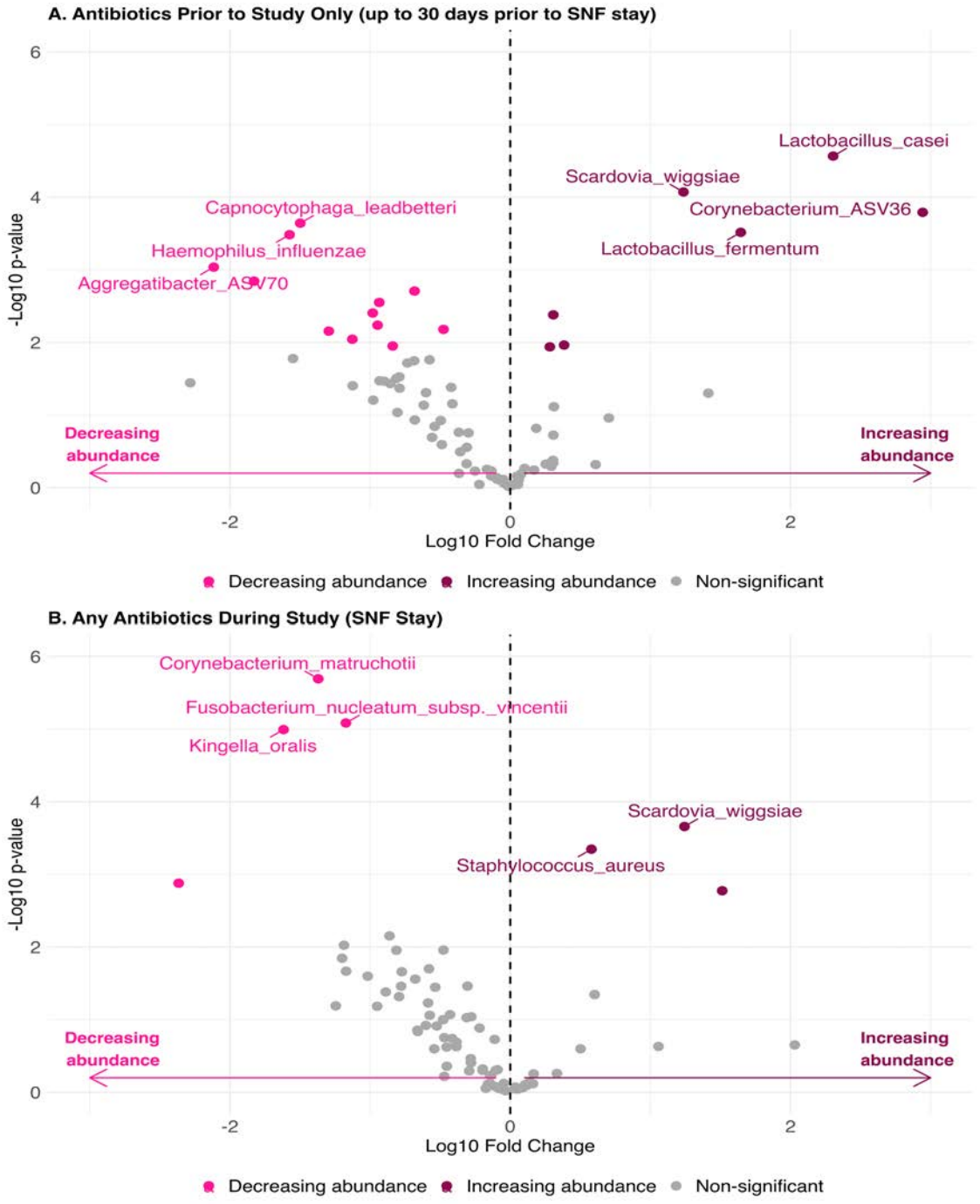
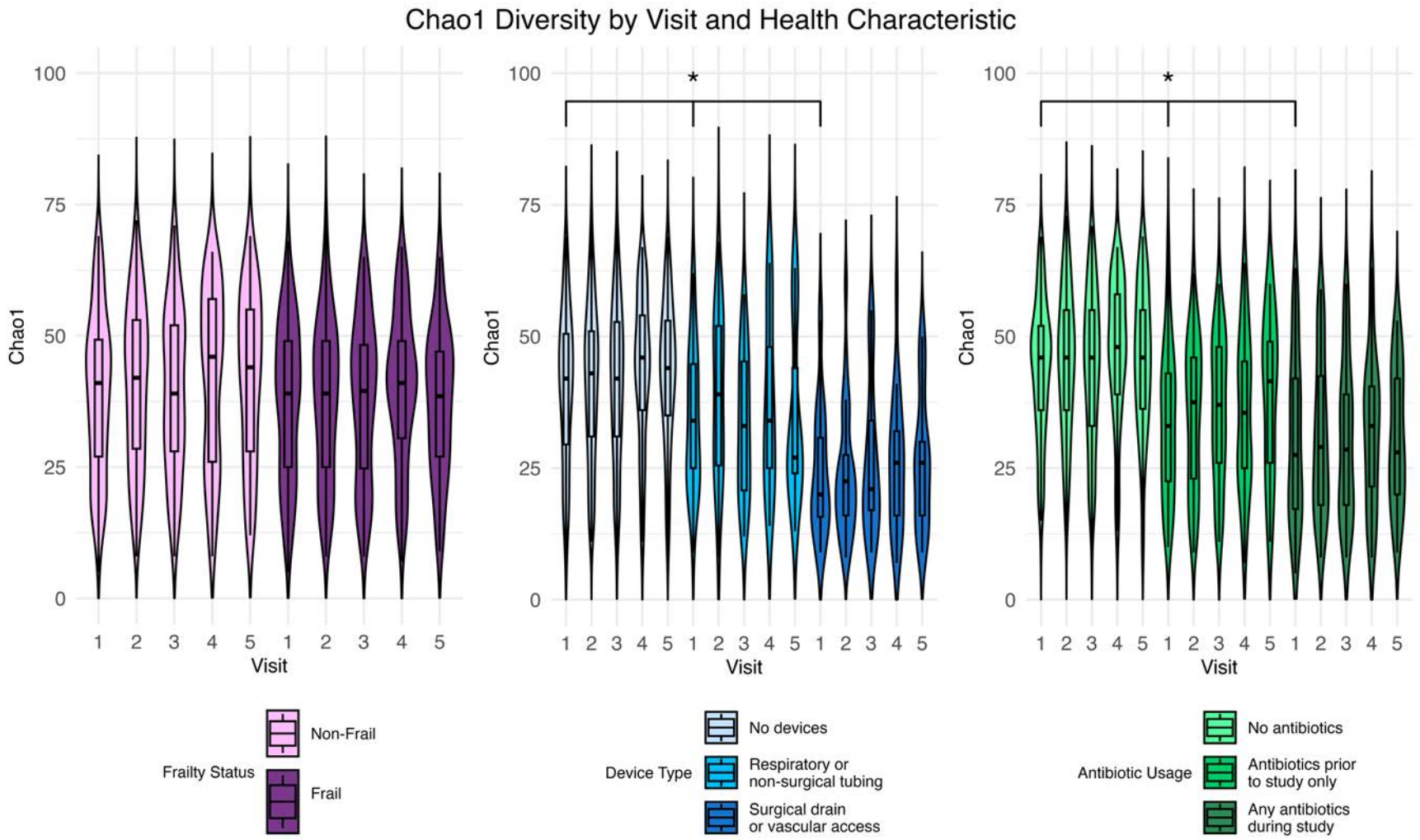
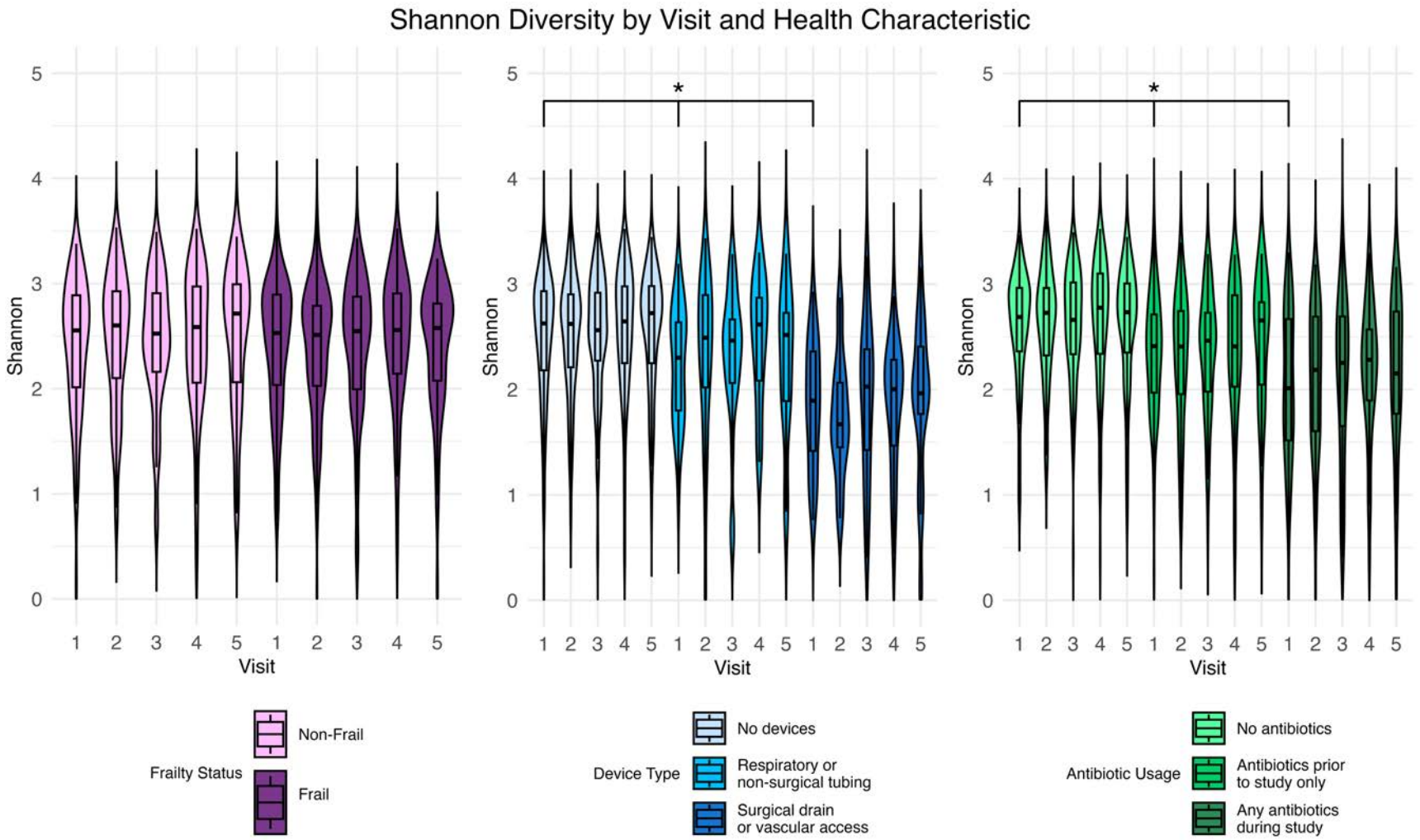


