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Exploration of the Oral Microbiome in Non-Ventilated Hospitalized Patients

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EXPLORATION OF THE ORAL MICROBIOME IN NON-VENTILATED HOSPITALIZED PATIENTS

by

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ABSTRACT

Non-ventilator hospital acquired pneumonia (NV-HAP) impacts 1 in 100 hospitalized patients and is a significant patient concern due to its negative clinical outcomes. Many factors play a role in NV-HAP development: oral health, oral care, microaspiration risk, and the oral microbiome. Little is known about how the oral microbiome alters during hospitalization. This study explored changes in the oral microbiome of non-ventilated hospitalized patients over time and analyzed the relationship between the oral microbiome, pre-hospital residence, and NV-HAP. A prospective, observational design was used to recruit 46 non-ventilated adults $\geq$ 65 years from a large medical center in central Florida (n=15 nursing home; n=31 home) within 72 hours of admission (baseline). Oral salivary specimens were collected, and an oral assessment was completed using the Oral Health Assessment Tool at four time points: baseline and hospital days 3, 5, and 7. Genomic DNA was extracted from oral samples for microbiome profiling by 16S rRNA sequencing. Taxonomic composition, relative abundance, alpha diversity (Shannon Index), and beta diversity (Bray-Curtis dissimilarity) of bacterial communities were determined. Data were analyzed using descriptive statistics, Chi-squared, independent t-test, repeated measures mixed effects modeling, two-way permutational ANOVA, ANOSIM, and multiple dispersion. Most participants were female (70%) and white (74%) with mean age of 78.7$\pm$9.1 years. Oral bacteria genera remained consistent across hospitalization: *Streptococcus*, *Rothia*, and *Prevotella*. Mean Shannon Index changed over time ($p<.001$) and over time by group ($p<.01$). Relative bacterial abundances were similar between groups; however, several less frequent oral bacteria genera were higher in the nursing home group. Mean baseline Shannon Index was lower in the nursing home group ($p<.001$). Mean Bray-Curtis dissimilarity at baseline

genus (p=.010) and ASV levels (p=.003) were higher in the nursing home group. Two patients developed probable NV-HAP (4%), at the time that *Neisseria* and *Streptococcus* increased. Although oral bacteria genera remained consistent, oral bacterial diversity changed across hospitalization and over time between groups. Several oral bacteria genera and oral bacterial diversity significantly differed between groups, emphasizing the importance of an individualized approach to oral care beginning at hospital admission. Specific bacteria genera may be meaningful indicators of NV-HAP development.

Keywords: older adult, non-ventilator, hospital-acquired pneumonia, oral microbiome

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TABLE OF CONTENTS

LIST OF FIGURES x
LIST OF TABLES xi
CHAPTER ONE: INTRODUCTION 1
Background and Significance 1
Manuscripts 4
Study Framework 5
References 7
CHAPTER TWO: ORAL MICROBES IN HOSPITAL-ACQUIRED PNEUMONIA: PRACTICE AND RESEARCH IMPLICATIONS 10
Abstract 10
Background 11
Etiology of HAP 12
Clinical Management of HAP 13
Purpose of Integrative Review 14
Methods 14
Results 15
Microbes in NV-HAP 17
Microbes in VAP 18
Discussion 19
Implications of Microbial Findings 19
Clinical Practice Recommendations 21
Research Recommendations 22
Limitations 23
Conclusion 24
References 24
CHAPTER THREE: EXPLORING THE ORAL MICROBIOME IN NON-VENTILATED HOSPITALIZED OLDER ADULTS: RESEARCH PROTOCOL FOR A PROSPECTIVE LONGITUDINAL STUDY 32
Abstract 32

Introduction 33
Prevention of NV-HAP 33
Oral Microbiome Alterations and Clinical Implications 36
Specific Aims 37
Methods 38
Design and Setting 38
Sample 38
Sample Size and Statistical Power 40
Outcome Measures 41
Research Procedures 45
Metadata Management 47
Data Analysis 48
Discussion 49
Study Strengths and Challenges 49
Important Takeaways 51
References 51
CHAPTER FOUR: FINDINGS 67
Abstract 67
Background 68
Methods 69
Study Design and Setting 69
Study Sample 70
Outcome Measures 71
Recruitment and Informed Consent 72
Data Collection 73
Metadata Management 73
16S rRNA Sequencing and Data Processing 74
Statistical Analysis 76
Results 77
Demographic Data 77
Longitudinal Changes in Oral Microbiome of Non-Ventilated Hospitalized Patients ... 81

Evaluation Relationship Between Pre-Hospital Residence and Oral Microbiome 91
Explore Relationship Between Oral Microbiome and NV-HAP Development 97
Discussion 99
Oral Microbiome Across Hospitalization 99
Importance of Pre-Hospital Residence/Environment 100
Combining a Clinical Picture of the Oral Microbiome, Clinical Variables, and Probable NV-HAP Development 102
Future Research Implications 106
Limitations 107
Conclusions 108
References 109
CHAPTER FIVE: NARRATIVE SUMMARY 116
Reflections and Summary of Research 116
Impact of Research 117
Future Research Plans and Trajectories 117
References 118
APPENDIX: IRB LETTER 119

LIST OF FIGURES

Figure 1. Concept Map of NV-HAP 6

Figure 2. Search Strategy for Integrative Literature Review 15

Figure 3. Concept Map of NV-HAP 35

Figure 4. Oral Bacteria Genera Changes Across Hospitalization (Grouped by Time) 82

Figure 5. Oral Bacteria Species Changes Across Hospitalization (Grouped by Time) 83

Figure 6. Mean Shannon Index for Combined Groups Significantly Changed Across Hospitalization 84

Figure 7. Mean Shannon Index Significantly Differed Between Groups Over Time Across Hospitalization 85

Figure 8. Baseline Oral Bacteria Genera Differences Between Groups 92

Figure 9. Baseline Oral Bacteria Species Differences Between Groups 93

Figure 10. Baseline Mean Shannon Index Significantly Lower in Nursing Home Group vs Home Group 94

Figure 11. Bray-Curtis Dissimilarity Mean and Variance Significantly Different Between Groups and Higher in Nursing Home Group at Genus Level 95

Figure 12. Bray-Curtis Dissimilarity Mean and Variance Significantly Different Between Groups and Higher in Nursing Home Group at ASV (Species) Level 96

LIST OF TABLES

Table 1. Details and Findings of Studies Included in the Review 16

Table 2. Associated Study Hypotheses 38

Table 3. Measurement of Demographic/Baseline Variables 42

Table 4. Measurement of Longitudinal Variables 43

Table 5. Measurement of Aims 44

Table 6. Baseline Variables of Study Sample 79

Table 7. Longitudinal Variables of Study Cohort Through Hospital Day 5 86

Table 8. Clinical Outcomes of Study Completers Through Hospital Day 5 87

Table 9. Longitudinal Variables of Study Cohort Through Hospital Day 7 88

Table 10. Clinical Outcomes of Study Completers Through Hospital Day 7 90

Table 11. Most Frequent Oral Bacteria Genera Relative Abundance Percentages Between Groups at Baseline 91

Table 12. Characteristics of Patients Who Developed NV-HAP 98

Table 13. Longitudinal Oral Bacteria Genera Relative Abundance Percentage Changes During Hospitalization in Patients who Developed Probable NV-HAP 98

CHAPTER ONE: INTRODUCTION

Non-ventilator hospital acquired pneumonia (NV-HAP) is a prevalent and costly healthcare-associated infection (HAI) with reported mortality rates ranging from 13.1% to 30.0%.[1] Though focus on pneumonia development within the hospital setting has primarily been on ventilator-associated pneumonia (VAP), NV-HAP has also emerged as a concern due to higher incidence rates and comparable mortality rates to VAP. [1,2] Several risk factors for NV-HAP exist; however, one primary modifiable risk factor is poor oral health. [3,4]

During the first week of hospitalization, dental plaque significantly worsens and builds in patients. [5] Dental plaque and oral microbe accumulation worsen oral health, placing hospitalized patients at a greater risk for NV-HAP development. [5,6] Patients may also reside in a nursing home prior to hospital admission, which further compounds their risk of developing NV-HAP. [7] Aspiration of oral secretions and dental plaque frequently occurs in patients, providing a pathway for pathogen migration into the pulmonary system. [3] Oral care provides an effective method of decreasing NV-HAP by targeting the oral microbial etiology in dental plaque; [3,8] however, oral care is frequently neglected in non-ventilated patients. [9]

The oral microbiome may be a factor in NV-HAP development. Although changes in the oral microbiome during hospitalization clinically impacts patients, [10] little is known about how the oral microbiome alters during hospitalization in non-ventilated patients.

Background and Significance

Pneumonia accounts for 25% of all HAIs, of which 60% are attributed to NV-HAP. [11] NV-HAP is a common HAI associated with poor patient outcomes and high costs per case ranging from $28,000 to $40,000. [1] Incidence rates of NV-HAP are also high, affecting

approximately 1.2 to 8.9 per 1,000 patients. [1] Unlike VAP, NV-HAP rates are not mandated to be reported, so incidence rates are likely underestimated. Incidence of NV-HAP is also significantly higher in older adults, as one study found a 30.4% NV-HAP rate among those ≥ 60 years of age. [12] NV-HAP increases hospital LOS from 4.0 to 15.9 days and mortality rates often exceed that of VAP. [1,2] A cluster randomized study conducted at our study site found the average hospital LOS for patients with NV-HAP was 16.5 days (median 12.0 days) and average time to NV-HAP diagnosis was 6.1 days (median 4.0 days). [13]

One method of NV-HAP prevention that has been explored in hospitalized patients is standardized oral care with toothpaste and a toothbrush. [3] Oral care reduces the oral colonization of respiratory pathogens, decreasing the likelihood of pneumonia development. [3,14] Standardized oral care implementation also decreases costs by avoiding NV-HAP cases, as one study found a return on investment of $1.6 million USD. [3] Despite its positive health protective effect, oral care is not uniformly performed in non-ventilated hospitalized patients and standardized oral care guidelines do not exist for this population. [9] In addition, other oral care products, such as oral chlorhexidine gluconate (CHG), have not been systematically studied in prevention of NV-HAP. Our study begins building a body of knowledge in an understudied population.

Oral microbiome alterations among different populations and environments hold important clinical implications. [10] The oral microbiome hosts a diverse community of microbiota that can play a role in disease development, including respiratory disease. [15,16] Our integrative review found that patients with NV-HAP had a greater dental plaque, oropharynx, and pulmonary colonization with gram-positive bacteria compared to gram-negative bacteria. [17] One prospective, observational study examined oral colonization in non-ventilated patients ≥ 65 years of age with lower limb fractures over two weeks. [10] The study found that those with oral

colonization of *Staphylococcus aureus* (*S. aureus*), *Escherechia coli* (*E. coli*), methicillin resistant *S. aureus* (MRSA), and *Pseudomonas aeruginosa* (*P. aeruginosa*) were nine times more likely to develop NV-HAP (OR=9.48, 95% CI=2.28-38.78, p=0.002), particularly on day 5 of hospitalization (OR=4.39, 95% CI=1.73-11.16, p=0.002). [10] As an individual pathogen, *S. aureus* increased the risk of NV-HAP development (OR=25.95, 95% CI=1.43-471.92, p=0.028). The colonization index of *E. coli* was also predictive of NV-HAP using Fisher's exact test (p=0.036), though not with univariate generalized linear modeling (OR=86.17, 95% CI=0.70-10680.08, p=0.070). [10]

To our knowledge, Ewan et al is the only researcher to examine the relationship between oral microbes and NV-HAP development. [10] They found that important oral microbes associated with NV-HAP were detected within 72 hours of hospitalization in 90% of study participants, but did not account for oral care. [10] Another study found that the oral microbiome remained relatively stable for the first three days of hospitalization. [18] Notably, the sample size was small, participants had a wide age range, and mechanical ventilation (MV) status was not specified. Findings suggest replication in a better-defined population and longer period of hospitalization. Our study sample will be well-defined ($\geq$ 65 years of age), adequately powered, and longitudinal (up to 7 days).

The oral microbiome may also alter depending on the environment. According to the National Institute on Aging, long-term care services include board/care homes, assisted living facilities, nursing homes (also referred to as SNFs), and continuing care retirement communities. [19] Research exploring the oral microbiome in long-term care has primarily been focused on the non-acute care nursing home setting. [20,21] A study found that oral salivary bacterial diversity was significantly lower in older, frail adults from a nursing home compared with healthy older adult

controls living independently.[21] Oral microbiota composition also significantly differed between groups.[21] Another study found bacterial colonization on the tongue with *Prevotella* and *Veillonella* were associated with increased mortality from pneumonia in nursing home residents.[20] Findings warrant further exploration when nursing home residents are admitted to hospital settings, which we intend to explore. Our study incorporates pre-hospital residence and oral care in the analyses to provide a novel clinical picture of non-ventilated patient's oral microbiome alterations during hospitalization.

In summary, prior research has not systematically explored the evolution of the oral microbiome over time during hospitalization, contributing to the lack of depth and rigor in the knowledge base of non-ventilated patients and NV-HAP development. In addition, to our knowledge, the relationship between being admitted to the hospital from a nursing home and baseline oral microbiota has not been examined. This prospective, observational study serves to address these research gaps using the following aims: (1) Longitudinally explore changes in the oral microbiome of non-ventilated hospitalized patients; (2) Explore the relationship between pre-hospital residence (nursing home versus home) and non-ventilated patient's baseline oral microbiome; and (3) Explore the relationship between the oral microbiome and NV-HAP development. Study findings will contribute to the long-term research goal of improving the health and outcomes of non-ventilated hospitalized patients.

Manuscripts

This non-traditional dissertation includes three manuscripts for publication. The first manuscript includes detailed background information on the bacteria that commonly cause NV-HAP (Chapter 2). This manuscript was published in *Critical Care Nurse* (Rathbun KP, Bourgault AM, Sole ML. Oral Microbes in Hospital-Acquired Pneumonia: Practice and

Research Implications. *Crit Care Nurse*. 2022 Jun 1;42(3):47-54. doi: 10.4037/ccn2022672). The second manuscript provides a description of the study protocol used for recruitment and data collection (Chapter 3). This protocol can be used by others to guide similar work. It will be submitted to *Research in Nursing and Health (RINAH).* The third manuscript is a comprehensive research article describing the study findings, guided by the three study aims (Chapter 4). The findings article will be submitted to *BMC Oral Health*, *Scientific Reports*, or *PLOS ONE*, and will likely be separated into at least two papers due to the large amount of data generated.