Figure 4.4 Differentially abundant species identified using ADAPT, comparing participants who did not use antibiotics (reference group) to participants with each antibiotic usage type (A) antibiotics prior to the study only (B) any antibiotics during the study, adjusting for age and *Candida albicans* colonization. Significance was determined based on an adjusted p-value < 0.05 correcting for multiple hypothesis testing using Benjamini-Hochberg correction; however, species are displayed by their -log10(p-value) for easier interpretability, with non-significant species shown in gray. Labeled points reflect species with p-values < 0.001. Log10 fold change (x-axis) indicates the direction of effect, with positive values representing species enriched in the device group (maroon) and negative values representing species depleted in the device group (pink).



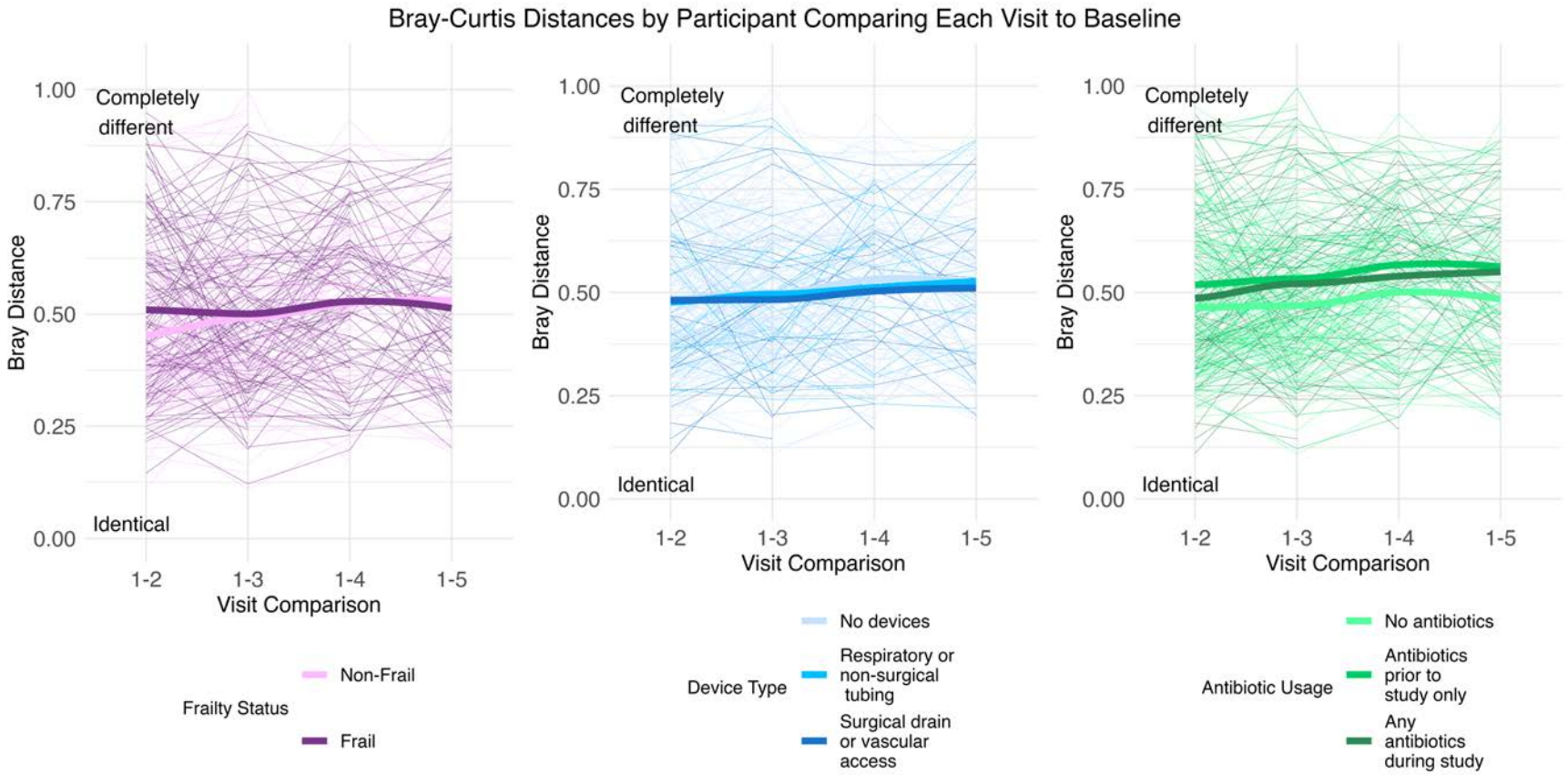
Supplemental Figure 4.1 Chao1 alpha diversity by study visit and (A) frailty status, (B) device type, and (C) antibiotic usage. Higher Chao1 values indicate greater microbial diversity. Kruskal-Wallis tests were performed to assess differences in Chao1 diversity across subgroups within each health characteristic and study visit independently. No significant differences were observed by frailty status. For device type and antibiotic usage, all subgroup comparisons were significant at each study visit. A single bracket is shown at visit 1 to illustrate significance (*) across device type subgroups and antibiotic subgroups, but similar significant differences were observed at all five study visits.



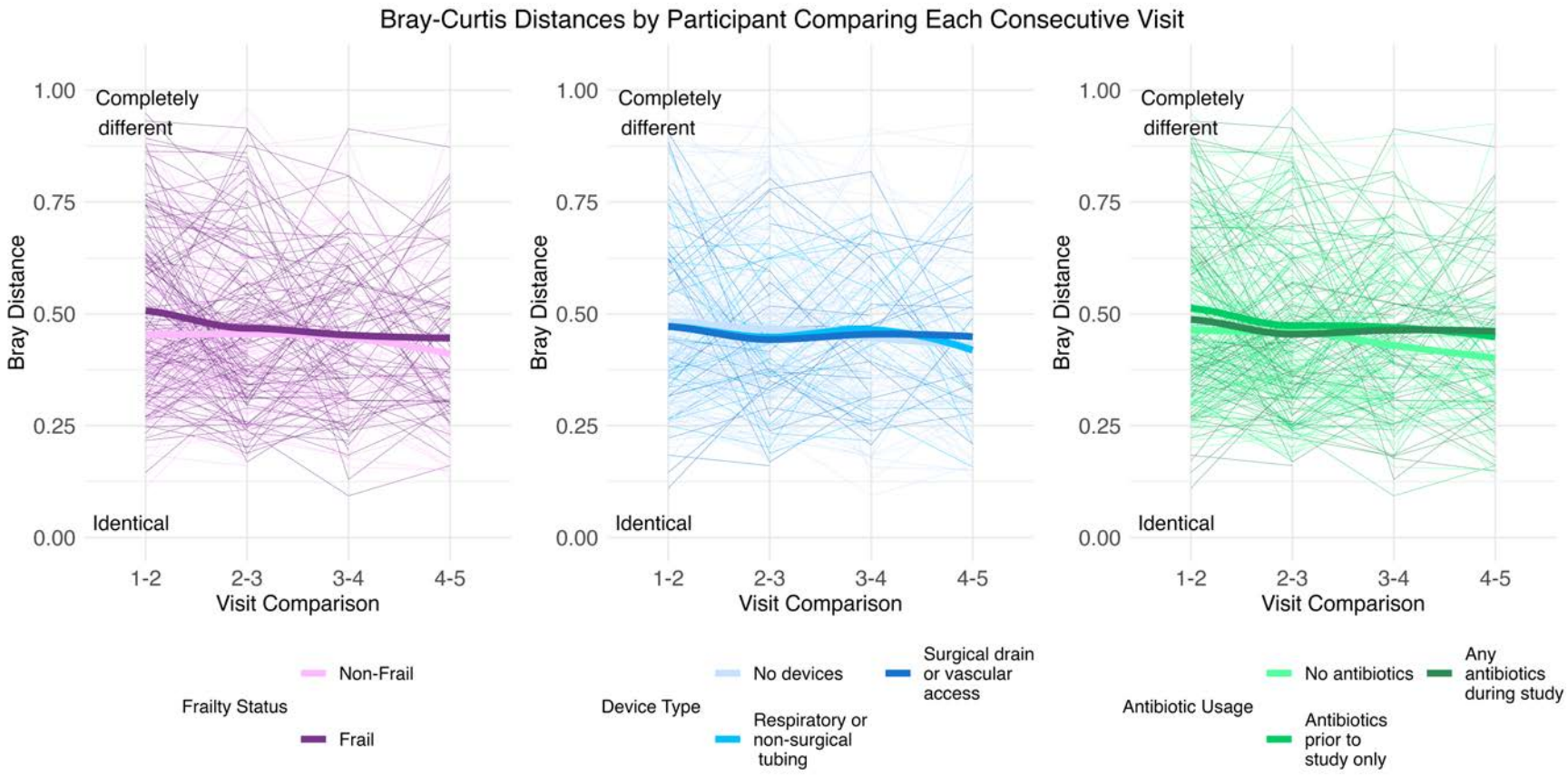
Supplemental Figure 4.2 Shannon alpha diversity by study visit and (A) frailty status (B) device type (C) antibiotic usage. Higher Shannon values indicate greater microbial diversity. Kruskal-Wallis tests were performed to assess differences in Shannon diversity across subgroups within each health characteristic and study visit independently. No significant differences were observed by frailty status. For device type and antibiotic usage, all subgroup comparisons were significant at each study visit. A single bracket is shown at visit 1 to illustrate significance (*) across device type subgroups and antibiotic subgroups, but similar significant differences were observed at all five study visits.

Supplemental Table 4.1 Mean and standard deviation (SD) of alpha diversity measures (Chao1 and Shannon) by frailty status, device type, antibiotic use and by study visit among 301 residents at a single skilled nursing facility (SNF) participating in the Tracking Acute Respiratory Illness in Older Adults (TARI) prospective cohort study enrolled April 5, 2021 through May 22, 2023. Kruskal-Wallis tests were performed to identify significant differences by Chao1 or Shannon across each health characteristic subgroup and study visit independently. Bold indicates those that were significantly different.

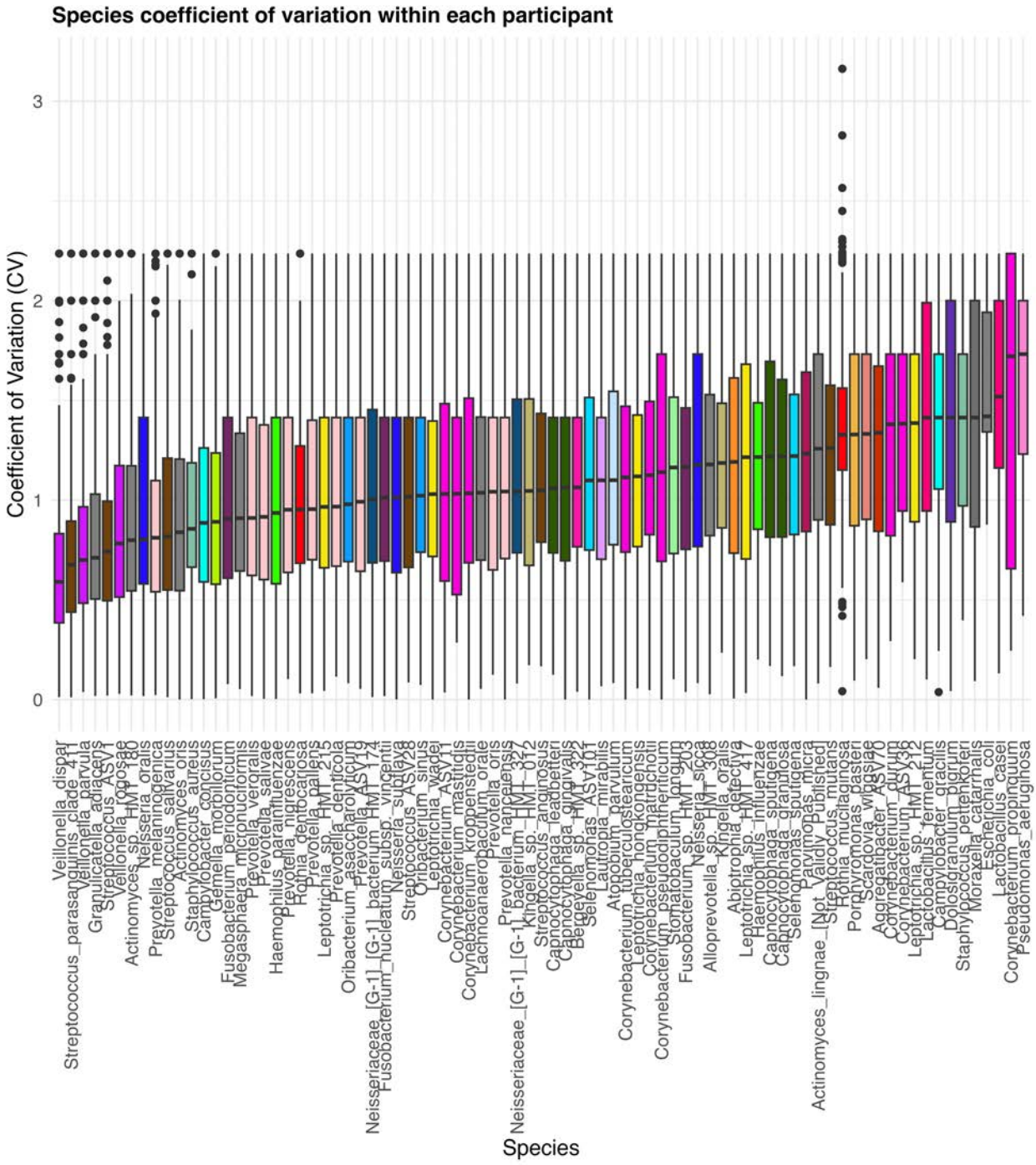
		Alpha Diversity Measures by Study Visit (Mean ± SD)				
Health Characteristics	**Measure**	**Study Visit 1**	**Study Visit 2**	**Study Visit 3**	**Study Visit 4**	**Study Visit 5**
FRAILTY STATUS						
Non-Frail	Chao1	38.8 ± 15.43	40.82 ± 15.56	39.63 ± 15.68	41.76 ± 16.77	42.07 ± 15.9
Frail	Chao1	37.71 ± 14.95	37.76 ± 14.98	37.88 ± 15.12	39.76 ± 14.38	37.4 ± 13.79
Non-Frail	Shannon	2.4 ± 0.65	2.47 ± 0.64	2.45 ± 0.62	2.46 ± 0.68	2.5 ± 0.67
Frail	Shannon	2.41 ± 0.61	2.37 ± 0.62	2.42 ± 0.65	2.48 ± 0.62	2.41 ± 0.62
DEVICE TYPE						
No devices	Chao1	**40.43 ± 14.74**	**41.09 ± 14.63**	**41.26 ± 14.77**	**43.52 ± 14.34**	**42.69 ± 13.63**
Respiratory or non-surgical tubing	Chao1	**34.18 ± 13.65**	**38.74 ± 15.53**	**33.08 ± 13.45**	**37.05 ± 16.51**	**34.27 ± 16.36**
Surgical drain or vascular access	Chao1	**24.18 ± 12.14**	**23.73 ± 12.24**	**25.88 ± 14.08**	**25.48 ± 12.65**	**26.12 ± 12.7**
No devices	Shannon	**2.49 ± 0.6**	**2.51 ± 0.57**	**2.52 ± 0.58**	**2.56 ± 0.62**	**2.57 ± 0.55**
Respiratory or non-surgical tubing	Shannon	**2.25 ± 0.55**	**2.32 ± 0.78**	**2.31 ± 0.67**	**2.48 ± 0.58**	**2.28 ± 0.71**
Surgical drain or vascular access	Shannon	**1.86 ± 0.59**	**1.75 ± 0.56**	**1.91 ± 0.75**	**1.86 ± 0.6**	**1.9 ± 0.76**
ANTIBIOTIC USAGE						
No antibiotics	Chao1	**43.71 ± 13.16**	**45.04 ± 13.85**	**43.96 ± 14.61**	**46.98 ± 13.52**	**45.5 ± 13.5**
Antibiotics prior to study only	Chao1	**33.61 ± 14.1**	**35.14 ± 13.98**	**35.92 ± 13.61**	**35.6 ± 14.57**	**38.68 ± 14.69**
Any antibiotics during the study	Chao1	**31.02 ± 15.94**	**30.9 ± 14.71**	**30.17 ± 14.63**	**32 ± 14.3**	**30.86 ± 12.9**
No antibiotics	Shannon	**2.6 ± 0.52**	**2.62 ± 0.52**	**2.63 ± 0.52**	**2.64 ± 0.6**	**2.61 ± 0.57**
Antibiotics prior to study only	Shannon	**2.33 ± 0.59**	**2.31 ± 0.63**	**2.34 ± 0.57**	**2.36 ± 0.65**	**2.47 ± 0.61**
Any antibiotics during the study	Shannon	**2.06 ± 0.72**	**2.1 ± 0.68**	**2.09 ± 0.77**	**2.19 ± 0.64**	**2.17 ± 0.72**