Study Framework

The framework that guided this study was a physiological one that predisposes a patient to developing NV-HAP (Figure 1). The framework is included in the protocol article (Chapter 3) as well. [22]

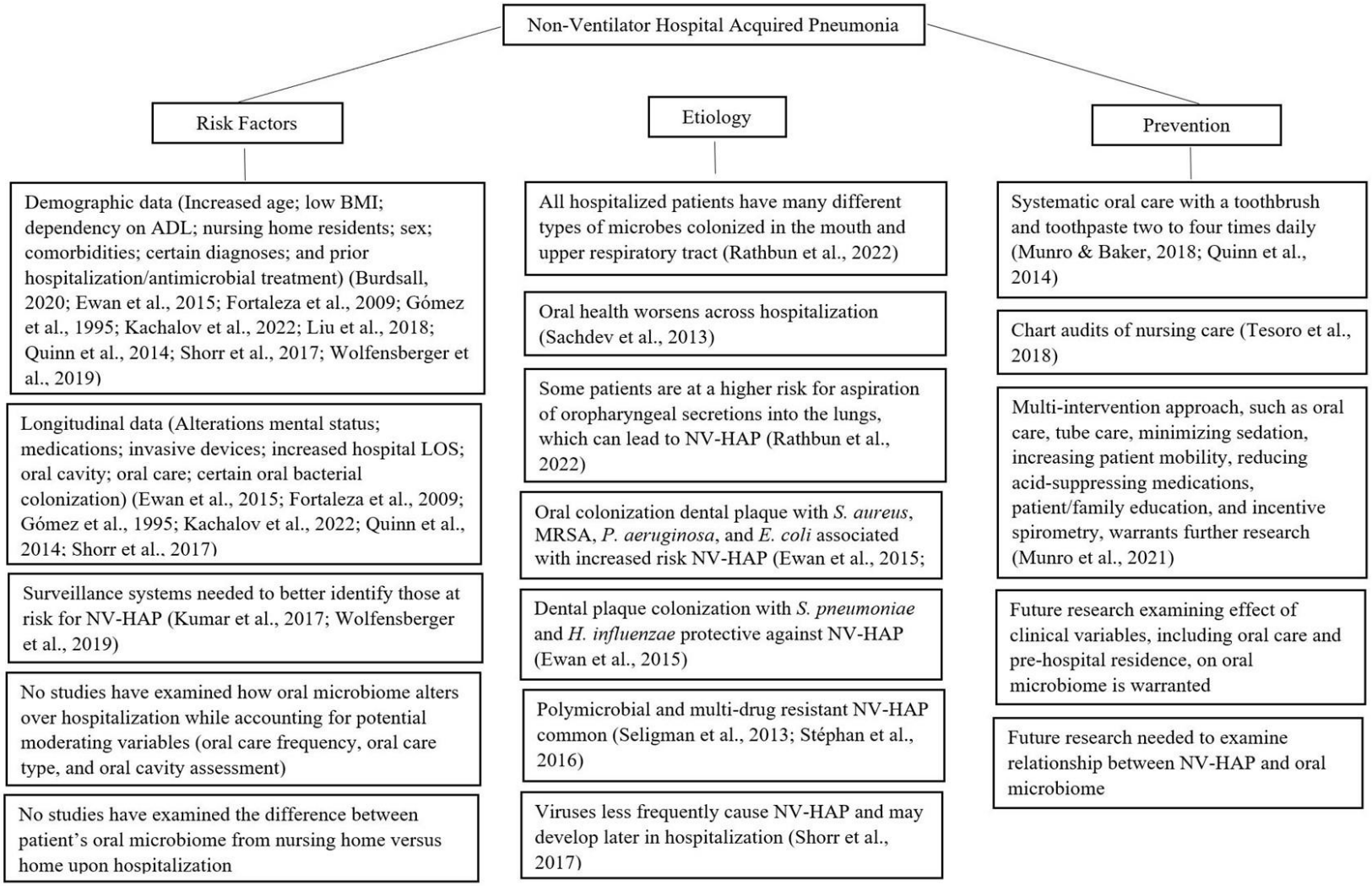

Figure 1. Concept Map of NV-HAP

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CHAPTER TWO: ORAL MICROBES IN HOSPITAL-ACQUIRED PNEUMONIA: PRACTICE AND RESEARCH IMPLICATIONS

Abstract

Background: Hospital-acquired pneumonia accounts for 25% of all health care–associated infections and is classified as either ventilator-associated or non–ventilator-associated pneumonia. Hospital-acquired pneumonia most frequently results from aspiration of oropharyngeal secretions into the lungs. Although preventive measures for ventilator-associated pneumonia are well established, few preventive measures exist for the nonventilator type.

Objective: To (1) explore oral microbes associated with ventilator-associated and non–ventilator-associated pneumonia in acutely ill, adult hospitalized patients, and (2) provide evidence-based recommendations for measures to prevent pneumonia in hospitalized patients.

Methods: A literature search was conducted using CINAHL, Academic Search Premier, Medline, and the Cochrane Library.

Results: Ten studies were found that identified common oral microbes in ventilator-associated and non–ventilator-associated pneumonia, including *Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* methicillin-resistant *Staphylococcus aureus, S aureus*, and *Streptococcus pneumoniae*. Collectively, oral colonization with *E coli, P aeruginosa,* methicillin-resistant *S aureus,* and *S aureus* increased the risk of nonventilator pneumonia. Findings also suggested microaspiration of colonized oral microbes into the lungs. Non–ventilator-associated pneumonia had similar colonization rates of gram-positive and gram-negative bacteria, whereas ventilator-associated pneumonia had greater colonization with gram-negative bacteria. The literature did not indicate a standard of oral care effective in all patient populations.

Discussion: Oral care is an effective intervention to prevent hospital-acquired pneumonia by reducing pathogenic oral microbial colonization. The impact of different methods and timing of oral care on oral microbes should be further explored, particularly in patients not receiving mechanical ventilation.

Conclusions: Findings reaffirm the importance of consistent oral care in hospitalized patients. In addition, practices should be different in patients receiving mechanical ventilation versus patients not receiving ventilation. Results may also provide knowledge to inform future preventive measures for pneumonia, particularly for nonventilator pneumonia.

Keywords: Oral bacteria; oral microbes; hospital-acquired pneumonia; non-ventilator hospital acquired pneumonia; ventilator-associated pneumonia

Background

Hospital-acquired pneumonia (HAP) is a common problem in health care, accounting for 25% of all health care–associated infections.[1] Hospital-acquired pneumonia develops after 48 hours of hospital admission and is typically categorized as either ventilator-associated pneumonia (VAP) or non–ventilator-associated hospital-acquired pneumonia (NV-HAP).[2,3] Ventilator-associated pneumonia occurs in critically ill, intubated patients and has been an important research focus owing to its high mortality rate, negative clinical outcomes, and high costs per case.[4,5] Unlike VAP, NV-HAP can affect any hospitalized patient, not just those who are critically ill. Interest in NV-HAP has increased owing to its high rate of occurrence, high mortality rate, and increased costs.[6] Currently, NV-HAP occurs in 1.2 to 8.9 patients per 1000 patient days, although rates are likely underestimated because hospitals are not required to report cases of NV-HAP, as they are for VAP.6 The costs of NV-HAP vary from $28 000 to $40 000 per case, and mortality rates among adults range from 13% to 30%.[6]

Etiology of HAP

Many different types of microbes colonize the mouth and upper respiratory tract in all individuals, including hospitalized patients.[7] Hospitalization itself changes the microbial colonization of the mouth and worsens oral health in adult patients.[5,8,9] Hospital-acquired pneumonia results from aspiration of oropharyngeal secretions into the lungs,[9,10] highlighting the importance of adequate oral health. Owing to the causal relationship between the oral microbial environment and the occurrence of HAP, it is useful to compare microbial colonization in the mouth versus the lungs.

Dental plaque, which is found in both natural teeth and dentures, is a biofilm of microbes that is frequently a source of pneumonia development.[8,11] Additional sources of microbial colonization associated with HAP include medical devices situated in the gastrointestinal or pulmonary systems (such as feeding tubes, gastric tubes, and endotracheal tubes), transfer of microbes between staff members (lack of adequate hand hygiene), host or treatment colonization risk factors (eg, antibiotics, surgery, underlying disease severity, invasive devices), and the environment.[10]

Bacteria are the main cause of HAP.[10] Viral and fungal causes of HAP are much less common and are typically seen in patients who are immunocompromised.[12] Most bacterial cases of HAP are caused by gram-negative bacteria, with only 20% to 30% of cases being caused by gram-positive bacteria.[13] Hospital-acquired pneumonia is also classified as either early onset or late onset. Early-onset HAP occurs within the first 4 days of hospitalization and is generally caused less frequently by drug-resistant bacteria compared with late-onset HAP.[13]

Multidrug-resistant (MDR) bacteria, such as methicillin-resistant Staphylococcus aureus (MRSA), are found more frequently in HAP compared with community-acquired pneumonia,

and MDR infections are increasing in both NV-HAP and VAP cases.[14] Immune suppression, antibiotic use and resistance, and hospitalization within the last 3 months are risk factors for experiencing an MDR infection.[14] In intubated patients, greater time receiving mechanical ventilation increases the likelihood of experiencing an MDR infection.[15] Early-onset HAP cases are generally associated with more positive clinical outcomes compared with late-onset HAP (owing to the virulence of the microbes found in the latter). In addition, late-onset HAP is often polymicrobial, making it more difficult for clinicians to identify and manage.[13]

Clinical Management of HAP

Diagnosis and management of HAP rely on understanding causative mechanisms and individualizing treatment on the basis of the causative microbes.[15,16] Evidence-based guidelines for HAP management suggest that patients with NV-HAP be managed in a similar manner to those with VAP by identifying risk for pneumonia infection with specific microbes (such as MDR pathogens).[10] Patients with NV-HAP should be treated in accordance with specific microbes identified from noninvasive samples.[16] Cultures are obtained from different specimen types including from the lungs and oropharyngeal or nasotracheal secretions.[10,13] Lung specimens are obtained using bronchoalveolar lavage (BAL) or protected BAL fluid. Bronchoalveolar lavage sampling is performed during bronchoscopy by instillation of sterile normal saline into a section of the lung and suctioning to collect the fluid for analysis.[17] Protected BAL uses a sterile protected brush to obtain the specimen from the lung.[18] Oropharyngeal secretions may be collected using a mouth swab and/or a sputum sample. Analyses of microbial colonization in dental plaque are also used in clinical research[19] but are not commonly performed in the clinical setting.

Purpose of Integrative Review

Oral microbes play an important role in the occurrence of HAP.[8] To our knowledge, no other published articles have explored the commonalities and differences among oral microbes found in NV-HAP and VAP. Identifying certain patterns of microbial colonization may also provide a foundation for development of a preventive regimen for NV-HAP. The purpose of this integrative review was to (1) explore common oral microbial species associated with NV-HAP and VAP in acutely ill, hospitalized adults and (2) provide evidence-based recommendations for prevention of HAP.

Methods

The databases used for this integrative review were CINAHL, Academic Search Premier, MEDLINE, the Cochrane Central Register of Controlled Trials, and the Cochrane Database of Systematic Reviews. The search strategy used was *pneumonia** AND *hospital acquired** OR *nosocomial infection** OR *cross infection** AND *oral microbe** OR *oral bacteria** OR *oral colonization**.

Articles were included if they were peer-reviewed research articles, were published in the English language, focused on adult hospitalized patients with either NV-HAP or VAP, and made mention of oral microbial colonization in relation to NV-HAP or VAP. Articles were excluded if they did not focus on the population described, did not include discussion of mechanical ventilation status, made no mention of oral microbe colonization in relation to HAP, or were literature reviews or evidence-based practice guidelines.

The results of the search process are shown in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses diagram in Figure 2. The initial search yielded 388 articles (305 articles after duplicates were removed and 2 additional articles were identified

through searches of references in relevant articles), of which 295 were excluded. Thus, 10 articles were included in this review. We completed a critical appraisal of each article using the Joanna Briggs appraisal tools specific to study design.[20] The levels of evidence found for the 10 articles were as follows: level I (experimental design), 1 article; level II (quasi-experimental design), 2 articles; level III (nonexperimental study design), 6 articles; and level V (case study), 1 article.[21]

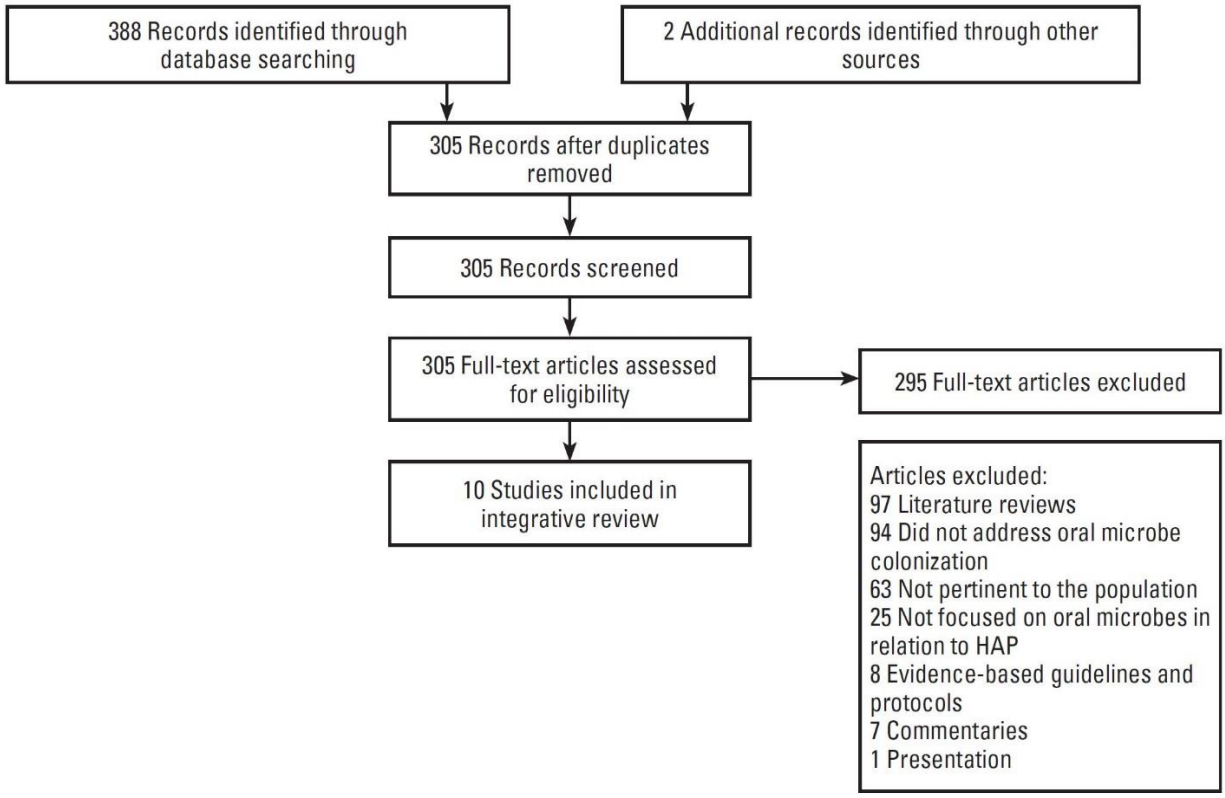

Figure 2. Search Strategy for Integrative Literature Review

Results

The 10 articles included in this review are listed in the Table.[8,11,19,22-28] Collectively, the studies provide an overview of commonalities and differences among oral microbes in the different types of pneumonia.

Table 1. Details and Findings of Studies Included in the Review

Reference	Design/Setting	Pneumonia Type	Colonization Site	Common Microbes	Level of Evidence[a]
Bonten et al (1996)[30]	Experimental (sub-analysis of RCT) in ICU	VAP	Oropharynx	Enteric gram-negative bacteria and Pseudomonadaceae	I[b]
Chen et al (2016)[24]	Nonexperimental (prospective observational) in emergency ICU	NV-HAP and VAP	Sputum (NV-HAP and lungs (VAP)	*A. baumannii* and MRSA	III[c]
El Attar et al (2010)[19]	Nonexperimental (case-control design) in a respiratory ICU	NV-HAP	Oropharynx, dental plaque, and lungs	*S. aureus*	III[c]
El-Solh et al (2004)[11]	Nonexperimental (prospective cohort study) in a critical care unit	VAP	Oropharynx, dental plaque, and lungs	*S. aureus*	III[c]
Ewan et al (2015)[8]	Nonexperimental (prospective cohort study) in orthopaedic units	NV-HAP	Dental plaque	*S. aureus*, MRSA, *P. aeruginosa*, and *E. coli* associated with increased risk of HAP (p=0.002)	III[c]
Gaber et al (2020)[26]	Nonexperimental (prospective observational) in University hospital	NV-HAP	Sputum, pleural fluid, and lungs	*P. aeruginosa* and *A. baumannii*	III[c]
Garrouste-Orgeas et al (1997)[29]	Nonexperimental (prospective observational) in medical-surgical ICU	VAP	Oropharynx	*S. aureus*, *A. baumannii*, and *P. aeruginosa*	III[c]
Mori et al (2006)[22]	Quasi-experimental trial with historical controls in medical-surgical ICU	VAP	Oropharynx	*P. aeruginosa*, MRSA, and *Candida*	II[d]
Nicolosi et al (2014)[23]	Quasi-experimental in patients undergoing cardiac surgery	VAP	Dental plaque	*K. pneumoniae*, *S. aureus*, and *P. aeruginosa*	II[d]
Ohkosh et al (2018)[28]	Nonexperimental (case study) in ICU	VAP	Sputum	*S. pneumoniae and H. influenzae*	V[e]

A baumannii; *Acinetobacter baumannii*; *E coli*, *Escherichia coli*; *H influenzae*, *Haemophilus influenzae*; ICU, intensive care unit; *K pneumoniae*, *Klebsiella pneumoniae*; MRSA, methicillin-resistant *Staphylococcus aureus*; NV-HAP, non–ventilator-associated hospital-acquired pneumonia; *P aeruginosa*, *Pseudomonas aeruginosa*; RCT, randomized controlled trial; *S aureus*, *Staphylococcus aureus*; *S pneumoniae*, *Streptococcus pneumoniae*; VAP, ventilator-associated pneumonia

[a]Adapted from Dearholt and Dang.21 Level I, experimental studies; level II, quasi-experimental studies; level III, nonexperimental studies; level II, quasi-experimental studies; and level V, case reports.

Microbes in NV-HAP

Patients with NV-HAP had similar colonization rates of gram-positive bacteria and gram-negative bacteria. The most common oral microbes in NV-HAP were *Acinetobacter baumannii, Pseudomonas aeruginosa,* and *S aureus*.[19,24] Patients who had a combination of oropharyngeal colonization with *Escherichia coli, P aeruginosa,* MRSA, and *S aureus* were more than 9 times as likely to develop NV-HAP (odds ratio, 9.48; 95% CI, 2.28-38.78; *P*= .002).[8] The presence of *E coli* and *S aureus* independently increased the risk of NV-HAP occurrence.8 In contrast, some oral microbes, including *Haemophilus influenzae* and *Streptococcus pneumoniae,* were actually protective against NV-HAP.[8] Findings for *S pneumoniae* were conflicting, as this bacterium was causative in 17% of NV-HAP cases in patients with moderate to severe chronic periodontitis.[19]

Microbes identified in dental plaque were also associated with the occurrence of NV-HAP.[8,19] In most patients with NV-HAP, dental plaque was colonized with 1 or more microbes, with *S aureus* being the most common.[19] Other bacteria identified in dental plaque included *Bacteroides* species, coagulase-negative staphylococci, and *S pneumoniae*.[19]

Specimens obtained by BAL from patients with NV-HAP contained similar microbes to those found in the oropharynx and dental plaque,[19] suggesting microaspiration of oropharyngeal secretions into the lungs. The most common microbe found in the lungs was *S aureus*.[19]

Different oral care regimens did not significantly change oral bacteria in patients with NV-HAP, aside from greater colonization with *Stenotrophomonas maltophilia* in patients who had oral care with 0.2% chlorhexidine gluconate (CHG) compared with patients who had oral care with 0.08% metronidazole.[23] Metronidazole is an antibacterial agent, whereas CHG is an antiseptic agent,[29] which could account for differences in oral microbial findings. Oral colonization with *S maltophilia* could also have been due to water contamination.

Microbes in VAP

Intubated patients with VAP had greater colonization with gram-negative bacteria than with gram-positive bacteria. The most common oral microbes found in VAP cases were *A baumannii, Klebsiella pneumoniae, P aeruginosa,* MRSA, and *S aureus*.[11,26,27] In a study in which 29% (14 of 49) of patients experienced VAP, *S aureus* was the most common microbe found in all specimen types.[11] One case study that examined the microbiological sputum profile of a patient with VAP found high degrees of colonization with *H influenza*e and *S pneumoniae*.[28]

In another study, gram-negative bacteria were primarily responsible for all 30 documented cases of VAP.[25] The most common gram-negative bacteria colonized in the oropharynx included *A baumannii* and *P aeruginosa*.[25] A similar study indicated that 18% (26 of 141) of patients with VAP had enteric gram-negative bacteria and Pseudomonadaceae in the oropharynx.[22]

Causative agents differed in early- versus late-onset VAP. In a small sample of 16 intubated patients, early-onset VAP was caused primarily by *P aeruginosa*.[26] Patients with late-onset VAP still had frequent colonization with *P aeruginosa*; however, they had a higher incidence of infections with more resistant microbes, including MRSA.

In patients who received oral care and experienced VAP, *K pneumoniae, P aeruginosa,* MRSA, and *S aureus* were frequently identified.[26,27] In patients who did not receive oral care and experienced VAP, *P aeruginosa* was predominant.[26,27] Several other gram-negative and gram-positive bacteria were identified in patients who did not receive oral care.[26,27] In addition, certain types of oral care influenced the type of bacteria found in patients with VAP.[23] Intubated patients who received oral care with 0.2% CHG had significantly greater colonization with gram-

negative bacteria in the lungs compared with patients who received oral care with 0.08% metronidazole (P = .02).[23]

Discussion

Implications of Microbial Findings

The studies in this review explored microbes found in the oropharynx, dental plaque, and lungs of patients with NV-HAP and VAP. Oral microbial findings were similar between pneumonia types, including *A baumannii, E coli, K pneumoniae, P aeruginosa,* MRSA, *S aureus,* and *S pneumoniae*. Gram-positive bacteria, such as *S aureus* and *S pneumoniae,* are common in the community setting and frequently found on the human body.[13] For instance, in healthy individuals, MRSA and *S aureus* are sometimes found in the nares and *S aureus* on the skin.[30,31] Hospital-acquired pneumonia infections caused by gram-positive bacteria (such as MRSA) are concerning owing to emerging resistant strands and high costs of treatment.[32] Patients at risk for development of *Staphylococcus* HAP infections include those with chronic conditions (such as diabetes) and immunocompromised patients who undergo invasive procedures.[30]

Cases of VAP are caused primarily by gram-negative bacteria, as reaffirmed in our review.[33] This finding may be due to the frequent colonization of the oropharynx and gut by gram-negative bacteria, followed by common mechanisms such as gastric reflux into the oropharynx, and through transmission by health care workers. Both of these situations could lead to VAP.[34] Gram-negative bacteria are associated with severe health consequences, including pneumonia, septicemia, meningitis, and surgical site or wound infections.[30] Many gram-negative bacteria are becoming resistant to antibiotics, which is a growing concern in the health care

setting owing to the serious infections that may result and limited antibiotic treatments available.[30,35]

A particularly concerning gram-negative bacterium found in both pneumonia types is *P aeruginosa,* which is often waterborne.[36] Common environmental reservoirs of *P aeruginosa* include sinks, sink faucets, respiratory therapy equipment, and portable water, among others.[37] *Pseudomonas aeruginosa* is of great concern in hospitals owing to its increasing presence in cases of VAP and antimicrobial resistance, making it difficult to treat.[38]

Another disconcerting bacterium found in the mouth in cases of both NV-HAP and VAP was *E coli*. Although *E coli* normally resides in the gut of healthy individuals, oropharyngeal colonization with *E coli* is rare in the community setting.[39] Oropharyngeal colonization with *E coli* is concerning because of its ability to cause HAP and associated negative health outcomes, including longer intensive care unit and hospital stays, high mortality and costs, and increased antibiotic use.[39] In addition, antibiotic-resistant strains of *E coli* have been emerging, which are associated with worse clinical outcomes.[39] Oropharyngeal colonization with *E coli* occurs more often in critically ill hospitalized patients, most likely owing to a multifactorial process.39 Factors that may increase oropharyngeal colonization with *E coli* include increased supine positioning, gastric reflux, gut-lung translocation, altered gastric pH from proton pump inhibitors, altered local immunity, and/or contamination from health care workers (resulting from poor hand hygiene).[39]

Few MDR pathogens were noted among both types of HAP. Our review found similar oral bacteria in early and late-onset VAP, with *P aeruginosa* being the most common. However, late-onset VAP cases had greater colonization with resistant bacteria (mainly MRSA).[26] Supporting literature shows that infecting microbes are more likely to respond to antibiotics in

early-onset than in late-onset VAP, which is frequently caused by resistant bacteria.[40] Multidrug-resistant pathogens were found in nearly all VAP cases regardless of when the pneumonia developed, suggesting that the microbial cause of early VAP may be shifting.[41]

Clinical Practice Recommendations

Our review found that a variety of potentially pathogenic microbes are associated with the development of HAP. Oral care is an effective preventive measure against pneumonia; however, review of the literature did not isolate a standard of oral care effective in all patient populations. Hospitalized patients may need different oral care regimens depending on their level of acuity and individualized risk factors for HAP. The oral care recommendations below are not inclusive but are evidence-based oral care practices.

Patients in Acute Care Settings Not Receiving Mechanical Ventilation

Toothbrushing and cleansing of gums and dentures may be effective methods of reducing plaque and microbe accumulation in the mouth, but further research is required to identify best practices that improve outcomes.[1,9,42] Recommendations regarding routine use of CHG in patients who are not receiving mechanical ventilation are conflicting and need further study.[43,44]

Patients Receiving Mechanical Ventilation

Ventilator-associated pneumonia prevention bundles often include oral care with CHG.[42,45] Chlorhexidine reduces the risk for VAP from 26% to 18%, but there is no evidence that it reduces mortality, duration of mechanical ventilation, or intensive care unit length of stay.[45] Concentrations of CHG vary and influence outcomes. A meta-analysis found that oral care with 2% CHG reduced the incidence of VAP (relative risk, 0.53; 95% CI, 0.31-0.91), but lower concentrations had no effect.46 Findings have been mixed regarding whether higher

concentrations of oral CHG may have adverse effects on the oral mucosa, such as lesions, ulcerations, and bleeding.[47,48] An increased risk of oral mucosal lesions was associated with mechanical ventilation, receiving 2% CHG for long periods of time, and severe illness.[47] A recent multisite study of 14,333 patients undergoing ventilation indicated that CHG was associated with increased odds of death and sepsis and had no effect on VAP.[49]

Hand Hygiene

Consistent hand hygiene is also important for patients and staff members to prevent oropharyngeal colonization with pathogens like *E coli,* which was commonly found in HAP cases. This organism is not normally found in the mouth but can be spread via the fecal-to-oral route through inadequate hand hygiene.

Research Recommendations

Future research should further explore oral microbes found in the hospitalized population not receiving mechanical ventilation, as the evidence on this topic is insufficient. Most articles included in our review focused on VAP, and many articles related to NV-HAP were outdated. Research should explore how oral microbes change over the course of hospitalization and with different treatment regimens. Understanding these changes will help clinicians individualize patient care, which will improve clinical outcomes. Oral bacteria may differ across patients, making it important to explore and better understand contributing factors. Other factors such as diet (eg, vegetarian) can also influence the mix of oral microbes in an individual patient.[50]

Second, future NV-HAP research should focus on the impact of different types of oral care on oral microbes. Our review found that specific oral microbes were associated with NV-HAP, including *E coli, P aeruginosa,* MRSA, and *S aureus*. We also found that certain types of oral microbes, such as *H influenzae* and *S pneumoniae,* may actually be protective against NV-

HAP,8 helping to maintain an equilibrium of the oral microbiome for both oral and systemic health.[51] Different oral care methods and/or products may have varying effects on oral microbial colonization. For instance, investigators in a randomized clinical trial found that 1% CHG oral care with a toothbrush reduced oral colonization with *S aureus* (one of the most common causes of HAP) by 42% during a 6-month period.[52] The frequency of oral care with CHG was not specified, although the concentration of CHG is a lower one than that used for VAP prevention in critically ill patients. The impact of different oral care regimens on the type of oral microbe development in different patient populations should be further explored. Different concentrations of CHG should be explored to determine which is most safe and effective.

Finally, aside from oral care, few prevention interventions have been systematically explored to prevent NV-HAP.[14] Future studies are needed to develop a comprehensive interdisciplinary approach to preventing NV-HAP.

Limitations

A limitation of this integrative review is the lack of studies that examined oral microbes associated with particular types of pneumonia, especially NV-HAP. Few studies focused solely on oral microbes in pneumonia, and they mainly provided descriptive statistics. Other studies not included in this review explore microbes found in the lungs of intubated patients and patients with VAP. However, the focus of this review was oral microbes, so these articles were not included. In addition, several studies had small sample sizes, limiting the generalizability of the findings. One study was specific to chronic periodontitis, limiting the generalizability of its findings to other NV-HAP cases. Finally, most articles included in the review were published more than 5 years ago. The prevalence of specific microbes may have changed over time; thus, the findings may not be applicable to the current clinical setting. Recent research has been

published on oral care for intubated patients; however, this research was not included because this topic was not the focus of this review.

Conclusion

Our review found common oral microbes among cases of NV-HAP and VAP. The former had similar rates of oral colonization with gram-positive and gram-negative bacteria, whereas the latter had greater colonization with gram-negative than with gram-positive bacteria. The findings provide a foundation for understanding oral microbes associated with pneumonia, particularly in patients not undergoing mechanical ventilation, which may inform future preventive measures and research trajectories. Microaspiration of oropharyngeal secretions, including oral microbes, was noted, reaffirming the importance of consistent and individualized oral care in all hospitalized patients. It is important for nurses to recognize that current evidence supports different oral care practices for patients receiving versus not receiving mechanical ventilation. Adherence to isolation protocols and proper hand hygiene are also essential in reducing the spread of pathogens.

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CHAPTER THREE: EXPLORING THE ORAL MICROBIOME IN NON-VENTILATED HOSPITALIZED OLDER ADULTS: RESEARCH PROTOCOL FOR A PROSPECTIVE LONGITUDINAL STUDY

Abstract

Non-ventilator hospital acquired pneumonia (NV-HAP) impacts 1 in 100 hospitalized patients and is associated with negative clinical outcomes including increased mortality rates up to 30%, increased hospital length of stay from 4.0 to 15.9 days, and high costs up to $40,000/case. NV-HAP develops from aspiration of oropharyngeal secretions, which occurs more frequently in patients with contributing clinical variables such as poor oral care and specific oral bacterial colonization. Consistent oral care is an effective preventive measure against NV-HAP; however, oral care is not a consistent standard of practice in the non-ventilated hospitalized population, and it is unknown how different types of oral care impact the oral microbiome. This prospective, observational study aims to explore the longitudinal oral microbiome changes in non-ventilated hospitalized older adults $\geq$ 65 years, as well as explore the relationship between the oral microbiome, pre-hospital residence (nursing home compared to home), and NV-HAP. A sample of 58 patients will be recruited (29 patients from a nursing home and 29 from home). Oral health status is rated using the Oral Health Assessment Tool prior to specimen collection. Baseline oral salivary specimens are obtained at enrollment (within 72 hours of hospital admission), and days 3, 5, and 7, or immediately prior to discharge. Genomic DNA will be extracted from oral specimens for microbiome profiling using 16S rRNA sequencing. Analyses metrics include bacterial taxonomy identification, alpha-diversity, and beta-diversity. Understanding how clinical variables alter the oral microbiome may help clinicians identify “high-risk” patients for NV-HAP and tailor prevention interventions accordingly.

Keywords: Oral microbiome; healthcare-associated pneumonia; non-ventilator; non-ventilator hospital acquired pneumonia; older adults

Introduction

Pneumonia accounts for 25% of all healthcare-associated infection (HAIs) in the United States, of which 60% are attributed to non-ventilator hospital acquired pneumonia (NV-HAP) (Munro & Baker, 2018). NV-HAP is a common HAI associated with poor patient outcomes and high costs per case ranging from $28,000 to $40,000 (Giuliano et al., 2018a). Incidence rates of NV-HAP are also high, affecting approximately 1.2 to 8.9 per 1,000 patients (Carey et al., 2022; Giuliano et al., 2018a). Unlike ventilator-associated pneumonia (VAP), NV-HAP rates are not mandated to be reported, so incidence rates are likely underestimated. NV-HAP rates are higher in older adults, as one study found a 30.4% NV-HAP rate among those $\geq$ 60 years of age (Xia et al., 2020). An Australian study reported a 78.2 median age for those with NV-HAP (Gardiner et al., 2022). NV-HAP increases hospital length of stay (LOS) from 4.0 to 15.9 days and mortality rates often exceed that of VAP (Davis & Finley, 2012; Giuliano et al., 2018a). Without resolution of NV-HAP, patients and hospitals will continue to experience unnecessarily high costs, hospital LOS, and mortality rates.

Prevention of NV-HAP

One strategy to prevent NV-HAP in hospitalized patients is standardized oral care with toothpaste and a toothbrush (Klompas et al., 2022; Quinn et al., 2014a). Oral care reduces oral colonization of potential respiratory pathogens, decreasing the likelihood of pneumonia development (Baker & Quinn, 2018; Raghavendran et al., 2007). Standardized oral care implementation also decreases costs by avoiding NV-HAP cases, as one study found a return on investment of $1.6 million USD (avoidance of 43 NV-HAP cases) within a 12-month oral care

intervention period (Quinn et al., 2014a). Despite its positive health protective effect, oral care is not performed consistently in non-ventilated hospitalized patients (Emery & Guido-Sanz, 2019). In addition, other oral care products, such as oral chlorhexidine gluconate (CHG), have not been systematically studied in prevention of NV-HAP.

Although we are aware that standardized oral care with a toothbrush and toothpaste (or with a suction toothbrush for those at a high risk for aspiration) two to four times per day decreases plaque buildup and oropharyngeal colonization (Munro & Baker, 2018; Quinn et al., 2014a), we do not yet know how the oral microbiome is altered secondary to oral care. Oral care frequency and products may alter the oral microbiome differently compared to one another and decrease bacteria more likely to cause NV-HAP. A randomized clinical trial (RCT) found that 0.12% oral CHG reduced *S. aureus* colonization in dental plaque of MV patients compared with the placebo group on hospital days 2 and 4 ($p=0.0065$ and $p=0.0201$, respectively) (Scannapieco et al., 2009). Another RCT found that oral care with 1% CHG reduced *S. aureus* from 66.7% to 33.3% over three months, and 25.0% at six months ($p < 0.010$) (Ab Malik et al., 2018). *S. aureus* has been associated with NV-HAP and is one of the most common causes of hospital-acquired pneumonia (Ewan et al., 2015; Rathbun et al., 2022). Various oral care regimens may reduce oral microbes specific to NV-HAP, such as *S. aureus* (Ewan et al., 2015), thereby reducing the likelihood of NV-HAP development.