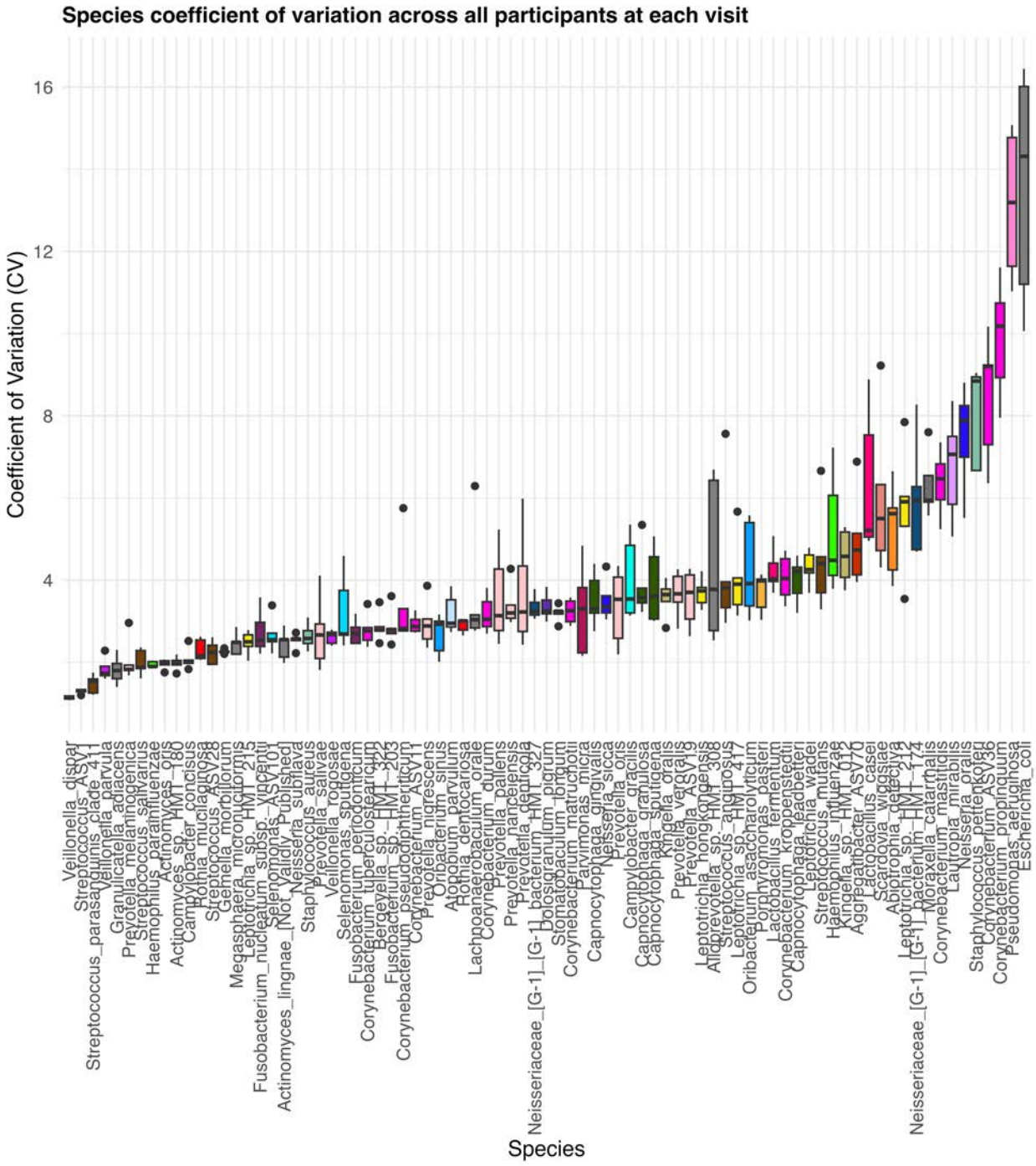
Supplemental Figure 4.3 Bray-Curtis dissimilarity distance by participant comparing each study visit to baseline (study visit 1) by (A) frailty status (B) device type (C) antibiotic usage. Summary line was generated using LOESS regression to capture the central trend across all participants.



Supplemental Figure 4.4 Bray-Curtis dissimilarity distance by participant comparing each study visit to the consecutive visit by (A) frailty status (B) device type (C) antibiotic usage. Summary line was generated using LOESS regression to capture the central trend across all participants.



Supplemental Figure 4.5 Coefficient of variation evaluating species stability within each participant colored by genus.



Supplemental Figure 4.6 Coefficient of variation evaluating species stability across all participants colored by genus

Supplemental Table 4.2 Reference taxa identified by independent ADAPT analysis for each study visit comparing those with no colonization (REF) to any colonization of Candida albicans or Staphylococcus aureus. Table summarizes which taxa were identified as reference for which study visits and details median coefficient of variation (CV) within and across participants for these taxa. Rows in bold are taxa that were identified as reference taxa across all independent analyses for each of the five study visits.

					Visits Where Identified as Reference Taxa[1]				
	Taxa	Species Name	Median CV Within Participants	Median CV Across Participants	Visit1	Visit2	Visit3	Visit4	Visit5
1	ASV1	*Streptococcus_ASV1*	0.743	1.311	Visit 1	-	-	Visit 4	Visit 5
2	**ASV4**	***Streptococcus_salivarius***	**0.817**	**1.871**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**
3	ASV6	*Veillonella_parvula*	0.700	1.728	Visit 1	-	-	Visit 4	Visit 5
4	**ASV7**	***Prevotella_melaninogenica***	**0.811**	**1.824**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**
5	ASV10	*Streptococcus_parasanguinis_clade_411*	0.676	1.533	Visit 1	Visit 2	-	Visit 4	Visit 5
6	ASV11	*Corynebacterium_ASV11*	1.031	2.872	Visit 1	-	-	-	-
7	ASV12	*Gemella_morbillorum*	0.891	2.257	Visit 1	-	Visit 3	Visit 4	Visit 5
8	ASV13	*Corynebacterium_pseudodiphtheriticum*	1.140	2.822	Visit 1	-	-	-	-
9	ASV14	*Dolosigranulum_pigrum*	1.414	3.243	Visit 1	-	-	-	-
10	ASV15	*Rothia_mucilaginosa*	1.328	2.155	Visit 1	-	-	Visit 4	Visit 5
11	ASV17	*Neisseria_sicca*	1.177	3.350	Visit 1	Visit 2	Visit 3	-	Visit 5

	Taxa	Species Name	Median CV Within Participants	Median CV Across Participants	Visits Where Identified as Reference Taxa[1]				
					Visit1	Visit2	Visit3	Visit4	Visit5
12	ASV19	*Prevotella_ASV19*	0.992	3.702	Visit 1	-	-	-	Visit 5
13	**ASV20**	***Granulicatella_adiacens***	**0.711**	**1.798**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**
14	ASV22	*Rothia_mucilaginosa*	1.328	2.155	Visit 1	-	-	-	-
15	ASV23	*Actinomyces_oris*	0.839	1.984	Visit 1	-	-	-	Visit 5
16	ASV27	*Capnocytophaga_gingivalis*	1.063	3.304	Visit 1	-	Visit 3	Visit 4	Visit 5
17	ASV29	*Actinomyces_sp._HMT_180*	0.799	1.995	Visit 1	Visit 2	-	Visit 4	Visit 5
18	ASV39	*Prevotella_nigrescens*	0.952	2.883	Visit 1	-	Visit 3	Visit 4	Visit 5
19	**ASV41**	***Megasphaera_micronuciformis***	**0.910**	**2.485**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**
20	ASV42	*Prevotella_veroralis*	0.911	3.666	Visit 1	Visit 2	Visit 3	-	Visit 5
21	ASV47	*Campylobacter_concisus*	0.886	2.027	Visit 1	-	-	-	Visit 5
22	ASV48	*Prevotella_salivae*	0.916	2.664	Visit 1	-	Visit 3	Visit 4	-
23	ASV54	*Oribacterium_sinus*	1.022	2.928	Visit 1	-	-	Visit 4	Visit 5
24	ASV59	*Kingella_sp._HMT_012*	1.046	4.578	Visit 1	-	Visit 3	-	-

	Taxa	Species Name	Median CV Within Participants	Median CV Across Participants	Visits Where Identified as Reference Taxa[1]				
					Visit1	Visit2	Visit3	Visit4	Visit5
25	ASV61	*Leptotrichia_sp._HMT_212*	1.386	5.907	Visit 1	-	-	-	Visit 5
26	ASV63	*Prevotella_pallens*	0.955	3.133	Visit 1	-	-	-	-
27	ASV68	*Capnocytophaga_granulosa*	1.220	3.574	Visit 1	-	-	Visit 4	Visit 5
28	ASV72	*Kingella_oralis*	1.186	3.647	Visit 1	-	Visit 3	Visit 4	Visit 5
29	ASV81	*Lachnoanaerobaculum_orale*	1.037	3.035	Visit 1	-	-	Visit 4	Visit 5
30	ASV84	*Leptotrichia_wadei*	1.030	4.254	Visit 1	-	-	Visit 4	Visit 5
31	ASV88	*Corynebacterium_kroppenstedtii*	1.034	4.040	Visit 1	-	-	-	-
32	ASV89	*Alloprevotella_sp._HMT_308*	1.179	3.773	Visit 1	Visit 2	-	Visit 4	-
33	ASV90	*Neisseriaceae_[G-1]_[G-1]_bacterium_ HMT_174*	1.004	5.940	Visit 1	-	-	-	-
34	ASV94	*Corynebacterium_durum*	1.381	3.044	Visit 1	-	-	Visit 4	-
35	ASV95	*Actinomyces_lingnae_[Not_Validly_ Published]*	1.258	2.551	Visit 1	Visit 2	Visit 3	-	Visit 5
36	ASV96	*Leptotrichia_sp._HMT_417*	1.215	3.900	Visit 1	-	-	-	Visit 5
37	ASV98	*Selenomonas_sputigena*	1.221	2.688	Visit 1	Visit 2	Visit 3	Visit 4	-