Refer to Figure 3 for further discussion of risk factors, etiologic development, and prevention strategies of NV-HAP.

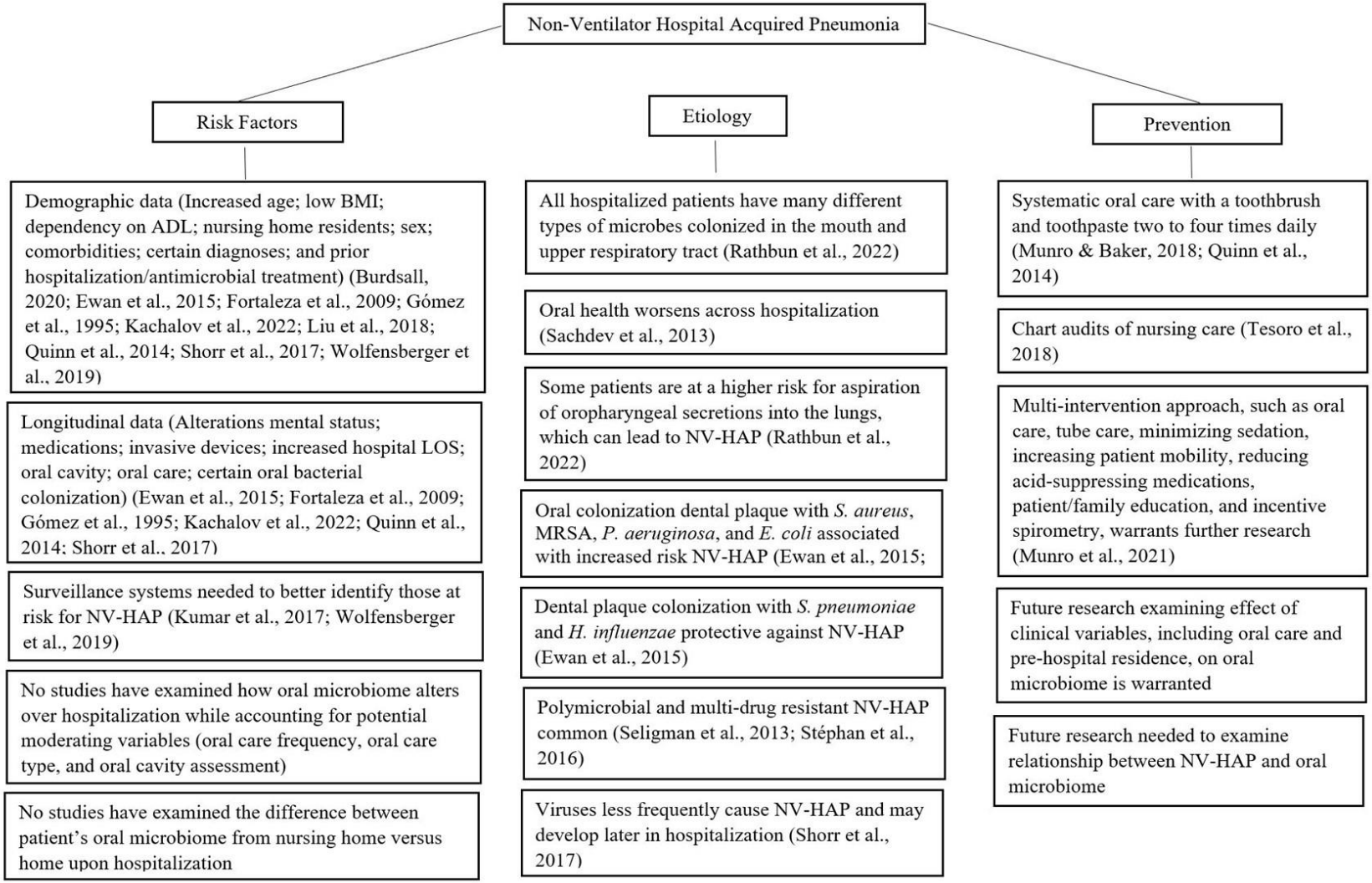

Figure 3. Concept Map of NV-HAP

Oral Microbiome Alterations and Clinical Implications

The oral microbiome hosts a diverse community of microbiota that can play a role in disease development, including respiratory disease (Gomes-Filho et al., 2010; Solbiati & Frias-Lopez, 2018). Specific oral microbe colonization holds important clinical implications, as our integrative review found that patients with NV-HAP had greater dental plaque, oropharynx, and pulmonary colonization with gram-positive bacteria compared to gram-negative bacteria (Rathbun et al., 2022). One prospective, observational study examined oral colonization in non-ventilated patients $\geq$ 65 years of age over a two-week period (Ewan et al., 2015). The study found that those with oral colonization of *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), methicillin resistant *S. aureus* (MRSA), and *Pseudomonas aeruginosa* (*P. aeruginosa*) were nine times more likely to develop NV-HAP (OR=9.48, 95% CI=2.28-38.78, p=0.002), particularly on day 5 of hospitalization (OR=4.39, 95% CI=1.73-11.16, p=0.002) (Ewan et al., 2015). As an individual pathogen, *S. aureus* increased the risk of NV-HAP development (OR=25.95, 95% CI=1.43-471.92, p=0.028). The colonization index of *E. coli* was also predictive of NV-HAP using Fisher's exact test (p=0.036), though not with univariate generalized linear modeling (OR=86.17, 95% CI=0.70-10680.08, p=0.070) (Ewan et al., 2015).

To our knowledge, Ewan et al. is the only study to examine the relationship between oral microbes and NV-HAP development (Ewan et al., 2015). They found that important oral microbes associated with NV-HAP were detected within 72 hours of hospitalization in 90% of study participants, but the researchers did not account for oral care (Ewan et al., 2015). Another study found that the oral microbiome remained relatively stable for the first three days of hospitalization (Cabral et al., 2017). Notably, the sample size in Cabral's study was small,

participants had a wide age range, and mechanical ventilation (MV) status was not specified. Findings suggest replication in a better-defined population and longer period of hospitalization.

The oral microbiome may also alter depending on the environment. According to the National Institute on Aging, long-term care services include board/care homes, assisted living facilities, nursing homes (also referred to as skilled nursing facilities), and continuing care retirement communities (National Institute on Aging, 2017). Research exploring the oral microbiome in long-term care has primarily been focused on the non-acute care nursing home setting (Kageyama et al., 2018; Taiji et al., 2018). A study found that oral salivary bacterial diversity was significantly lower in older, frail adults from a nursing home compared with healthy older adults living independently (Taiji et al., 2018). Oral microbiota composition also significantly differed between these groups (Taiji et al., 2018). Another study reported bacterial colonization on the tongue with *Prevotella* and *Veillonella* were associated with increased mortality from pneumonia in nursing home residents (Kageyama et al., 2018). Findings warrant further exploration when nursing home residents are admitted to hospital settings, as this phenomenon of interest has not yet been explored.

Specific Aims

Our study aims to:

1. Longitudinally explore changes in the oral microbiome of non-ventilated hospitalized patients.
2. Explore the relationship between pre-hospital residence (nursing home versus home) and non-ventilated patient's baseline oral microbiome.
3. Explore the relationship between the oral microbiome and NV-HAP development.

Associated study hypotheses are discussed in Table 2.

Table 2. Associated Study Hypotheses

Aim	Hypotheses
Aim 1	*Hypothesis 1:* Oral bacterial taxonomy, alpha-diversity, and/or beta-diversity will alter over time during hospitalization in non-ventilated patients.
Aim 2	*Hypothesis 2:* Patients from a nursing home will have different oral bacterial taxonomy and less oral microbial diversity within 72 hours of hospitalization compared to patients admitted to the hospital from home.
Aim 3	*Hypothesis 3:* NV-HAP development will be associated with one or more predominant oral microbes

Methods

This protocol is in accordance with Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for reporting observational studies (Network, 2021). Institutional Review Board (IRB) approval of the study was obtained in November of 2020 from both the university and study site.

Design and Setting

A prospective, observational, longitudinal study design is used. The study setting is a large academic medical center located in central Florida. Patients are recruited from one of three progressive care units (PCUs) at the study site. PCUs, also referred to as intermediate or step-down units, provide care to patients with acuity levels between medical-surgical and intensive care units (ICUs) (Stacy, 2011). Medical-surgical units were not chosen since patients generally have a shorter hospital length of stay and we are collecting longitudinal data across hospitalization.

Sample

Convenience sampling is used to recruit 58 patients (29 patients from a nursing home and 29 patients from home).

Inclusion criteria consist of:

1. Admission to a progressive care unit (PCU);

2. Not requiring MV;
3. Enrolled within < 72 hours of hospital admission from either home or a nursing home;
4. Age ≥ 65 years;
5. Negative COVID-19 test or screening.

Our age cutoff was chosen as older adults ≥ 65 years since the older adult population has a significantly different oral microbial composition compared to younger groups ($p<0.05$) (Feres et al., 2016), which could influence study outcomes. Older adults are more likely to reside in a nursing home (Aim 2) and have more risk factors for NV-HAP development (Aim 3). This age group promotes a more homogenous sample of those at highest risk for NV-HAP.

Exclusion criteria consist of:

1. Diagnosis of pneumonia < 48 hours after admission to the hospital (which would be categorized as community-acquired pneumonia);
2. Mechanical ventilation;
3. Hospice care;
4. Immunosuppression within the past 3 months (chemotherapy, radiotherapy, immunosuppressive medications, or ≥10 mg prednisolone per day) (Belstrøm, 2020; Ewan et al., 2015; Passaro et al., 2016a; Quinn et al., 2014a);
5. Prisoners.

Study endpoints include any of the exclusion criteria met: mortality, day 7 of hospitalization, and/or discharge/transfer from the hospital. Diagnosis of NV-HAP was not chosen to be a study endpoint due to limited literature regarding how the oral microbiome alters during pneumonia progression.

Sample Size and Statistical Power

According to our prior work, the time effect on both oral microbial diversity (Shannon Index) and dissimilarity (Bray-Curtis [BC] Index) of hospitalized patients was significant (Sole et al., 2021). In a similar study to ours, Sole et al. (2021) found alpha-diversity (Shannon Index) in the oral microbiome of mechanically ventilated patients significantly decreased over time. They detected a large effect size ($\eta 2$ =0.185, *Ptime*=0.02) on the time factor (i.e., within-effect).

The large within-effect, equivalent to a Cohen's f of 0.476 ($f = \sqrt{\eta 2/(1 - \eta 2)}$ (Cohen, 1988), will allow us to detect a meaningful difference in oral microbial diversity over time in this study (Aim 1). To achieve a comparable effect size with a power of 80% and a significance level of 0.05, we performed sample size estimation using 2-Way repeated ANOVA. The power analysis calculation was based on measuring each patient's oral microbiome at four different time points (Aim 1) and baseline microbiome sampling based on pre-hospital residence (nursing home vs. home; Aim 2). We assumed the microbiome indices would be normally distributed with homogeneity of variances for all combinations of time and patient residence. However, in case this assumption is not satisfied, and non-parametric Friedman's 2-Way ANOVA be used for data analysis, we increased the sample size estimation by 15%. This is a commonly accepted estimate, as no sample size estimation model exists for Friedman's 2-way ANOVA (Prism, n.d.).

We estimated the need to recruit 29 patients from each of the two groups to meet both Aims 1 and 2. We will aim to over-enroll by 20% to account for attrition, with a targeted sample size of 70 patients (35 patients per group). This sampling plan will allow us to obtain 35 independent observations for each potential combination of time and patient residence. We believe that the number of independent observations will allow us to control for the effect of 4-5 confounding covariates (oral care/assessment, frailty, antibiotics, and Comorbidity Index). If we

are only able to obtain samples at 3 different time points instead of 4, we will still be able to detect a level of significance with 66 patients (including over-enrollment by 20%). Sample size analyses were performed using Stata/MP 15.1 (SataCorp LLC, 2019) and R package (WebPower, 2019).

Outcome Measures

All data are collected by the Principal Investigator (PI) either through a) abstraction from the electronic medical record (EMR) at the site (ORMC); or b) directly from the patients, legally authorized representative (LAR), or nursing staff (Tables 3, 4 and 5).

Table 3. Measurement of Demographic/Baseline Variables

Variable	Measurement	Type
Sex	Male or female	Dichotomous
Age	Numerical value	Continuous
Race	White, Black/African American, Asian, Native Hawaiian/Pacific Islander, American Indian/Alaska Native, Other (Centers for Disease Control and Prevention, 2018b)	Categorical
Ethnicity	Hispanic/Latino or not Hispanic/Latino (Centers for Disease Control and Prevention, 2018a)	Dichotomous
Cognitive impairment	"Mini-Mental State Examination" (Tsoi et al., 2015)	Continuous
Delirium	"Confusion Assessment Method" (Schuurmans MJ et al., 2003; Sharon et al., 1990)	Dichotomous
Mortality	Charlson Comorbidity Index	Continuous
Frailty	Validated "Canadian Study of Health and Aging Clinical Frailty Scale" (Rockwood et al., 2005)	Categorical
Body mass index	Underweight, normal weight, overweight, obese	Categorical
Diagnoses	Yes/no: Cardiovascular, surgical, general medical	Dichotomous for each category
Dental device	Yes/no: Dentures, bridge, retainer	Dichotomous for each category
Smoking history	Yes or no	Dichotomous
Current smoker	Yes or no	Dichotomous
Pre-hospital residence	Home or nursing home	Dichotomous
Oral care barrier(s)	Yes/no: Inadequate supplies; no help; lack energy	Dichotomous for each category
Readmitted hospital within 30-days	Yes or no	Dichotomous
30-day readmit post-study	Yes or no	Dichotomous

Table 4. Measurement of Longitudinal Variables

Variable	Measurement	Type
Consciousness	Glasgow Coma Scale	Continuous
Oral assessment	"Oral Health Assessment Tool" (Chalmers et al., 2005)	Continuous
Invasive device	Yes/no: Urinary catheter, central venous catheter, nasoenteral tube	Dichotomous for each category
Medications	Yes/no: Central nervous system depressants, antibiotics, antacids, corticosteroids	Dichotomous for each category
Antibiotics name	Name of antibiotics	Categorical
Antibiotics date	Date antibiotics began	Continuous
Oral care frequency	Times/day oral care performed	Continuous
Oral care type	Toothbrushing/toothpaste, chlorhexidine, other	Categorical
Oral care personnel	Staff member, patient, family	Categorical
Oxygen	Yes or no: Oxygen	Dichotomous
Mode	Mode of oxygen delivery (mask or nasal)	Dichotomous
Flow	Flow of oxygen (LPM)	Continuous
Non-invasive ventilation	Delivery of positive pressure ventilation (CPAP or BiPAP)	Dichotomous
CPAP cm/H_2O	CPAP pressure (measured in cm/H20)	Continuous
PS cm/H_2O	Pressure support (measured in cm/H20)	Continuous
FiO_2	Fraction of inspired oxygen delivered	Continuous
COVID-19 diagnosis	Yes or no	Dichotomous
Date diagnosed	Yes/no: Date diagnosed with COVID-19	Dichotomous
Hospitalization	Yes/no: Hospitalization with COVID-19	Dichotomous
Complications	Yes/no: Complications associated with COVID-19	Dichotomous
NV-HAP diagnosis	Yes or no: Diagnosis with NV-HAP	Dichotomous
Date diagnosed	Date diagnosed with NV-HAP	Continuous
Hospital LOS	Number of days in hospital	Continuous
Discharge disposition	Long-term care facility, home, death	Categorical

Table 5. Measurement of Aims

Variable	Measurement	Type
Aim 1		
Oral microbiome		
Bacterial taxonomy identification	Oral microbe type across up to four time points	Categorical
α-diversity	Within-sample diversity (Shannon and Simpson diversity indices)	Continuous
β-diversity	Between-sample diversity (UniFrac distances and BC dissimilarity)	Continuous
Aim 2		
Pre-hospital residence	Nursing home or home	Dichotomous
Bacterial taxonomy identification	Oral microbe type in baseline sample	Categorical
α-diversity	Within-sample diversity (Shannon and Simpson diversity indices)	Continuous
β-diversity	Between-sample diversity (UniFrac distances and BC dissimilarity)	Continuous
Aim 3		
NV-HAP	Yes or no (defined by CDC criteria)	Dichotomous

Primary outcome (Aim 1): Oral bacterial taxonomy and bacterial diversity measures will be assessed over time during hospitalization.

Secondary outcome (Aim 2): Pre-hospital residence of each patient is recorded from the EMR. Oral bacterial taxonomy and bacterial diversity measures will be compared at enrollment based on pre-hospital residence (nursing home versus home).

Secondary outcome (Aim 3): NV-HAP is defined using Centers for Disease Control and Prevention (CDC) criteria as occurring on or after day 3 of hospital admission to an inpatient location (Centers for Disease Control and Prevention, 2019, 2020).The patient must present with a minimum of fever, leukopenia/leukocytosis, and/or altered mental status with no other cause for those > 70 years of age. At least two of the following signs must also be noted: change in respiratory sputum/secretions; new onset/ worsened cough, dyspnea, or tachypnea; rales/bronchial breath sounds; and/or worsening gas exchange. Two or more chest x-rays must be

positive for either infiltrate, consolidation, and/or cavitation. One definitive imaging test is sufficient in those without underlying heart or lung disease. NV-HAP criteria are collected daily from the EMR. A physician or an advanced practice nurse will independently verify all NV-HAP diagnoses.

Research Procedures

Recruitment and Informed Consent

The PI uses the EMR to screen patients using our inclusion criteria to determine patient eligibility for study participation. For those who meet inclusion criteria and agree to study participation, the PI obtains written informed consent from either the patient (if able to consent) or the LAR. Before obtaining consent from a patient, the PI assesses the patient's cognitive status and ability to consent using the Mini-Mental State Examination (MMSE) and Confusion Assessment Method (CAM) Tool. The MMSE is a validated tool used to assess cognitive status for dementia in hospital, primary care, clinical, and community settings (Tsoi et al., 2015). The CAM is a validated tool used to screen for delirium (Schuurmans MJ et al., 2003; Sharon et al., 1990). A patient cannot consent for themselves if the MMSE score is < 25 (score must be $\underline{\geq}$ 25) and/or the presence of delirium is confirmed on the CAM with positive features 1 and 2 plus either 3 or 4 (total of at least 3 confirmed features). If a patient meets either of these criteria and is not able to consent for themselves, a LAR may consent on behalf of the patient. The LAR is educated on the study in the same manner as though they were the patient.

Data Collection

Demographic/baseline data are collected upon patient enrollment into the study. Longitudinal data are collected both upon enrollment and each study day (unless otherwise specified) until a study endpoint is met. The oral cavity is assessed immediately before oral

specimen collection using the "Oral Health Assessment Tool," which assesses several aspects of the oral cavity: lips, tongue, gums/tissues, saliva, natural teeth, dentures, cleanliness, and pain (Chalmers et al., 2005). The PI obtains a baseline oral sample within 72 hours of hospital admission (sample 1). Our aim is to recruit and obtain sample 1 as soon as possible after admission, but no later than 72 hours. After sample 1 is obtained, oral samples will be collected by the PI on days 3, 5, and 7, or immediately before patient discharge (total of 4 samples per participant). Saliva was chosen instead of dental plaque to better represent the collective microbial composition of the oral cavity (Mira, 2018). The salivary microbiota reflects bacterial alterations in both supragingival and subgingival microbiotas (Belstrøm, 2020).

We chose our data collection time frames based on the following: First, we want to explore the relationship between the patient's baseline oral microbiome and pre-hospital residence (Aim 2). We chose 72 hours as the cutoff as prior research found that the oral microbiome remains relatively stable for the first three days of hospitalization and 90% of significant initial microbial colonization is detected within this window (Cabral et al., 2017; Ewan et al., 2015). Second, oral specimens are collected until a study endpoint is met, including day 7 of hospitalization. A study found a significant association between oral bacterial colonization and NV-HAP on day 5 of hospitalization, suggesting we will see significant oral microbial changes within the first 7 days of hospitalization (Ewan et al., 2015). Recent data from our study site found the average time to NV-HAP diagnosis was 6.1 days (median 4.0 days) (Giuliano et al., 2021). The average LOS for older adults $\geq$ 65 years of age on our study units was 6.4 days. Using a cutoff of 7 days for oral sampling allows us to detect oral microbiome changes associated with NV-HAP and capture NV-HAP cases, while remaining feasible. Given

the average LOS is only 6.4 days, following the patients for a longer period would not likely allow us to detect any significant changes after day 7 due to the small sample size.

Before collecting the oral salivary sample, the PI ensures the participant has not had anything to eat, drink, or oral hygiene care within the past 30 minutes (Cabral et al., 2017). The PI asks the patient to spit one milliliter of unstimulated saliva into a standardized collection kit (Cabral et al., 2017; Gomar-Vercher et al., 2018). Unstimulated saliva provides a more diverse representation of the oral cavity, while stimulated saliva has a microbial profile similar to the tongue (Mira, 2018). No significant differences in microbial diversity were found using differing methods of unstimulated saliva collection including spit, drool, or oral rinse (Lim et al., 2017). The spitting method was chosen since it is less complex of a process for patients.

Lab Analysis of Oral Specimens

The PI will analyze specimens using 16S rRNA sequencing during which genomic DNA is first extracted from the oral samples. The 16S rRNA gene is amplified (targeting the V3-V4 variable region) (Cabral et al., 2017; Deo & Deshmukh, 2019), and the sample libraries barcoded. Sequence data are generated from the pooled libraries using the Illumina MiSeq platform.

Metadata Management

Clinical data associated with samples are de-identified, coded numerically, and entered into Research Electronic Data Capture (REDCap^{TM}). All data will be stored on a dedicated, password-protected computer kept in the PI's locked office at the university. Efforts will be made to minimize missing data. The extent and pattern of missing data will be evaluated and handled accordingly, if needed.

Data Analysis

16S rRNA Sequence Data Processing

Analyses metrics will include bacterial taxonomy identification, alpha diversity, and beta-diversity (Moon & Lee, 2016; Morgan & Huttenhower, 2012). The microbiome samples (fastq files) will be processed using the QIIME2 pipeline to identify Amplicon Sequence Variants (ASVs) which will be used to generate a microbial taxa profile for each sample (Caporaso et al., 2010). The ASV data will also be used to generate various sample diversity measures, including alpha diversity (Shannon and Simpson diversity indices) and beta-diversity (UniFrac distances and BC dissimilarity).

Aim 1 Analyses

We will assess how diversity and community structure change over time between groups. If the measures are normally distributed, 2-Way repeated ANOVA otherwise non-parametric Friedman's 2-Way ANOVA, will be used. Multiple mixed effect models will be used to evaluate the effects of time and between groups through controlling for confounding variables if they show a significant difference between groups. The overall bacterial community structures will be compared using multivariate analysis including exploratory ordination approach, MANCOVA, if the measures show normality or non-parametric permutational multivariate ANOVA (PERMANOVA) otherwise. It is unknown if sex as a biological variable will influence findings, thus this relationship will be explored.

Aim 2 Analyses

Statistical differences in baseline oral microbial diversity based on pre-hospital residence will be tested using 2 sample t-test, if the measure demonstrates normality, otherwise Wilcoxon rank-sum test. We will also compare the overall bacterial community structures of those admitted

to the hospital from a nursing home (Group 1) with those from home (Group 2) using multivariate analysis including exploratory ordination approach and MANCOVA if the measures show normality or non-parametric PERMANOVA otherwise.

Both Aims 1 and 2 Analyses

Methods appropriate for dealing with compositional data will be used (Aitchison, 1981), since the 16S rRNA gene based taxonomic profiles reflect relative abundances of taxa. Non-parametric multidimensional scaling (NMDS) based on BC dissimilarity will be used to evaluate the bacterial community structure difference between groups after appropriate log-transformation of the relative abundance data. Multinomial logistic regression modeling will be developed to model these relative abundance data and adjust for potential confounding variables (e.g., oral care/assessment, frailty, antibiotics, and Comorbidity Index).

Aim 3 Analyses

Descriptive statistics will be used to summarize the incidence of NV-HAP and the bacterial community structure in those diagnosed with NV-HAP.

Discussion

Study Strengths and Challenges

Currently, we have enrolled 47 patients in our study (32 patients from home and 15 patients from a nursing home). Of the 47 patients, 28 had data collected across all four time points, which is a strength, and 38 had data collected for the minimum of three time points. Study protocol strengths also include using an interdisciplinary approach to pair clinical data with bioinformatics; short enrollment window; longitudinal data collection; and coupling of oral microbiome with clinical data including pre-hospital residence, oral health status, and oral care.

Conducting a clinical study has posed several challenges that we are continuously adapting to due to the nature of clinical research, particularly amid the coronavirus 2019 (COVID-19) pandemic. Data collection began in May of 2021, so we have adjusted our inclusion criteria over the past year and a half to reflect current COVID-19 testing practices at the study site. The study site currently screens each patient for COVID-19; however, does not routinely test each patient. Having a diagnosis of COVID-19 may alter the oral microbiome, which could influence study outcomes. Current inclusion criteria were adjusted to reflect patients having either a negative COVID-19 test and/or screening.

Next, we found that many patients met exclusion criteria during screening, particularly re-admission to the hospital within 30 days and diagnosis of pneumonia within 48 hours of hospital admission. It is unknown whether hospital re-admission within 30 days alters the oral microbiome, which is why we initially included this as part of our exclusion criteria. We removed this as part of our exclusion criteria and now instead include hospital re-admission within 30 days as a recorded clinical demographic variable. Due to the nature of our study focusing on NV-HAP, hospital admission with pneumonia is considered community-acquired pneumonia, therefore we could not change this exclusion criterion.

In addition, it has been challenging to recruit nursing home patients due to a) there being fewer patients admitted to the hospital study units from a nursing home compared to home; b) most require a proxy (which presents its own set of challenges); c) many are admitted to the hospital with pneumonia, and d) many are not able to provide a saliva specimen due to cognitive impairment (e.g., agitation). Proxies often are not physically present at the hospital and are difficult to get in contact with, which also presents challenges to communication. We have been proactive in reaching out to proxies via phone and leaving messages with the study PI's contact

information. Each proxy that has responded to study inquiry has agreed to study participation. We have also observed that enrolled nursing home patients have a decreased hospital length of stay compared to patients from home. Thus, fewer nursing home patients have data collected at all four time points.

Important Takeaways

Data collection within the clinical setting can be rather unpredictable (particularly amid a pandemic) emphasizing the importance of a team approach, flexibility, and having a backup plan (Sole et al., 2018). Clinical data collection over multiple time points has been challenging (but feasible for this specific study protocol) with only one researcher (the PI) collecting data. However, researchers should consider the benefits and consequences of using one versus multiple individuals for data collection. This will likely depend on several factors including (but not limited to) study enrollment window; number of data collection time points; time and type of data collection; and cost constraints.

Future related clinical studies exploring the oral microbiome and/or oral health should consider these points as well as consider adding a dental perspective to their study protocol to allow for more of an interdisciplinary approach (Lupi et al., 2022). While we incorporated bioinformatics, biological, and biostatistical expertise in our protocol, we lacked the ability to add a dental perspective due to limited study resources, as this protocol is part of a funded fellowship grant supporting dissertation research. We recommend dental perspective be considered in future like research protocols.

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CHAPTER FOUR: FINDINGS

Abstract

Background: Non-ventilator hospital acquired pneumonia (NV-HAP) impacts 1 in 100 hospitalized patients and is a significant patient concern due to its negative clinical outcomes. Many factors play a role in NV-HAP development: oral health, oral care, microaspiration risk, and the oral microbiome. Little is known about how the oral microbiome alters during hospitalization. This study explored changes in the oral microbiome of non-ventilated hospitalized patients over time and analyzed the relationship between the oral microbiome, pre-hospital residence, and NV-HAP.

Methods: A prospective, observational design was used to recruit 46 non-ventilated adults $\geq$ 65 years from a large medical center in central Florida (n=15 nursing home; n=31 home) within 72 hours of admission (baseline). Oral salivary specimens were collected, and an oral assessment was completed using the Oral Health Assessment Tool at four time points: baseline and hospital days 3, 5, and 7. Genomic DNA was extracted from oral samples for microbiome profiling by 16S rRNA sequencing. Taxonomic composition, relative abundance, alpha diversity (Shannon Index), and beta diversity (Bray-Curtis dissimilarity) of bacterial communities were determined. Data were analyzed using descriptive statistics, Chi-squared, independent t-test, repeated measures mixed effects modeling, two-way permutational ANOVA, ANOSIM, and multiple dispersion.

Results: Most participants were female (70%) and white (74%) with mean age of 78.7$\pm$9.1 years. Oral bacteria genera remained consistent across hospitalization: *Streptococcus*, *Rothia*, and *Prevotella*. Mean Shannon Index changed over time ($p<.001$) and over time by group ($p<.01$). Relative bacterial abundances were similar between groups; however, several less

frequent oral bacteria genera were higher in the nursing home group. Mean baseline Shannon Index was lower in the nursing home group (p<.001). Mean Bray-Curtis dissimilarity at baseline genus (p=.010) and ASV levels (p=.003) were higher in the nursing home group. Two patients developed probable NV-HAP (4%), at the time that *Neisseria* and *Streptococcus* increased. **Conclusions:** Although oral bacteria genera remained consistent, oral bacterial diversity changed across hospitalization and over time between groups. Several oral bacteria genera and oral bacterial diversity significantly differed between groups, emphasizing the importance of an individualized approach to oral care beginning at hospital admission. Specific bacteria genera may be meaningful indicators of NV-HAP development.

Keywords: oral health; oral care; oral microbiome; nursing home; older adult; non-ventilator hospital acquired pneumonia

Background

Non-ventilator hospital acquired pneumonia (NV-HAP) impacts 1 in 100 hospitalized patients and incidence rates are significantly higher in older adults. [1] NV-HAP is associated with high mortality rates up to 30%, increased hospital length of stay from 4.0 to 15.9 days, increased 30-day hospital re-admissions, and high costs per case ranging from $28,000 to $40,000. [2-4] NV-HAP also develops shortly after hospitalization. One study found the mean time to NV-HAP development was less than one week. [5]

NV-HAP is currently an urgent patient safety concern in the United States (US). [3] Pneumonia comprises 25% of healthcare-associated infections, of which 60% are categorized as NV-HAP. [6] Although initiatives in the US have been developed through the Hospital-Acquired Condition Reduction Program to reduce several healthcare-associated infections, NV-HAP is not

currently included. [3] It is imperative to lower NV-HAP rates to reduce the associated mortality rates and to alleviate the high financial burden placed on patients and healthcare systems.

A primary method to prevent NV-HAP that has been explored in non-ventilated patients is consistent oral care, which reduces the oral colonization of respiratory pathogens, thereby reducing likelihood of pneumonia development. [7-9] Many clinical factors, such as oral health, oral care, microaspiration risk, and the oral microbiome, collectively influence a patient's risk of developing NV-HAP. [10] The oral microbiome plays an important role in maintaining both oral and systemic health. [11-13] Disequilibrium within the oral microbiome may cause oral and/or systemic disease, including respiratory disease such as pneumonia. [10,13,14] Although changes in the oral microbiome clinically impact patients, [10] little is known about how the oral microbiome alters during hospitalization and with clinical variables unique to non-ventilated patients.

The primary aim of the study was to longitudinally explore changes in the oral microbiome of non-ventilated hospitalized patients (Aim 1). Secondary study aims were to explore the relationship between pre-hospital residence/environment (nursing home vs. home) and non-ventilated patient's baseline oral microbiome (Aim 2), as well as explore the relationship between the oral microbiome and NV-HAP development (Aim 3).

Methods

Refer to our protocol article for additional study details. [15]

Study Design and Setting

A prospective, observational study design was conducted from May of 2021 to August of 2022. Institutional Review Board approval of the study was obtained in November of 2020 from both the University and study site. The study setting was a large academic medical center located

in central Florida. Hospitalized patients were recruited from one of three progressive care units (PCUs; also referred to as intermediate or step-down units).

Study Sample

Convenience sampling was used to recruit a target enrollment of 58 patients (29 patients from a nursing home and 29 patients from home). Inclusion criteria consisted of not requiring mechanical ventilation, age ≥ 65 years, admission to a PCU, enrolled within 72 hours of hospital admission from either a nursing home or home, and negative COVID-19 test/screening. Nursing homes are also commonly referred to as skilled nursing facilities and are a type of long-term care facility. [16] Exclusion criteria consisted of pneumonia diagnosis within 48-hours of hospital admission (considered community-acquired pneumonia), mechanical ventilation, hospice care, immunosuppression within the past 3 months (chemotherapy, radiotherapy, immunosuppressive medications, or ≥10 mg prednisolone per day) [7,10,17,18], and prisoners. Study endpoints included exclusion criteria being met, mortality, day 7 of hospitalization, and/or discharge or transfer from the hospital.