					Visits Where Identified as Reference Taxa[1]				
	Taxa	Species Name	Median CV Within Participants	Median CV Across Participants	Visit1	Visit2	Visit3	Visit4	Visit5
38	ASV107	*Stomatobaculum_longum*	1.164	3.244	Visit 1	-	Visit 3	Visit 4	-
39	ASV108	*Campylobacter_gracilis*	1.414	3.540	Visit 1	-	-	Visit 4	Visit 5
40	ASV110	*Parvimonas_micra*	1.233	3.297	Visit 1	Visit 2	-	-	Visit 5
41	ASV21	*Rothia_dentocariosa*	0.954	3.009	-	Visit 2	-	-	Visit 5
42	ASV35	*Corynebacterium_tuberculostearicum*	1.114	2.762	-	Visit 2	-	-	-
43	ASV49	*Leptotrichia_hongkongensis*	1.119	3.738	-	Visit 2	-	Visit 4	-
44	ASV57	*Capnocytophaga_leadbetteri*	1.060	4.215	-	Visit 2	-	Visit 4	-
45	ASV62	*Neisseriaceae_[G-1]_[G-1]_bacterium_HMT_327*	1.043	3.243	-	Visit 2	Visit 3	Visit 4	-
46	ASV101	*Selenomonas_ASV101*	1.098	2.547	-	Visit 2	-	Visit 4	Visit 5
47	ASV105	*Atopobium_parvulum*	1.100	2.949	-	Visit 2	-	Visit 4	Visit 5
48	ASV55	*Prevotella_oris*	1.042	3.532	-	-	Visit 3	Visit 4	Visit 5
49	ASV80	*Staphylococcus_pettenkoferi*	1.414	8.846	-	-	Visit 3	Visit 4	Visit 5
50	ASV85	*Bergeyella_sp._HMT_322*	1.064	2.782	-	-	Visit 3	Visit 4	Visit 5

	Taxa	Species Name	Median CV Within Participants	Median CV Across Participants	Visits Where Identified as Reference Taxa[1]				
					Visit1	Visit2	Visit3	Visit4	Visit5
51	ASV2	*Veillonella_dispar*	0.590	1.139	-	-	-	Visit 4	Visit 5
52	ASV8	*Haemophilus_parainfluenzae*	0.936	1.896	-	-	-	Visit 4	-
53	ASV51	*Fusobacterium_nucleatum_subsp._vincentii*	1.012	2.540	-	-	-	Visit 4	Visit 5
54	ASV52	*Fusobacterium_sp._HMT_203*	1.166	2.799	-	-	-	Visit 4	-
55	ASV60	*Capnocytophaga_sputigena*	1.218	3.605	-	-	-	Visit 4	Visit 5
56	ASV66	*Porphyromonas_pasteri*	1.329	3.982	-	-	-	Visit 4	-
57	ASV79	*Streptococcus_mutans*	1.261	4.402	-	-	-	Visit 4	-
58	ASV99	*Oribacterium_asaccharolyticum*	0.979	3.914	-	-	-	Visit 4	-
59	ASV33	*Haemophilus_influenzae*	1.216	4.482	-	-	-	-	Visit 5
60	ASV40	*Lautropia_mirabilis*	1.099	7.062	-	-	-	-	Visit 5
61	ASV43	*Prevotella_denticola*	0.967	3.223	-	-	-	-	Visit 5
62	ASV83	*Neisseria_oralis*	0.802	7.885	-	-	-	-	Visit 5

Supplemental Table 4.3 Differentially abundant taxa identified by ADAPT for (A) any device use [REF: no devices], (B) respiratory or non-surgical tubing [REF: no devices], and (C) surgical drains or vascular access devices [REF: no devices], (D) any antibiotics use [REF: no antibiotics], (E) only prior antibiotics [REF: no antibiotics], (F) any antibiotics during study [REF: no antibiotics] adjusting for age and *Candida albicans* colonization. The log10 fold change informs the direction of effect and the adjusted p-value informs significance; this adjusts for multiple hypothesis testing using the Benjamini-Hochberg correction with values <0.05 being significant. Species are ordered by significance level beginning with the most significant.

A. No devices (REF) vs. any device (respiratory, non-surgical tubing, surgical drain, or vascular access)

	Species Name	Log10 fold change	Adjusted p-value	Direction of Effect
1	*Corynebacterium_matruchotii**	-1.79	0.0001	Decreasing abundance
2	*Lactobacillus_casei**	3.10	0.0001	Increasing abundance
3	*Fusobacterium_periodonticum**	-1.76	0.002	Decreasing abundance
4	*Staphylococcus_aureus**	0.67	0.008	Increasing abundance
5	*Capnocytophaga_leadbetteri*	-1.65	0.008	Decreasing abundance
6	*Aggregatibacter_ASV70*	-2.78	0.008	Decreasing abundance
7	*Corynebacterium_durum**	-1.35	0.008	Decreasing abundance
8	*Porphyromonas_pasteri**	-1.47	0.013	Decreasing abundance
9	*Lactobacillus_fermentum*	1.83	0.019	Increasing abundance
10	*Staphylococcus_pettenkoferi*	2.46	0.022	Increasing abundance
11	*Capnocytophaga_granulosa**	-1.48	0.029	Decreasing abundance
12	*Actinomyces_oris**	-0.65	0.034	Decreasing abundance
13	*Veillonella_rogosae**	-1.48	0.038	Decreasing abundance
14	*Kingella_oralis**	-1.22	0.039	Decreasing abundance
15	*Pseudomonas_aeruginosa**	2.65	0.047	Increasing abundance

*Species also differentially abundant in one of split independent analyses

B. No devices (REF) vs. respiratory or non-surgical tubing

	Species Name	Log10 fold change	Adjusted p-value	Direction of Effect
1	*Actinomyces_sp._HMT_180***	-0.66	0.032	Decreasing abundance
2	*Fusobacterium_periodonticum*	-1.99	0.032	Decreasing abundance
3	*Corynebacterium_matruchotii*	-1.42	0.032	Decreasing abundance
4	*Veillonella_rogosae*	-2.27	0.045	Decreasing abundance
5	*Porphyromonas_pasteri*	-1.85	0.045	Decreasing abundance
6	*Capnocytophaga_granulosa*	-1.84	0.045	Decreasing abundance
7	*Lactobacillus_casei*	1.75	0.045	Increasing abundance

**Species was differentially abundant only in this device type.

C. No devices (REF) vs. surgical drain or vascular access

	Species Name	Log10 fold change	Adjusted p-value	Direction of Effect
1	*Lactobacillus_casei*	3.04	0.002	Increasing abundance
2	*Streptococcus_ASV1***	-0.56	0.009	Decreasing abundance
3	*Staphylococcus_aureus*	0.73	0.009	Increasing abundance
4	*Actinomyces_oris*	-0.97	0.009	Decreasing abundance
5	*Corynebacterium_matruchotii*	-1.89	0.009	Decreasing abundance
6	*Oribacterium_sinus***	-1.98	0.009	Decreasing abundance
7	*Kingella_oralis*	-2.46	0.009	Decreasing abundance
8	*Selenomonas_ASV101***	-1.40	0.009	Decreasing abundance
9	*Corynebacterium_durum*	-1.64	0.024	Decreasing abundance
10	*Streptococcus_ASV28***	-1.41	0.024	Decreasing abundance
11	*Leptotrichia_sp._HMT_417***	-2.76	0.024	Decreasing abundance
12	*Pseudomonas_aeruginosa*	3.52	0.025	Increasing abundance
13	*Atopobium_parvulum***	-1.22	0.025	Decreasing abundance
14	*Fusobacterium_periodonticum*	-1.89	0.026	Decreasing abundance
15	*Streptococcus_mutans***	-1.61	0.026	Decreasing abundance
16	*Parvimonas_micra***	-1.28	0.030	Decreasing abundance
17	*Granulicatella_adiacens***	-0.41	0.030	Decreasing abundance
18	*Fusobacterium_nucleatum_subsp._vincentii***	-1.07	0.0496	Decreasing abundance

**Species was differentially abundant only in this device type.

Supplemental Table 4.4 Differentially abundant taxa identified by ADAPT for (A) any antibiotics use [REF: no antibiotics], (B) only prior antibiotics [REF: no antibiotics], (C) any antibiotics during study [REF: no antibiotics] adjusting for age and *Candida albicans* colonization. The log10 fold change informs the direction of effect and the adjusted p-value informs significance; this adjusts for multiple hypothesis testing using the Benjamini-Hochberg correction with values <0.05 being significant. Species are ordered by significance level beginning with the most significant.