Power Analysis

Our prior work showed a significant time effect on both oral microbial alpha diversity (Shannon Index) and beta diversity (Bray-Curtis dissimilarity) of hospitalized patients. [19] A large effect size was detected ($\eta 2$ =0.185, *Ptime*=0.02) on the time factor (i.e., within-effect). We estimated that the large within-effect, equivalent to a Cohen's f of 0.476 ($f = \sqrt{\eta 2}/(1 - \eta 2)$ [20], would allow for detection of a meaningful difference in oral microbial diversity over time (Aim 1). To achieve a comparable effect size with a power of 80% and a significance level of 0.05, sample size estimation was completed using 2-Way repeated ANOVA. The power analysis calculation was based on measuring each patient's oral microbiome at four different time points

(Aim 1) and baseline microbiome between groups (nursing home vs. home; Aim 2). It was assumed that microbiome indices would be normally distributed with homogeneity of variances for all combinations of time and pre-hospital residence. However, to ensure equivalent power in case this assumption was not satisfied, a non-parametric Friedman's 2-Way ANOVA would be used for data analysis and we increased the sample size estimation by 15%, which is a commonly accepted estimate. [21]

To meet Aims 1 and 2, we estimated the need to recruit 29 patients from each group (total 58 patients). We aimed to over-enroll by 20% to account for attrition with a targeted sample size of 70 patients. The number of independent observations would allow us to control the effect of four to five confounding covariates. A level of significance would still be detectable with 66 patients, including over-enrollment by 20%, if samples could only be obtained at three instead of four time points. Sample size analyses were completed using Stata/MP 15.1 (SataCorp LLC, 2019) and R package. [22]

Outcome Measures

All study data were collected by the Principal Investigator (PI) either through a) abstraction from the electronic medical record (EMR); or b) from the patients, legally authorized representative (LAR), or nursing staff.

Aim 1

Oral bacterial taxonomy and alpha diversity (Shannon Index) were assessed over time during hospitalization. Longitudinal and outcome clinical variables were also recorded from the EMR.

Aim 2

Pre-hospital residence was recorded from the EMR for each patient. Oral bacterial taxonomy, alpha diversity (Shannon Index), and beta diversity (Bray Curtis dissimilarity) were compared at enrollment based on pre-hospital residence (nursing home versus home). Baseline demographic and clinical variables were also recorded from the EMR.

Aim 3

NV-HAP was defined according to Centers for Disease Control and Prevention (CDC) criteria as occurring on or after day 3 of hospital admission to an inpatient location. [23,24] NV-HAP criteria were collected from the EMR. A physician or an advanced practice nurse independently documented all probable NV-HAP diagnoses in the EMR. In patients diagnosed with probable NV-HAP, oral bacterial taxonomy and alpha diversity (Shannon Index) were assessed across hospitalization. Clinical variables were also recorded from the EMR in patients who developed probable NV-HAP.

Recruitment and Informed Consent

The PI used the EMR to screen patients using inclusion criteria to determine patient eligibility to participate in the study. For patients who met inclusion criteria and agreed to participate in the study, the PI obtained written informed consent from either the patient (if able to consent) or the LAR. Prior to obtaining informed consent from the patient, the PI assessed the patient's ability to consent using both the Mini-Mental State Examination (MMSE) and Confusion Assessment Method (CAM) tool (assessed cognitive status for dementia and delirium, respectively). [25-27] The presence of delirium on the CAM tool was considered positive with features 1 and 2 plus either 3 or 4 (total minimum of 3 confirmed features). Patients could independently sign consent if their MMSE score was $\geq$ 25 and no delirium was noted using the

CAM tool. A LAR consented on behalf of the patient if the patient's MMSE score was < 25 and/or presence of delirium was confirmed.

Data Collection

Demographic and baseline data were collected upon patient enrollment into the study. Longitudinal data were collected both upon enrollment and each study day (unless otherwise specified) until a study endpoint was met.

Prior to specimen collection, the PI assessed the oral cavity using the Oral Health Assessment Tool (OHAT). The OHAT assesses several basic aspects of the oral cavity; higher scores indicate worse oral health (score range 0 to 16). [28] The PI also obtained a baseline oral salivary sample within 72 hours of hospital admission (sample 1). Our aim was to recruit and obtain sample 1 as soon as possible after hospital admission, but no later than 72 hours. After sample 1 was obtained, the PI collected oral samples on days 3, 5, and 7, or immediately before patient discharge (total of 4 samples per participant). If the baseline sample was obtained closer to 72 hours (day 3), it would still be considered an independent specimen from the day 3 (second) specimen. All patients had the number of hospital admission hours recorded when they enrolled in the study. Before collecting the oral salivary sample, the PI ensured the participant did not have anything to drink, eat, or oral hygiene within the past 30 minutes. [29] The PI then asked the patient to spit one milliliter of unstimulated saliva into a standardized collection kit. [29,30]

Metadata Management

Clinical data associated with oral salivary samples were de-identified, coded numerically, and entered into Research Electronic Data Capture (REDCap^{TM}). All data were stored on a

password-protected computer in the PI's locked office at the University. Signed consent forms were also stored in a locked filing cabinet in the PI's locked office.

16S rRNA Sequencing and Data Processing

DNA Extraction and Targeted Library Preparation

The oral salivary samples were processed and analyzed with the ZymoBIOMICS® Service: Targeted Metagenomic Sequencing (Zymo Research, Irvine, CA). ZymoBIOMICS®-96. MagBead DNA Kit was used for DNA extraction with a 50µl elution volume. The DNA samples were prepared for targeted sequencing with the Quick-16S™ Primer Set V3-V4 next-generation sequencing (NGS) Library Prep Kit. The sequencing library was prepared using an innovative library preparation process in which PCR reactions were performed in real-time PCR machines to control cycles and therefore limit PCR chimera formation. The final PCR products were quantified with qPCR fluorescence readings and pooled together based on equal molarity. The final pooled library was cleaned up with the Select-a-Size DNA Clean & Concentrator™, then quantified with TapeStation® (Agilent Technologies, Santa Clara, CA) and Qubit® (Thermo Fisher Scientific, Waltham, WA).

Control Samples

The ZymoBIOMICS® Microbial Community Standard was used as a positive control for each DNA extraction. The ZymoBIOMICS® Microbial Community DNA Standard was used as a positive control for each targeted library preparation. Negative controls (e.g., blank extraction control, blank library preparation control) were included to assess the level of bioburden carried by the wet-lab process.

Sequencing

The final library was sequenced on Illumina® MiSeq™ with a v3 reagent kit (600 cycles). The sequencing was performed with 10% PhiX spike-in.

Bioinformatics Analysis

Unique amplicon sequences were inferred from raw reads using the Dada2 pipeline. [31] Chimeric sequences were also removed with the Dada2 pipeline. The number of chimera-free sequences that underwent further amplicon size filtration and were included in the final data analyzed in Qiime v.1.9.1 ranged from 18,830 to 46,212. Taxonomy assignment was performed using Uclust from Qiime v.1.9.1. Taxonomy was assigned with the Zymo Research Database, a 16S database that is internally designed and curated, as reference. Composition visualization, alpha-diversity, and beta-diversity analyses were performed with Qiime v.1.9.1. [32] Taxonomy that had significant abundance among different groups were identified by LEfSe using default settings. [33] Other analyses were performed with internal scripts.

Absolute Abundance Quantification

A quantitative real-time PCR was set up with a standard curve. The standard curve was made with plasmid DNA containing one copy of the 16S gene and one copy of the fungal ITS2 region prepared in 10-fold serial dilutions. The primers used were the same as those used in Targeted Library Preparation. The equation generated by the plasmid DNA standard curve was used to calculate the number of gene copies in the reaction for each sample. The PCR input volume (2 µl) was used to calculate the number of gene copies per microliter in each DNA sample. The number of genome copies per microliter DNA sample was calculated by dividing the gene copy number by an assumed number of gene copies per genome. The value used for 16S copies per genome is 4. The value used for ITS copies per genome is 200. The amount of DNA per microliter DNA sample was calculated using an assumed genome size of 4.64 x 106

bp, the genome size of *Escherichia coli*, for 16S samples, or an assumed genome size of 1.20 x 107 bp, the genome size of *Saccharomyces cerevisiae*, for ITS samples. This calculation is the following: Calculated Total DNA = Calculated Total Genome Copies × Assumed Genome Size (4.64 × 106 bp) × Average Molecular Weight of a DNA bp (660 g/mole/bp) ÷ Avogadro's Number (6.022 x 1023/mole).

Statistical Analysis

SPSS version 26.0 and R Programming language were used for data analysis.

16S rRNA Sequence Data Processing

Analyses metrics included bacterial taxonomy identification, alpha diversity (Shannon Index), and beta diversity (Bray-Curtis dissimilarity).

Aim 1

Descriptive statistics were used to summarize the most frequent oral bacterial taxonomies (genus and species) using relative abundance percentages across hospitalization. For alpha diversity (Shannon Index), repeated measures mixed effects model was used to compare the longitudinal changes of the oral microbiome.

Aim 2

Descriptive statistics were used to summarize the most frequent bacterial community structures (genus and species) using relative abundance percentages between groups. Independent samples t-test was used to assess differences in oral bacterial genera relative abundance percentages between groups. For alpha diversity, two-way permutational ANOVA was used to compare Shannon Index values between the two groups. For beta diversity (Bray-Curtis dissimilarity), the Analysis of Similarities (ANOSIM) was used to compare the group

difference in mean, and the Multivariate dispersion with permutation test was used to compare the group difference in variance.

Aim 3

Descriptive statistics were used to summarize the incidence, oral bacterial community structure, and alpha diversity (Shannon Index) in those diagnosed with NV-HAP, because only two participants developed probable NV-HAP.

Results

A total of 49 patients/proxies were approached by the PI regarding study participation; 47 patients (or their proxies) consented to participate. One patient was unable to provide a saliva specimen and refused an oral swab after consenting to study participation; therefore, was excluded from our final analyses. Forty-six patients were included in our baseline analysis (15 patients admitted from nursing home vs. 31 patients admitted from home). Although the window for recruitment was 72 hours, 45 of 46 patients were enrolled within 48 hours of hospital admission. Regarding longitudinal data collection, 38 patients had data collected across three time points (through Day 5); and 28 patients had data collected across all four time points (through Day 7).

Demographic Data

Table 6 depicts demographic and clinical information for the sample. The majority of participants were female (70%) and white (74%) with mean age of 78.7 $\pm$ 9.1 years. Racial (26%) and Hispanic ethnic minorities (11%) were represented. Participants had high levels of comorbidities (mean Charlson Comorbidity Index score of 6.8), indicating higher likelihood of mortality risk. Most patients had cardiac (96%) diagnoses. Around half of the patients had a history of smoking (54%) and dental device (48%). Few patients were re-admitted to the hospital

within 30 days prior to study enrollment (13%). Compared to home group, the nursing home group had significantly higher levels of cognitive impairment ($p<.001$), delirium ($p=.010$), frailty scores ($p<.001$), and Charlson Comorbidity Index scores ($p=.039$).

Table 6. Baseline Variables of Study Sample

Variable	Total Group (*n*=46)	Nursing Home Group (*n*=15)	Home Group (*n*=31)	P-Value
Sex, *n* (%)				
Female	32 (69.6)	13 (86.7)	19 (61.3)	.080[a]
Male	14 (30.4)	2 (13.3)	12 (38.7)	
Age, mean (SD)	78.7 (9.1)	81.4 (8.9)	77.3 (9.1)	.158[b]
Race, *n* (%)				
White	34 (73.9)	11 (73.3)	23 (74.2)	.488[a]
Black	6 (13.0)	2 (13.3)	4 (12.9)	
Other	5 (10.9)	1 (6.7)	4 (12.9)	
Asian	1 (2.2)	1 (6.7)	0 (0)	
Ethnicity, *n* (%)				
Not Hispanic/Latino	41(89.1)	15 (100)	26 (83.9)	.099[a]
Hispanic/Latino	5 (10.9)	0 (0)	5 (16.1)	
Cognitive Impairment, *n* (%)	11 (23.9)	10 (66.7)	1 (3.2)	<.001[a*]
Delirium, *n* (%)	3 (6.5)	3 (20.0)	0 (0)	.010[a*]
GCS score, mean (SD)	14.7 (0.6)	14.1 (0.7)	15.0 (0)	<.001[b*]
Frailty Score, *n* (%)				
3 (Well, treated comorbidity	4 (8.7)	0 (0)	4 (12.9)	<.001[a*]
4 (Apparently vulnerable)	16 (34.8)	1 (6.7)	15 (48.4)	
5 (Mildly frail)	10 (21.7)	1 (6.7)	9 (29.0)	
6 (Moderately frail)	14 (30.4)	11 (73.3)	3 (9.7)	
7 (Severely frail)	2 (4.3)	2 (13.3)	0 (0)	
BMI, *n* (%)				
Underweight	6 (13.0)	3 (20.0)	3 (9.7)	.512[a]
Normal weight	9 (19.6)	4 (26.7)	5 (16.1)	
Overweight	13 (28.3)	4 (26.7)	9 (29.0)	
Obese	18 (39.1)	4 (26.7)	14 (45.2)	
Charlson Comorbidity Score, mean (SD)	6.8 (2.2)	7.7 (1.3)	6.3 (2.5)	.039[b*]
Diagnosis, *n* (%)				
Cardiac	44 (95.7)	14 (93.3)	30 (96.8)	.592[a]
Dental Device, *n* (%)	22 (47.8)	8 (53.3)	14 (45.2)	.603[a]
Smoking History, *n* (%)	25 (54.3)	9 (60.0)	16 (51.6)	.592[a]
Current Smoker, *n* (%)	3 (6.5)	1 (6.7)	2 (6.5)	.978[a]
Re-Admitted Within 30 Days Prior to Study, *n* (%)	6 (13)	3 (20.0)	3 (9.7)	.330[a]
OHAT Score, mean (SD)	6.6 (3.6)	10.6 (2.6)	4.6 (2.0)	<.001[b*]
Oral Care Frequency/Day, mean (SD)	0.5 (0.8)	0.1 (0.4)	0.7 (0.9)	.025[b*]
Oxygen, *n* (%)	11 (23.9)	5 (33.3)	6 (19.4)	.297[a]
Non-Invasive Ventilation, *n* (%)	1 (2.2)	1 (6.7)	0 (0)	.146[a]
Oral Care Barriers, *n* (%)				
No supplies	22 (47.8)	5 (33.3)	17 (54.8)	.171[a]
No help	16 (34.8)	11 (73.3)	5 (16.1)	<.001[a*]
Lack energy	14 (30.4)	3 (20)	11 (35.5)	.285[a]
None	8 (17.4)	0 (0)	8 (25.8)	.030[a*]
Invasive Devices, *n* (%)				
Urinary catheter	2 (4.3)	1 (6.7)	1 (3.2)	.592[a]
Central venous catheter	2 (4.3)	2 (13.3)	0 (0)	.038[a*]
Medications, *n* (%)				

Variable	Total Group (*n*=46)	Nursing Home Group (*n*=15)	Home Group (*n*=31)	P-Value
CNS depressants	7 (15.2)	2 (13.3)	5 (16.1)	.805[a]
Antacids	18 (39.1)	7 (46.7)	11 (35.5)	.466[a]
Corticosteroids	4 (8.7)	2 (13.3)	2 (6.5)	.437[a]
Antibiotics	11 (23.9)	7 (46.7)	4 (12.9)	.012[a*]

CNS, central nervous system; *GCS*, Glasgow Coma Scale; *OHAT*, Oral Health Assessment Tool; *SD*, standard deviation

[a] Chi-Squared test; [b] t-test

*Statistically significant $p < 0.05$

Longitudinal Changes in Oral Microbiome of Non-Ventilated Hospitalized Patients

Oral Bacterial Taxonomy

Refer to Tables 7 through 10 for longitudinal clinical variables. Tables 7 and 8 include study completers through day 5 of hospitalization (*n*=38), while Tables 9 and 10 include study completers through day 7 of hospitalization (*n*=28). At baseline, the oral bacteria genera with the highest relative abundance were *Streptococcus, Rothia*, *Prevotella, Veillonella,* and *Actinomyces*. On hospital day 3, *Streptococcus*, *Rothia*, *Prevotella*, *Actinomyces*, and *Veillonella* were most common. On hospital day 5, *Streptococcus, Prevotella, Rothia, Neisseria,* and *Veillonella* were frequently noted. On hospital day 6 or 7 (depending on patient discharge), *Streptococcus, Prevotella, Rothia, Neisseria,* and *Actinomyces* were most common. See Figures 4 and 5 for differences between groups in oral bacterial genus and species taxonomies, respectively.

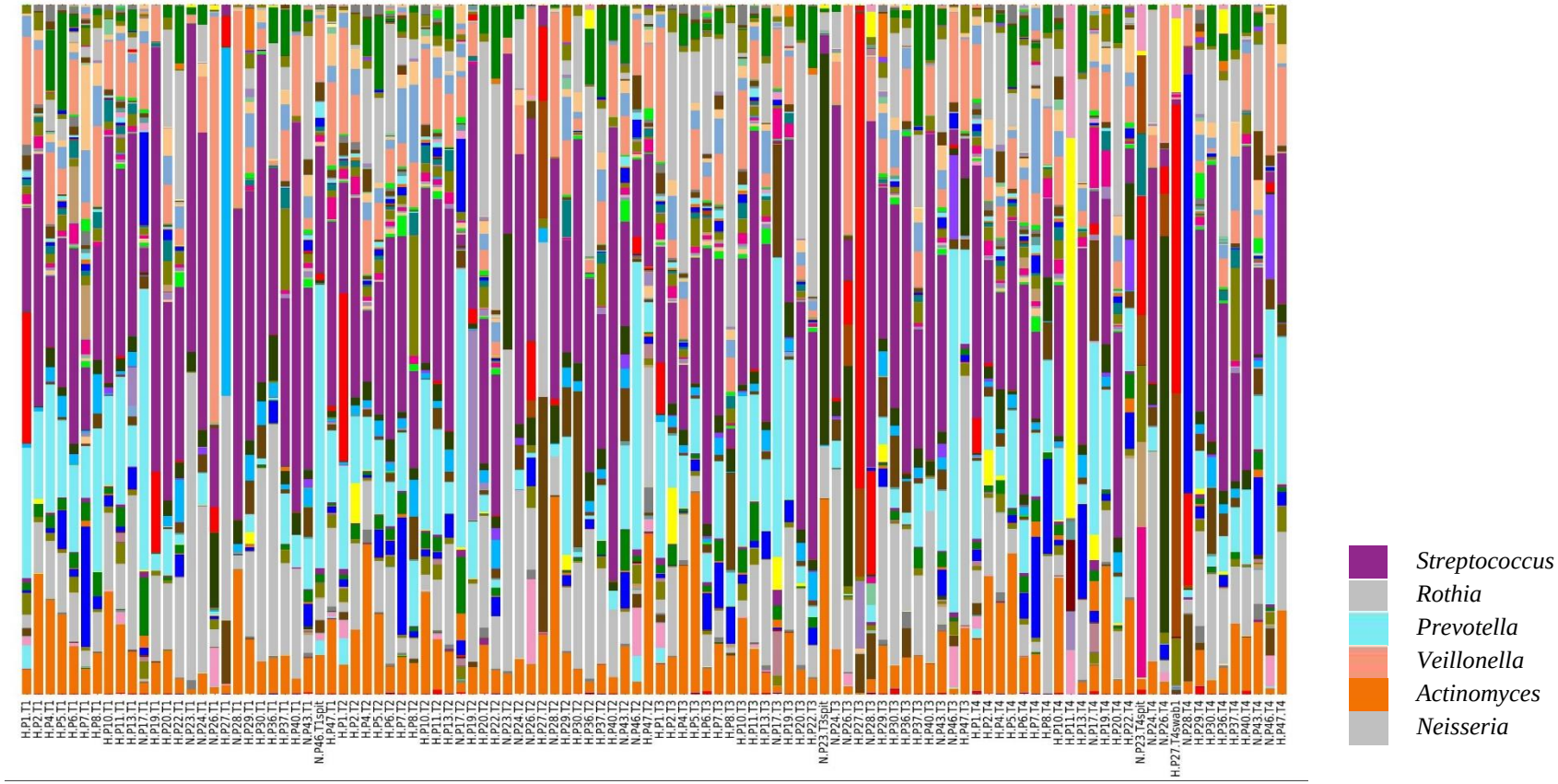

Figure 4. Oral Bacteria Genera Changes Across Hospitalization (Grouped by Time)

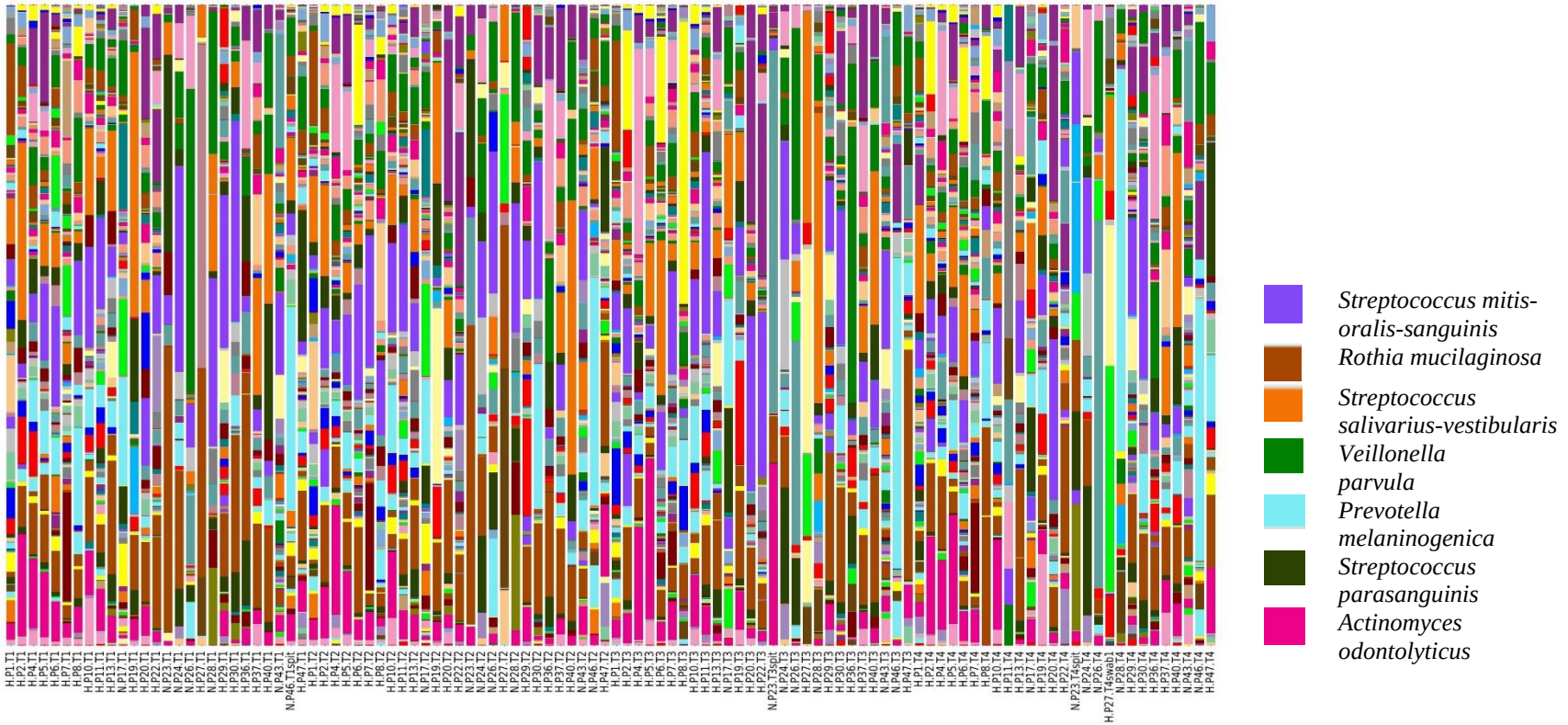

Figure 5. Oral Bacteria Species Changes Across Hospitalization (Grouped by Time)

Alpha Diversity

Shannon Index changed significantly across four time points (p<.001), as mean values ranged across hospitalization from 4.5 (SD=1.0), 4.7 (SD=0.9), 4.5 (SD=0.9), 4.6 (SD=1.0) (Figure 6). There was also a significant interaction between groups over time (p<.010) (Figure 7). The nursing home group mean Shannon Index values changed over time from 4.3 (SD=1.1), 4.5 (SD=1.0), 4.0 (SD=1.0), and 4.0 (SD=1.1). The home group mean Shannon Index values were 4.6 (SD=1.0), 4.8 (SD=0.9), 4.7 (SD=0.8), and 4.9 (SD=0.8). Repeated measures mixed effects modeling was computed with five predictors as confounders. There was no influence of sex (p=.532), Charlson Comorbidity Index (p=.794), cognitive impairment (p=.404), frailty score (p=.794), and oral assessment score (p=.088) on Shannon Index values. After adjusting for covariates, there was still a significant time and group by time interaction.

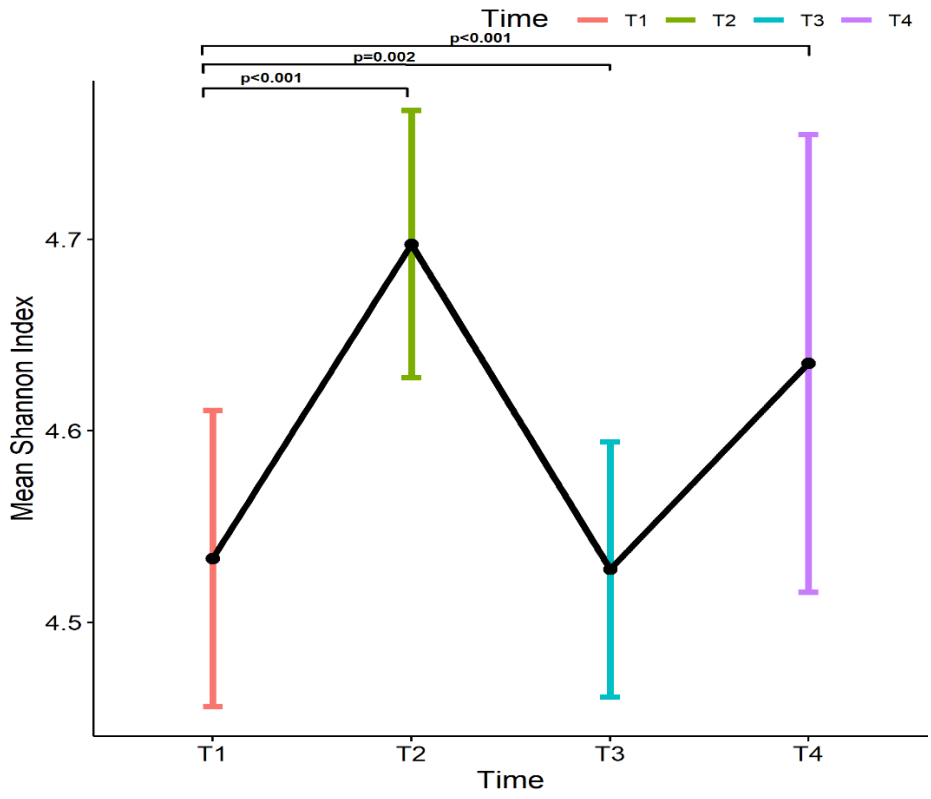

Figure 6. Mean Shannon Index for Combined Groups Significantly Changed Across Hospitalization

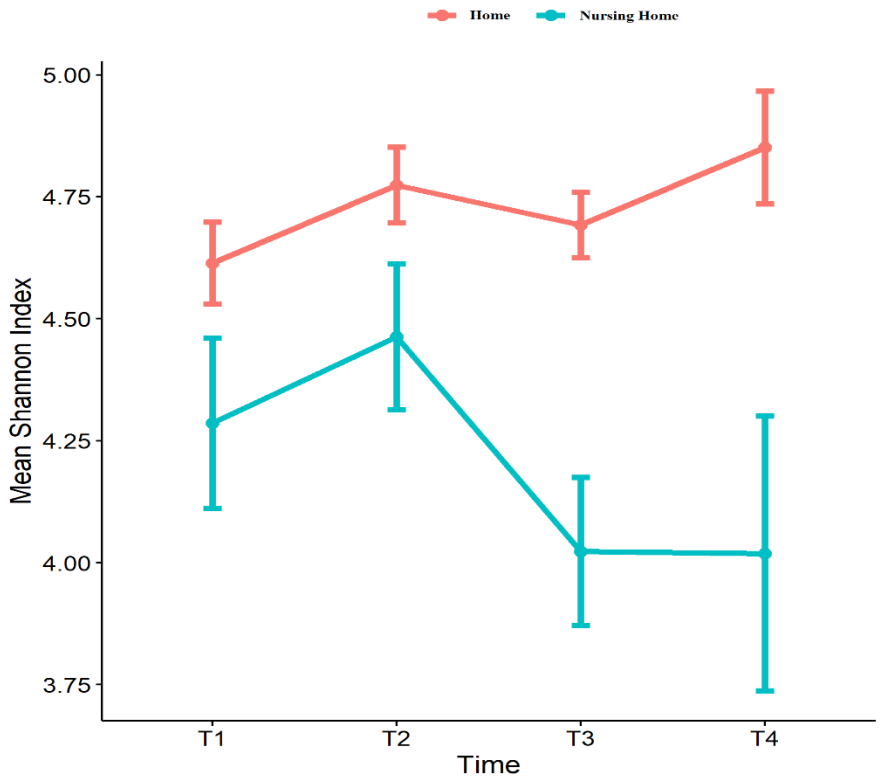

Figure 7. Mean Shannon Index Significantly Differed Between Groups Over Time Across Hospitalization

Table 7. Longitudinal Variables of Study Cohort Through Hospital Day 5

Variable	Time Point 1 (Baseline; Within 48 hours)			Time Point 2 (Hospital Day 3)			Time Point 3 (Hospital Day 5)		
	Nursing Home (*n*=9)	Home (*n*=29)	P-Value	Nursing Home (*n*=9)	Home (*n*=29)	P-Value	Nursing Home (*n*=9)	Home (*n*=29)	P-Value
GCS Score, mean (SD)	14.1 (0.8)	15.0 (0)	<.001[a*]	14.0 (0.7)	15.0 (0)	<.001[a*]	13.1 (2.5)	15.0 (0)	<.001[a*]
Invasive Devices, *n* (%)									
Urinary catheter	1 (11.1)	1 (3.4)	.368[b]	1 (11.1)	1 (3.4)	.368[b]	0 (0)	0 (0)	NA
Central venous catheter	2 (22.2)	0 (0)	.009[b*]	1 (11.1)	0 (0)	.069[b]	1 (11.9)	0 (0)	.069[b]
Medications, *n* (%)									
CNS depressants	1 (11.1)	4 (13.8)	.835[b]	3 (33.3)	4 (13.8)	.186[b]	2 (22.2)	7 (24.1)	.906[b]
Antacids	4 (44.4)	11 (37.9)	.727[b]	5 (55.6)	11 (37.9)	.350[b]	6 (66.7)	12 (41.4)	.184[b]
Corticosteroids	1 (11.1)	1 (3.4)	.368[b]	2 (22.2)	2 (6.9)	.191[b]	1 (11.1)	1 (3.4)	.368[b]
Antibiotics	5 (55.6)	3 (10.3)	.004[b*]	4 (44.4)	4 (13.8)	.049[b*]	2 (22.2)	8 (27.6)	.750[b]
OHAT Score, mean (SD)	10.3 (2.4)	4.7 (2.0)	<.001[a*]	10.6 (1.8)	5.2 (2.3)	<.001[a*]	10.7 (3.0)	6.4 (2.2)	<.001[a*]
Oral Care Frequency/Day, mean (SD)	0.1 (0.3)	0.7 (0.9)	.066[a]	0.2 (0.4)	0.9 (0.8)	.035[a*]	0.6 (0.5)	0.7 (0.7)	.512[a]
Oral Care Type, *n* (%)									
Toothbrushing/ toothpaste	1 (11.1)	13 (44.8)	.067[b]	1 (11.1)	18 (62.1)	.008[b*]	4 (44.4)	15 (51.7)	.703[b]
Chlorhexidine gluconate	0 (0)	0 (0)	NA	0 (0)	0 (0)	NA	1 (11.1)	2 (6.9)	.682[b]
Other	0 (0)	0 (0)	NA	1 (11.1)	0 (0)	.069[b]	0 (0)	0 (0)	NA
Oral Care Personnel, *n* (%)									
Staff member	1 (100)	0 (0)	<.001[b*]	1 (50)	1 (5.6)	.047[b*]	3 (60)	0 (0)	<.001
Patient	0 (0)	13 (100)	<.001[b*]	1 (50)	17 (94.4)	.047[b*]	2 (40)	17 (100)	<.001
Oxygen, *n* (%)	4 (44.4)	6 (20.7)	.157[b]	3 (33.3)	8 (27.6)	.740[b]	2 (22.2)	7 (24.1)	.906[b]
Non-Invasive Ventilation, *n* (%)	1 (11.1)	0 (0)	.069[b]	1 (11.1)	0 (0)	.069[b]	1 (11.1)	0 (0)	.069[b]
NV-HAP Diagnosis, *n* (%)	0 (0)	0 (0)	NA	1 (11.1)	0 (0)	.069[b]	1 (11.1)	1 (3.4)	.368[b]

CNS, central nervous system; *GCS*, Glasgow Coma Scale; *NV-HAP*, non-ventilator hospital acquired pneumonia; *OHAT*, Oral Health Assessment Tool; *SD*, standard deviation