A. No antibiotics (REF) vs. any antibiotics (prior only or any study)

	Species Name	Log10 fold change	Adjusted p-value	Direction of Effect
1	*Corynebacterium_matruchotii**	-1.61	0.00000004	Decreasing abundance
2	*Lactobacillus_casei**	2.39	0.00005	Increasing abundance
3	*Gemella_morbillorum**	-0.67	0.0001	Decreasing abundance
4	*Kingella_oralis**	-1.45	0.0001	Decreasing abundance
5	*Aggregatibacter_ASV70**	-2.27	0.0002	Decreasing abundance
6	*Haemophilus_parainfluenzae**	-0.75	0.0004	Decreasing abundance
7	*Haemophilus_influenzae**	-1.57	0.0004	Decreasing abundance
8	*Scardovia_wiggsiae**	1.16	0.0005	Increasing abundance
9	*Corynebacterium_ASV36**	2.76	0.001	Increasing abundance
10	*Fusobacterium_nucleatum_subsp._vincentii**	-0.88	0.001	Decreasing abundance
11	*Capnocytophaga_leadbetteri**	-1.21	0.001	Decreasing abundance
12	*Fusobacterium_sp._HMT_203**	-0.99	0.002	Decreasing abundance
13	*Lactobacillus_fermentum**	1.54	0.003	Increasing abundance
14	*Alloprevotella_sp._HMT_308*	-0.85	0.003	Decreasing abundance
15	*Leptotrichia_sp._HMT_417**	-1.37	0.003	Decreasing abundance
16	*Fusobacterium_periodonticum*	-1.12	0.006	Decreasing abundance
17	*Oribacterium_asaccharolyticum**	-0.81	0.006	Decreasing abundance
18	*Prevotella_nigrescens*	-0.82	0.008	Decreasing abundance
19	*Leptotrichia_sp._HMT_212*	-1.61	0.008	Decreasing abundance
20	*Prevotella_nanceiensis*	-1.02	0.008	Decreasing abundance
21	*Corynebacterium_durum*	-0.80	0.008	Decreasing abundance
22	*Leptotrichia_sp._HMT_215*	-0.98	0.009	Decreasing abundance
23	*Prevotella_pallens*	-0.85	0.013	Decreasing abundance
24	*Porphyromonas_pasteri**	-0.97	0.016	Decreasing abundance
25	*Neisseria_subflava*	-0.75	0.016	Decreasing abundance
26	*Oribacterium_sinus**	-0.78	0.016	Decreasing abundance
27	*Campylobacter_concisus*	-0.49	0.017	Decreasing abundance
28	*Pseudomonas_aeruginosa*	1.97	0.020	Increasing abundance

29	*Dolosigranulum_pigrum*	-0.88	0.027	Decreasing abundance
30	*Selenomonas_ASV101*	-0.54	0.027	Decreasing abundance
31	*Prevotella_oris*	-0.72	0.029	Decreasing abundance
32	*Rothia_mucilaginosa*	-0.88	0.033	Decreasing abundance

*Species also differentially abundant in one of split independent analyses

B. No antibiotics (REF) vs. prior antibiotics only

	Species Name	Log10 fold change	Adjusted p-value	Direction of Effect
1	*Corynebacterium_matruchotii*	-1.54	0.00002	Decreasing abundance
2	*Lactobacillus_casei*	2.30	0.001	Increasing abundance
3	*Scardovia_wiggsiae*	1.24	0.002	Increasing abundance
4	*Corynebacterium_ASV36*	2.94	0.003	Increasing abundance
5	*Capnocytophaga_leadbetteri*	-1.50	0.004	Decreasing abundance
6	*Haemophilus_influenzae*	-1.57	0.004	Decreasing abundance
7	*Lactobacillus_fermentum*	1.65	0.004	Increasing abundance
8	*Aggregatibacter_ASV70*	-2.12	0.009	Decreasing abundance
9	*Kingella_sp._HMT_012***	-1.83	0.013	Decreasing abundance
10	*Haemophilus_parainfluenzae*	-0.68	0.016	Decreasing abundance
11	*Oribacterium_asaccharolyticum*	-0.93	0.020	Decreasing abundance
12	*Veillonella_dispar***	0.31	0.026	Increasing abundance
13	*Kingella_oralis*	-0.98	0.026	Decreasing abundance
14	*Oribacterium_sinus*	-0.95	0.033	Decreasing abundance
15	*Gemella_morbillorum*	-0.48	0.035	Decreasing abundance
16	*Leptotrichia_sp._HMT_417*	-1.29	0.035	Decreasing abundance
17	*Porphyromonas_pasteri*	-1.13	0.043	Decreasing abundance
18	*Staphylococcus_aureus*	0.38	0.046	Increasing abundance
19	*Streptococcus_parasanguinis_clade_411***	0.28	0.046	Increasing abundance
20	*Fusobacterium_sp._HMT_203*	-0.84	0.046	Decreasing abundance

**Species was differentially abundant only in this antibiotic group.

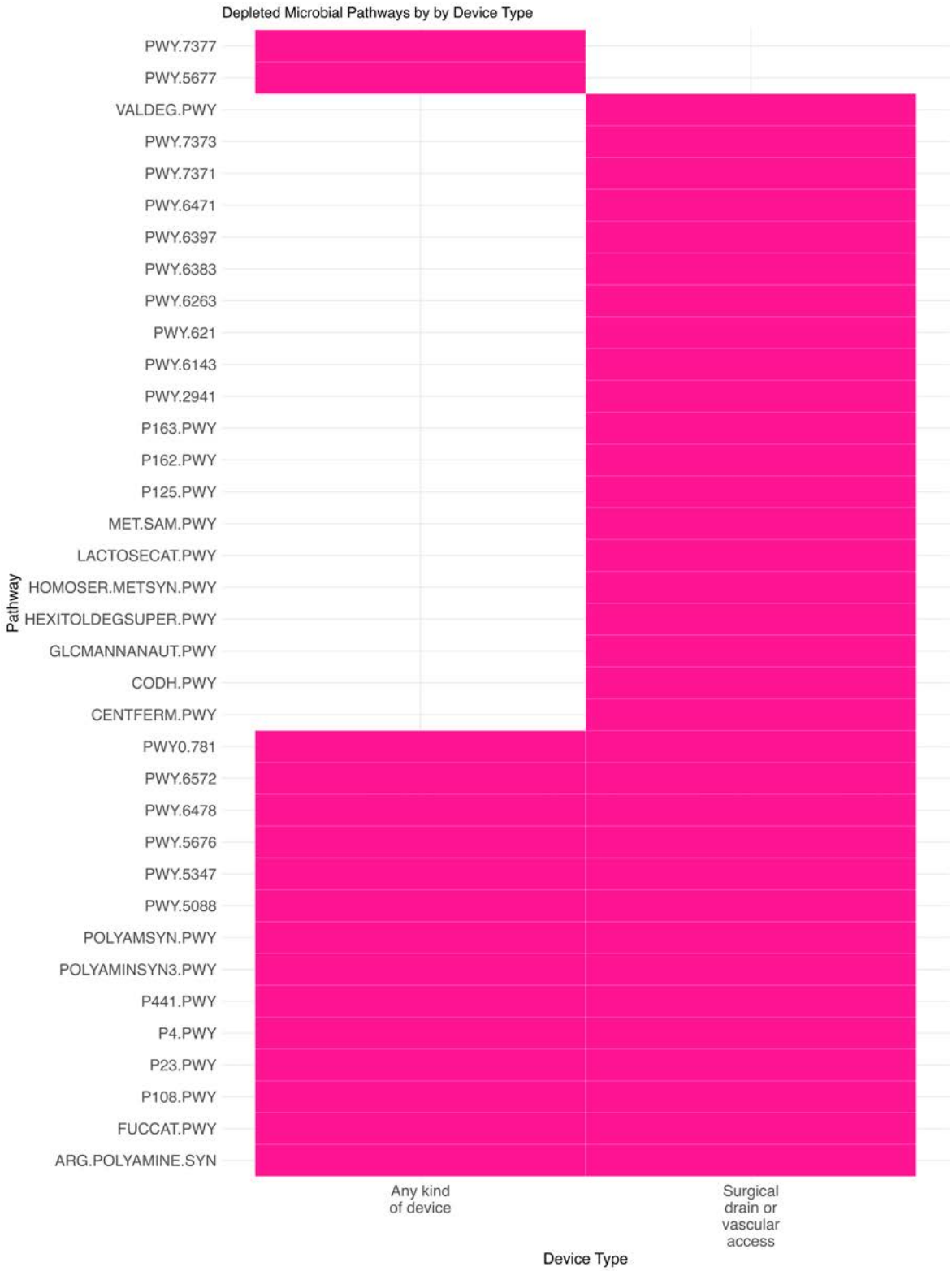
C. No antibiotics (REF) vs. any antibiotics during study

	Species Name	Log10 fold change	Adjusted p-value	Direction of Effect
1	*Lactobacillus_casei*	2.81	0.0001	Increasing abundance
2	*Corynebacterium_matruchotii*	-1.37	0.0001	Decreasing abundance
3	*Fusobacterium_nucleatum_subsp._vincentii*	-1.17	0.0002	Decreasing abundance
4	*Kingella_oralis*	-1.62	0.0002	Decreasing abundance
5	*Scardovia_wiggsiae*	1.24	0.004	Increasing abundance

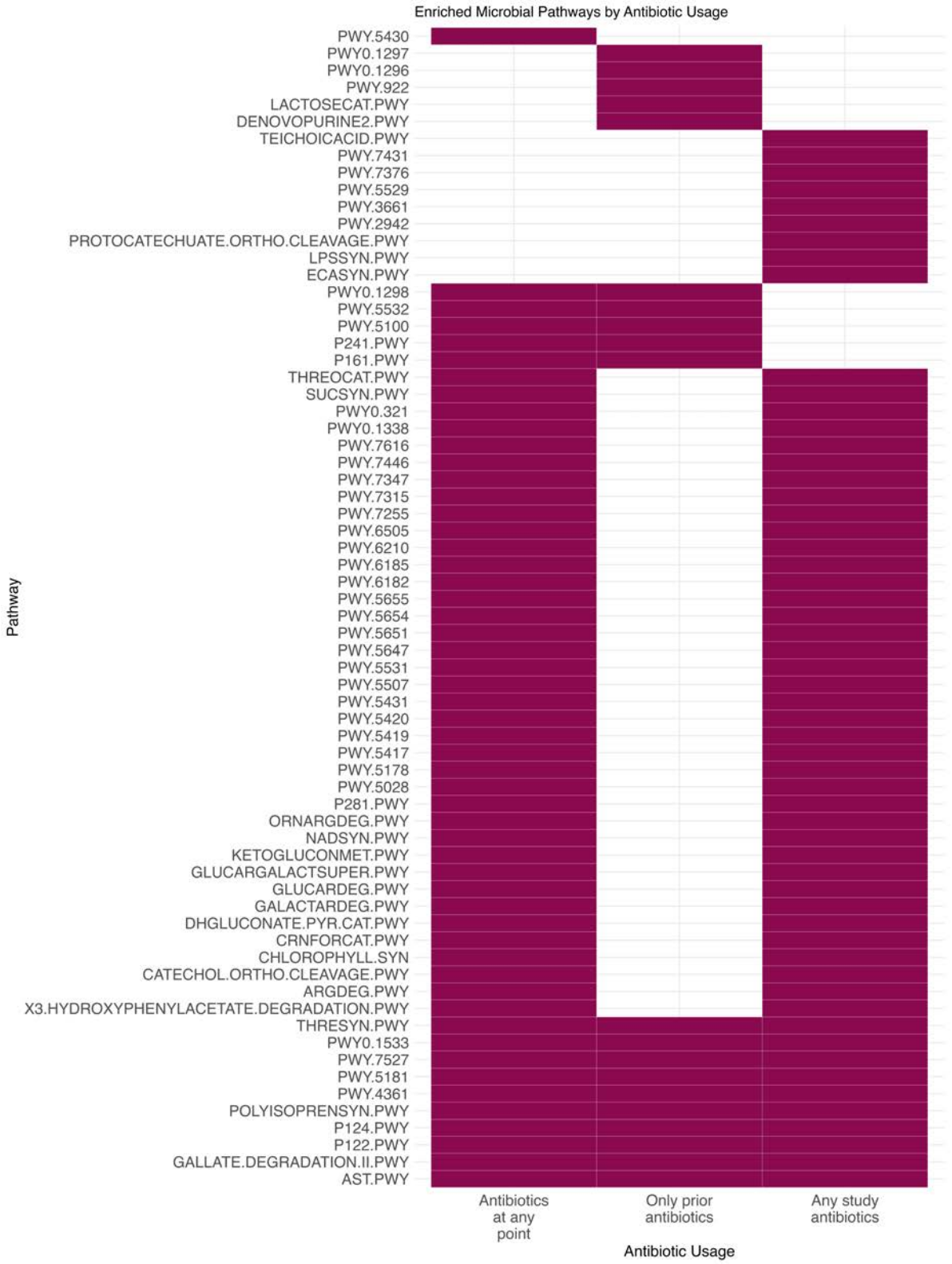
6	*Staphylococcus_aureus*	0.58	0.006	Increasing abundance
7	*Corynebacterium_ASV36*	3.56	0.012	Increasing abundance
8	*Aggregatibacter_ASV70*	-2.37	0.013	Decreasing abundance
9	*Lactobacillus_fermentum*	1.51	0.015	Increasing abundance



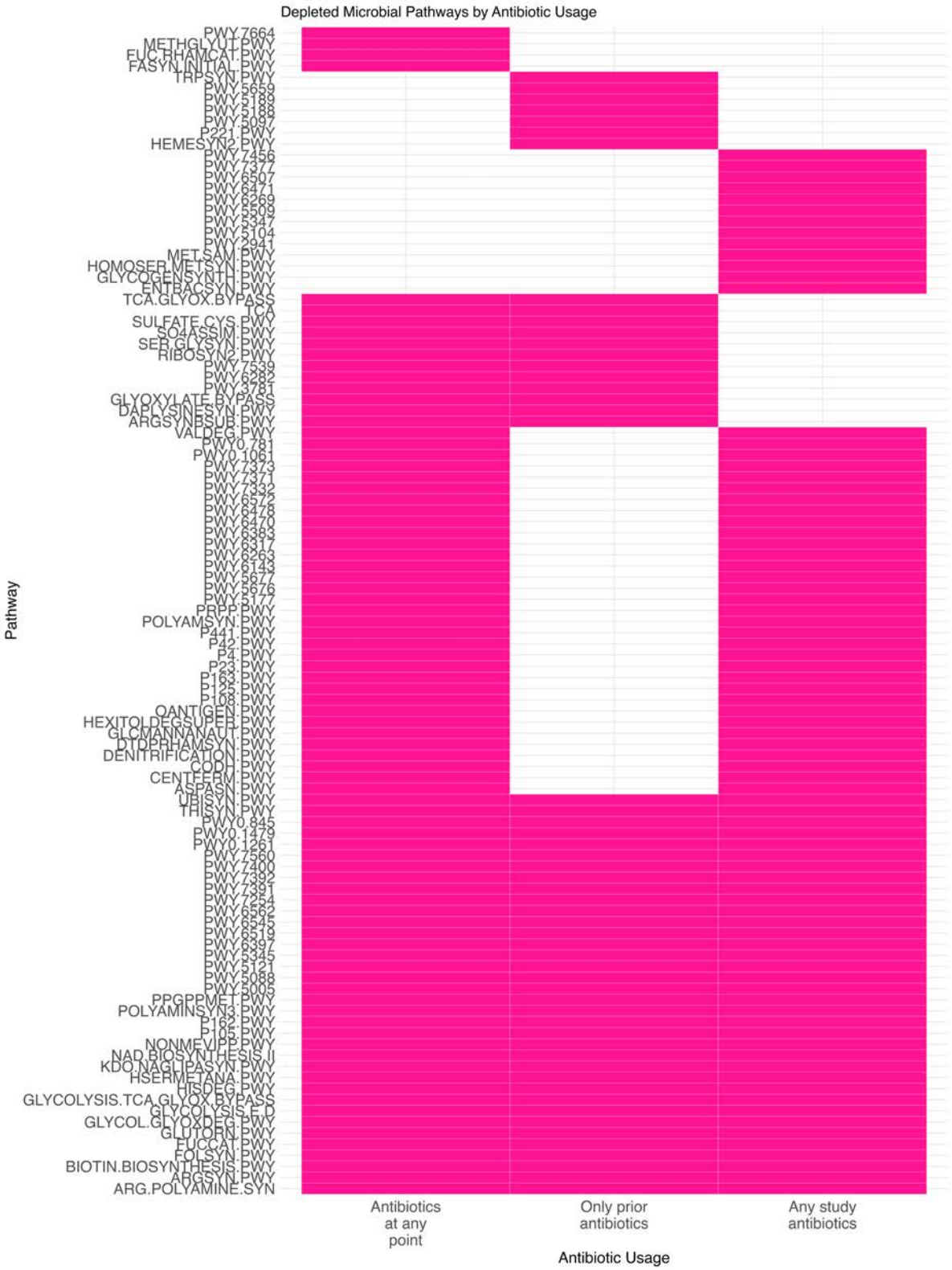
Supplemental Figure 4.7 Microbial functional pathways inferred using PICRUSt2 and identified as enriched using ADAPT differential abundance based on timing of antibiotic usage and adjusted for age and *Candida albicans* colonization. Significance is based on adjusted p-value < 0.05 correcting for multiple hypothesis testing using Benjamini-Hochberg correction. Log10 fold change informs if enriched or depleted.



Supplemental Figure 4.8 Microbial functional pathways inferred using PICRUSt2 and identified as depleted using ADAPT differential abundance based on timing of antibiotic usage and adjusted for age and *Candida albicans* colonization. Significance is based on adjusted p-value < 0.05 correcting for multiple hypothesis testing using Benjamini-Hochberg correction. Log10 fold change informs if enriched or depleted.



Supplemental Figure 4.9 Microbial functional pathways inferred using PICRUSt2 and identified as enriched using ADAPT differential abundance based on device type and adjusted for age and *Candida albicans* colonization. Significance is based on adjusted p-value < 0.05 correcting for multiple hypothesis testing using Benjamini-Hochberg correction. Log10 fold change informs if enriched or depleted.



Supplemental Figure 4.10 Microbial functional pathways inferred using PICRUSt2 and identified as depleted using ADAPT differential abundance based on device type and adjusted for age and Candida albicans colonization. Significance is based on adjusted p-value < 0.05 correcting for multiple hypothesis testing using Benjamini-Hochberg correction. Log10 fold change informs if enriched or depleted.

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4.10 Supplemental Methods

4.10.1 Sequencing

Sequencing Samples were sequenced over 4 runs (4 plates for the first 3 runs and 3 plates for the last run). Each plate included a positive mock control, ZymoBIOMICS Microbial Community DNA Standard (Zymo Research cat# D6306; which is a mixture of genomic DNA extracted from pure cultures of eight bacterial and two fungal strains) and 2 negative controls (extraction control and water control). We processed sequenced reads. Sequencing was done on the Illumina MiSeq platform, using a MiSeq Reagent Kit V2 500 cycles (Illumina cat# MS102-2003), according to the manufacturer's instructions with modifications found in the Schloss SOP. Accuprime High Fidelity Taq (Life Technologies cat # 12346094) was used instead of Accuprime Pfx supermix. PCR products are visualized using an E-Gel 96 with SYBR Safe DNA Gel Stain, 2% (Life technologies cat# G7208-02). Libraries are normalized using SequalPrep Normalization Plate Kit (Life technologies cat # A10510-01) following the manufacturer's protocol for sequential elution. The concentration of the pooled samples is determined using Kapa Biosystems Library Quantification kit for Illumina platforms (KapaBiosystems KK4824). The sizes of the amplicons in the library are determined using the Agilent Bioanalyzer High Sensitivity DNA analysis kit (cat# 5067-4626). The final library consists of equal molar amounts from each of the plates, normalized to the pooled plate at the lowest concentration. Library Preparation for Sequencing and Sequencing Libraries are prepared according to Illumina's protocol for Preparing Libraries for Sequencing on the MiSeq (part# 15039740 Rev. D). PhiX (15%) was added in 16s amplicon sequencing to create diversity. Sequencing reagents are prepared according to the Schloss SOP, custom read 1, read 2 and index primers are added to the reagent cartridge. FASTQ files are generated for paired end reads. DNA was quantified using Quant-iT PicoGreen dsDNA Assay kit (cat#: P7589).

4.10.2 DADA2 pipeline

Reads were processed to amplicon sequence variants (ASVs) using DADA2 (version 1.30.1) and the expanded Human Oral Microbiome Database (eHOMD). After examining quality plots for forward and reverse reads of each run, reads were trimmed at the 240th and 230th nucleotide position respectively for run one, 240th and 200th for run two, 245th and 235th for run three, and 245th and 225th for run four, or at the first instance of a quality score <= to 11.

The following parameters were used for filtering reads: a maximum of 2 expected errors for both forward and reverse reads and no ambiguous bases. Errors were learned (independently for each run) using 1 million bases. The forward and reverse reads were denoised and then forward and reverse reads were merged together. At this point, the runs were merged together, and chimeras were removed using DADA2s removeBimeraDenovo command using the consensus method. Subsequently, reads were assigned taxonomic names to the species level using the assignTaxonomy and the addSpecies functions of "dada2" package.

Chapter 5 Summary and Closing Thoughts

5.1 Public Health Significance

As the American population continues to rapidly age, the demand for short-term, subacute care, particularly skilled nursing facilities (SNFs), is steadily rising. This trend is not only driven by demographic shifts but also changes in Medicare policies and broader efforts to reduce healthcare costs. Hospitalizations are expensive, and prolonged stays increase a patient's exposure to hospital-acquired infections. As a result, many patients are now discharged from hospitals to SNFs for continued recovery in a lower-cost setting. SNFs provide essential transitional care, especially in cases where a partner or caregiver is unable to fully manage care without trained professionals. These facilities are often supported through Medicare Part A, which covers a 100-day stay in a SNF following a qualifying hospital stay. This dissertation spans multiple dimensions of health among older adults recovering at a SNF and highlights opportunities to enhance care planning, reduce hospital readmissions, and improve health outcomes in this vulnerable population.

In Chapter 2, I examined the population characteristics of SNF residents and found that respiratory symptoms, particularly cough and body aches, were common upon entry but declined over time. These non-specific symptoms may reflect the physiological stress and trauma of recent hospitalization rather than acute illness, underscoring the need for careful clinical interpretation rather than immediate intervention. I also identified that dependence for activities of daily living (ADL) and the presence of a vascular access or surgical drain were the strongest predictors of a longer length of stay. Understanding how characteristics influence length of stay can inform discharge planning for families and resource allocation for facilities. Notably, I introduced a new hypothesis into the literature, a potential "healthy-elder" bias where older adults who utilize SNF care may represent a healthier subset of older adults who have higher baseline health, potentially influencing how we understand recovery trajectories in this setting.

In Chapter 3, I found that poor self-assessed oral health was significantly associated with frailty and modified by age, chronic respiratory disease, ADL dependence, and antidepressant

use. This subjective measure was consistent with findings using dental examination, suggesting it may serve as a simple, non-invasive screening tool to identify residents at risk for poor oral health, frailty, and related outcomes. Given limited access to dental care in SNFs, due to resource contracts and short lengths of stay, this tool could be integrated into routine care to improve early detection and inform individualized care plans.

In Chapter 4, I evaluated the short-term dynamics of the nose/throat microbiome and found that microbial composition remained relatively stable over time. This represents a novel contribution to the literature, as few microbiome studies have focused on older adults, and none have examined changes every few days. I found that microbial diversity was lowest among those who were frail, had a device, or were taking antibiotics; these factors, all highly prevalent exposures in SNFs, also influenced overall composition with discriminatory taxa identified by device type and timing of antibiotic usage. Additionally, changes in microbial function were observed including depletion of the denitrification, polyamine synthesis, and sulfate-cysteine biosynthesis pathways that are essential among older adults due to their roles in biofilm formation, immune response, cardiovascular health, and oxidative stress. These findings highlight the potential importance of microbiome resilience in supporting recovery and suggest that preserving microbial balance and function may play a critical role in recovery outcomes.

Together, these chapters contribute important insights into the health of SNF residents and offer practical tools and considerations for clinicians, caregivers, and policymakers. By deepening our understanding of resident characteristics, oral health, and the microbiome, this dissertation supports more effective, personalized care planning and discharge strategies. A key strength of this work is the use of a prospective cohort study, with over 74% of participants completed at least study visits. We collected detailed socio-demographic data, health behaviors, and medical histories using Minimum Data Set (MDS), ensuring a comprehensive dataset with minimal missing information. Frailty was assessed using the validated Frailty Short Interview, strengthening the reliability of our functional assessments. We also incorporated fungal-bacterial interactions, specifically the role of *Candida albicans,* into our analysis of the nose/throat microbiome, providing a more holistic view of the microbial ecology. Ultimately these efforts can contribute to improving quality of life, reducing unnecessary hospital readmissions, and lowering the overall costs associated with post-acute care.

5.2 Future Directions

Research on older adults in SNFs remains limited, especially compared to studies on community-dwelling and long-term nursing home residents. As demand for post-acute care continues to grow and national investment in these care facilities continues to increase, additional research is needed to better understand the unique health needs and recovery trajectories of this population. Our study focused on a single, non-profit SNF in Southeast Michigan, located in a predominantly White community. Given known disparities in access and quality of skilling nursing care, future research should investigate whether our findings hold across diverse SNF populations nationwide. Furthermore, with the ongoing shift from non-profit to for-profit SNF ownership, it is critical to assess how these structural changes may influence patient outcomes and potentially widen existing health disparities.

5.3 Personal Reflection

Pursuing my doctoral degree has been a challenging, yet exceptionally rewarding, experience that has cultivated personal and professional growth. One of the most important lessons I take from my time at Michigan is the value of challenging myself and leaning into the uncomfortable. As a first-time R user coming from nearly a decade in industry, transitioning back into the role of full-time student was not easy. I hadn't thought about calculus in more than a decade, and my coding skills were sub-par. My advisor, Betsy, pushed me far beyond what I thought I was capable of; I took advanced biostatistics courses and human genetics courses, returned to the wet lab, and ran complex microbiome analyses independently. These experiences reminded me that I enjoy the challenge and that the skills I develop through an obstacle are always valuable. Often, the harder something is, the more rewarding it becomes.

Another lesson I've learned (or probably always knew) is that academic writing is a struggle for me. My high school teacher, Mr. Enders, always said "practice makes permanent" and when it comes to writing, he couldn't have been more right. Betsy was patient and always connected me with opportunities to improve; from her detailed reviews, to sessions at the SPH Writing Lab, Summer Session in Epidemiology writing course, and participating in writing groups. I now understand that I need to write as often as possible, start with small goals, and be in the right mindset and environment. Writing is hard, and probably always will be, but every day it feels a little less intimidating.

My dissertation was rooted in developing skills in epidemiologic methods, laboratory work, and microbiome analysis among an aging cohort. When I look back to my first semester in the program, I'm struck with how little I knew and how many people helped me grow along the way. There's always more to learn, but most importantly, I've come to value the importance of paying it forward. I loved my time as a GSI, engaging with undergraduate public health students and playing a small role in their journeys was deeply meaningful. I hope to continue seeking out these opportunities to pass on my experience, skills, and knowledge; whether through collaborative projects with students or stepping back into the classroom, these moments bring me just as much joy as publishing a manuscript.

Finally, this program has shown me the importance of a strong network. Life and work are not always easy, but a good community makes it better. My family and friends offered me unwavering support. With my fellow EpiDocs, I always had people to celebrate the highs and push through the lows. The Foxman Lab and Department of Epidemiology consistently cheered me on and were rooting for my success. The broader Michigan community fostered cross-functional collaboration and emphasized the power of a diverse team. At conferences, I met passionate public health professionals who inspire and remind me why I chose this field. In challenging times, I believe that science will prevail and that together, we can accomplish so much more.