[a] t-test; [b] Chi-Squared test

*Statistically significant $p < 0.05$

Table 8. Clinical Outcomes of Study Completers Through Hospital Day 5

Clinical Outcomes	Nursing Home Group (n=9)	Home Group (n=29)	P-Value
Hospital LOS days, mean (SD)	6.8 (1.0)	9.1 (4.4)	.141[a]
Discharge Disposition, *n* (%)			
Home	1 (11.1)	25 (86.2)	<.001[b*]
LTC facility	8 (88.9)	3 (10.3)	
Inpatient rehab	0 (0)	1 (3.4)	
30-Day Hospital Re-admission Post-Study, *n* (%)	4 (44.4)	7 (24.1)	.241[b]

LOS, length of stay; *LTC*, long-term care; *SD*, standard deviation

[a] t-test; [b] Chi-Squared test

*Statistically significant $p < 0.05$

Table 9. Longitudinal Variables of Study Cohort Through Hospital Day 7

Variable	Time Point 1 (Baseline; Within 48 hours)			Time Point 2 (Hospital Day 3)			Time Point 3 (Hospital Day 5)			Time Point 4 (Hospital Day 6 or 7)		
	Nursing Home (*n*=8)	Home (*n*=20)	P-Value	Nursing Home (*n*=8)	Home (*n*=20)	P-Value	Nursing Home (*n*=8)	Home Group (*n*=20)	P-Value	Nursing Home (*n*=8)	Home (*n*=20)	P-Value
GCS Score, mean (SD)	14.0 (0.8)	15.0 (0)	<.001[a*]	14.0 (0.8)	15.0 (0)	<.001[a*]	13.0 (2.6)	15.0 (0)	.002[a*]	13.1 (2.3)	15.0 (0)	<.001[a*]
Invasive Device, *n* (%)												
Urinary catheter	1 (12.5)	0 (0)	.107[b]	1 (12.5)	0 (0)	.107[b]	0 (0)	0 (0)	NA	0 (0)	0 (0)	NA
Central venous catheter	2 (25.0)	0 (0)	.020[b*]	1 (12.5)	0 (0)	.107[b]	1 (12.5)	0 (0)	.107[b]	1 (12.5)	0 (0)	.107[b]
Medications, *n* (%)												
CNS depressants	0 (0)	4 (20.0)	.172[b]	2 (25.0)	3 (15.0)	.533[b]	2 (25.0)	6 (30.0)	.791[b]	2 (25.0)	5 (25.0)	1.000[b]
Antacids	4 (50.0)	6 (30.0)	.318[b]	5 (62.5)	6 (30.0)	.112[b]	6 (75.0)	7 (35.0)	.055[b]	6 (75.0)	6 (30.0)	.030[b*]
Corticosteroids	1 (12.5)	1 (5.0)	.486[b]	2 (25.0)	1 (5.0)	.112[b]	1 (12.5)	1 (5.0)	.486[b]	0 (0)	1 (5.0)	.520[b]
Antibiotics	5 (62.5)	2 (10.0)	.004[b*]	4 (50.0)	3 (15.0)	.053[b]	2 (25.0)	7 (35.0)	.609[b]	1 (12.5)	6 (30.0)	.334[b]
OHAT score, mean (SD)	10.3 (2.6)	4.6 (2.3)	<.001[a*]	10.4 (1.8)	5.0 (2.5)	<.001[a*]	10.4 (3.0)	6.2 (2.4)	<.001 [a*]	11.3 (2.8)	6.2 (2.3)	<.001[a*]
Oral Care Frequency/Day, mean (SD)	0.1 (0.4)	1.0 (0.9)	.025[a*]	0.3 (0.5)	1.1 (0.9)	.024[a*]	0.6 (0.5)	0.8 (0.8)	.560[a]	0.1 (0.4)	1.2 (0.9)	.003[a*]
Oral Care Type, *n* (%)												
Toothbrushing/ toothpaste	1 (12.5)	12 (60.0)	.023[b*]	1 (12.5)	14 (70.0)	.006[b]*	4 (50.0)	11 (55.0)	.811[b]	1 (12.5)	14 (70)	.006[b*]
Chlorhexidine gluconate	0 (0)	0 (0)	NA	0 (0)	0 (0)	NA	1 (12.5)	1 (5.0)	.486[b]	0 (0)	3 (15.0)	.246[b]
Oral Care Personnel, *n* (%)												
Staff member	1 (100)	0 (0)	<.001[b*]	1 (50)	0 (0)	.006[b*]	3 (60)	0 (0)	.003[b]	0 (0)	0 (0)	NA
Patient	0 (0)	12 (100)	<.001[b*]	1 (50)	14 (100)	.006[b*]	2 (40)	12 (100)	.003[b]	1 (100)	16 (100)	NA
Oxygen, *n* (%)	4 (50.0)	4 (20.0)	.112[b]	3 (37.5)	5 (25.0)	.508[b]	2 (25.0)	5 (25.0)	1.000 [b]	2 (25.0)	3 (15.0)	.533[b]
Non-Invasive Ventilation, *n* (%)	1 (12.5)	0 (0)	.107[b]	1 (12.5)	0 (0)	.107[b]	1 (12.5)	0 (0)	.107[b]	1 (12.5)	0 (0)	.107[b]

Variable	Time Point 1 (Baseline; Within 48 hours)			Time Point 2 (Hospital Day 3)			Time Point 3 (Hospital Day 5)			Time Point 4 (Hospital Day 6 or 7)		
	Nursing Home (*n*=8)	Home (*n*=20)	P-Value	Nursing Home (*n*=8)	Home (*n*=20)	P-Value	Nursing Home (*n*=8)	Home Group (*n*=20)	P-Value	Nursing Home (*n*=8)	Home (*n*=20)	P-Value
NV-HAP Diagnosis, *n* (%)	0 (0)	0 (0)	NA	1 (12.5)	0 (0)	.107[b]	1 (12.5)	1 (5.0)	.486[b]	1 (12.5)	1 (5.0)	.486[b]

CNS, central nervous system; *GCS*, Glasgow Coma Scale; *NV-HAP*, non-ventilator hospital acquired pneumonia; *OHAT*, Oral Health Assessment Tool; *SD*, standard deviation

[a] t-test; [b] Chi-Squared test

*Statistically significant $p < 0.05$

Table 10. Clinical Outcomes of Study Completers Through Hospital Day 7

Clinical Outcomes	Nursing Home Group (n=8)	Home Group (n=20)	P-Value
Hospital LOS days, mean (SD)	6.9 (1.0)	9.9 (4.1)	.054[a]
Discharge Disposition, *n* (%)			
Home	1 (12.5)	16 (80.0)	.001[b*]
LTC facility	7 (87.5)	3 (15.0)	
Inpatient rehab	0 (0)	1 (5.0)	
30-Day Hospital Re-admission Post-Study, *n* (%)	4 (50.0)	7 (35.0)	.463[b]

LOS, length of stay; *LTC*, long-term care; *SD*, standard deviation

[a] t-test; [b] Chi-Squared test

*Statistically significant $p < 0.05$

Evaluation Relationship Between Pre-Hospital Residence and Oral Microbiome

Oral Bacterial Taxonomy

Both nursing home and home groups had similar oral bacteria genera with the highest relative abundance at hospital admission, including *Streptococcus*, *Rothia*, *Prevotella*, *Veillonella*, and *Actinomyces*, which comprised nearly 70% of oral colonization. As relative abundance percentages decreased, bacteria genera began differing between groups (Table 11). The relative abundance of several oral bacteria genera was significantly higher in the nursing home compared to home group including *Anaeroglobus* (p=.032), *Bifidobacterium* (p=.029), *Burkholderia-Paraburkholderia* (p=.040), *Mycoplasma* (p=.029), *Propionibacterium* (p=.002), *Pseudomonas* (p=.003), and *Pseudoramibacter* (p=.030). One bacterial genus, *Stomatobaculum* (p=.036), was more common in the home group. See Figures 8 and 9 for differences between groups in oral bacteria genera and species, respectively.

Table 11. Most Frequent Oral Bacteria Genera Relative Abundance Percentages Between Groups at Baseline

Nursing Home Baseline Oral Bacterial Genus Taxonomy	Mean (%)	Home Baseline Oral Bacterial Genus Taxonomy	Mean (%)
Streptococcus	27.8	*Streptococcus*	30.7
Rothia	14.6	*Rothia*	14.4
Prevotella	10.2	*Prevotella*	8.5
Veillonella	9.9	*Actinomyces*	6.7
Actinomyces	5.4	*Veillonella*	6.1
Neisseria	5.0	*Neisseria*	4.6
Granulicatella	2.5	*Haemophilus*	4.0
Fusobacterium	2.1	*Gemella*	3.4
Haemophilus	1.9	*Porphyromonas*	2.0
Peptostreptococcus	1.6	*Granulicatella*	2.0

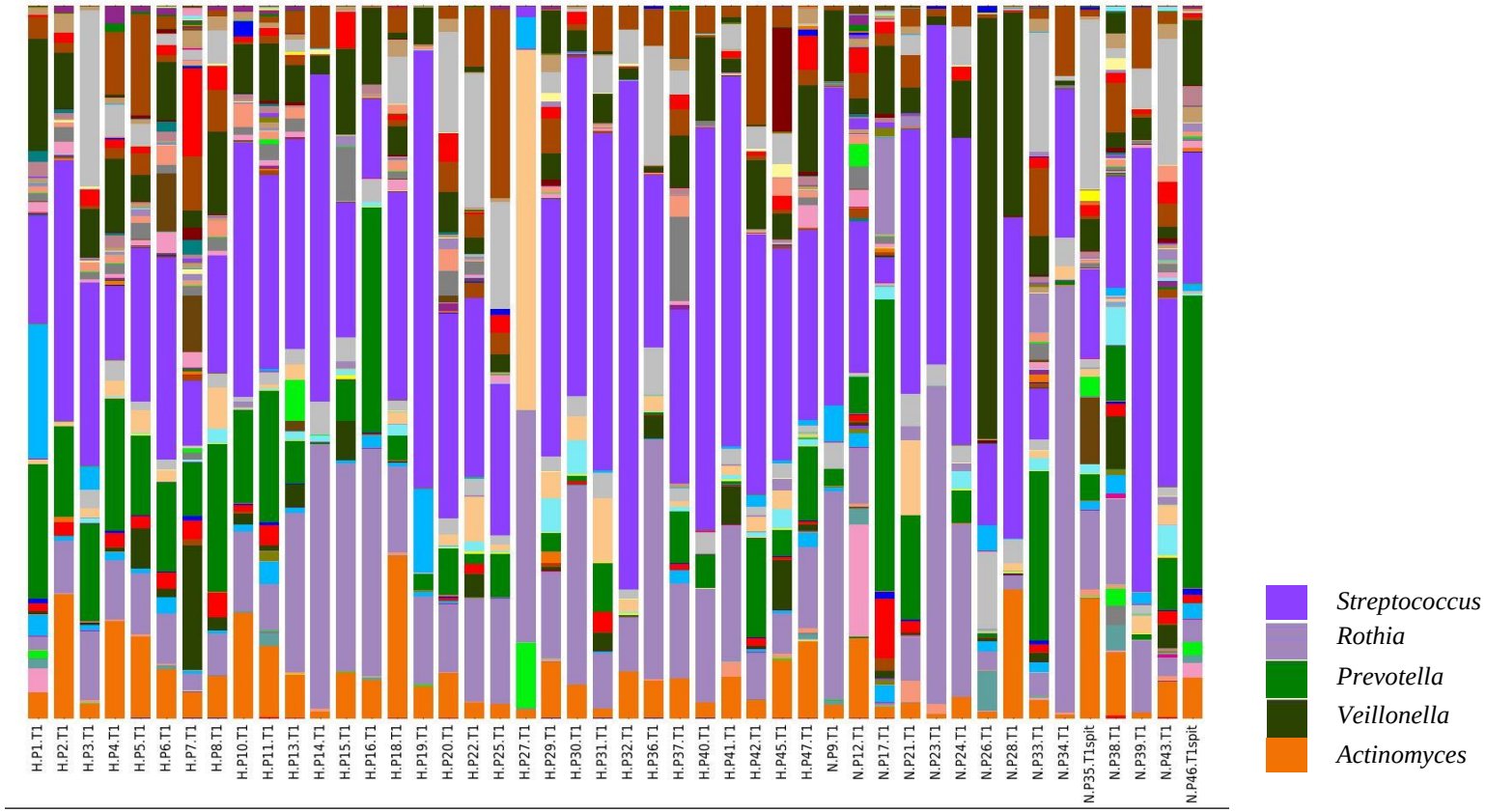

Figure 8. Baseline Oral Bacteria Genera Differences Between Groups

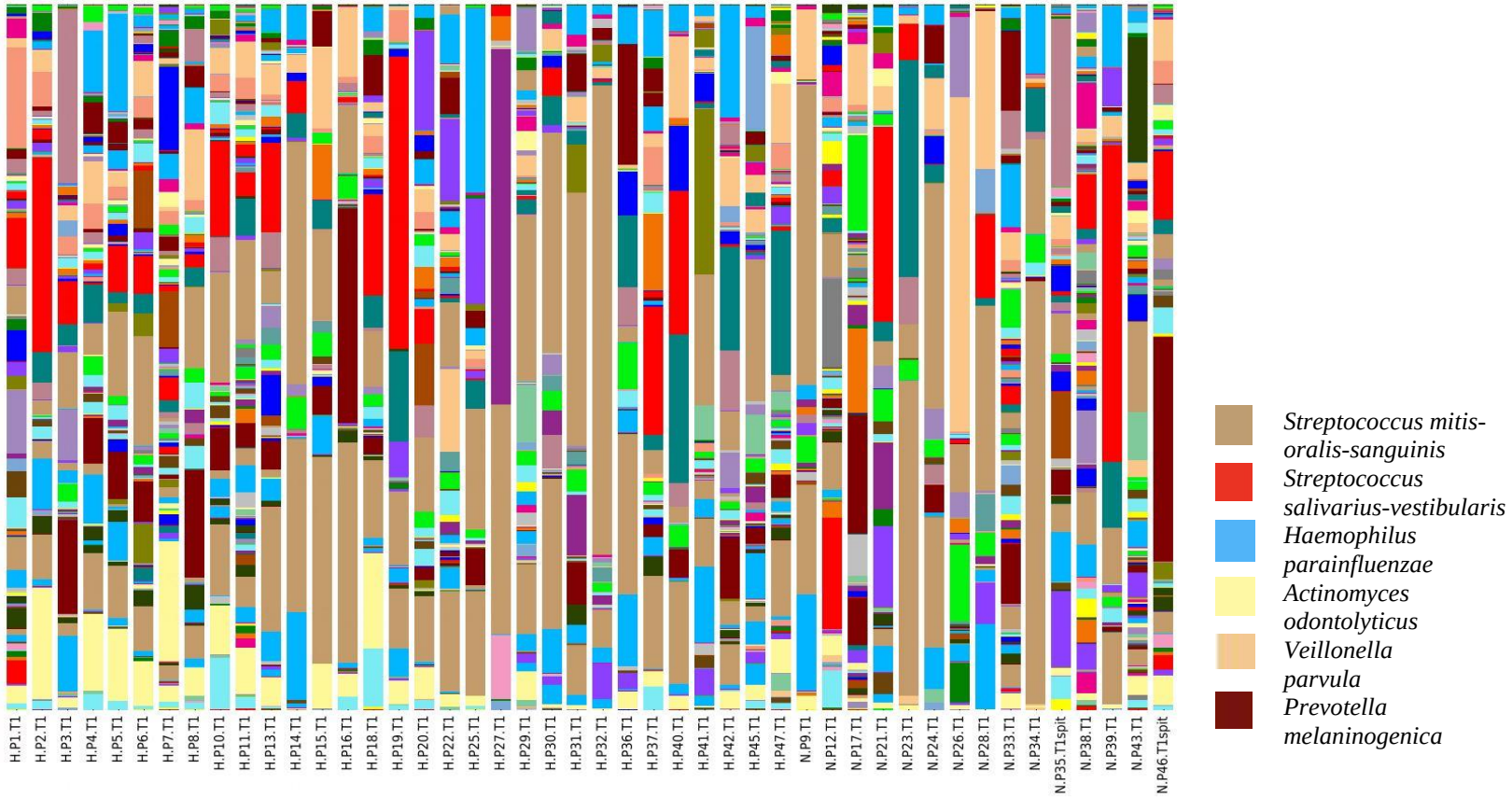

Figure 9. Baseline Oral Bacteria Species Differences Between Groups

Alpha Diversity

Mean Shannon Index values were significantly lower in the nursing home compared to home group (4.2, SD 1.2 vs 4.5, SD 0.9; p<.001) (Figure 7).

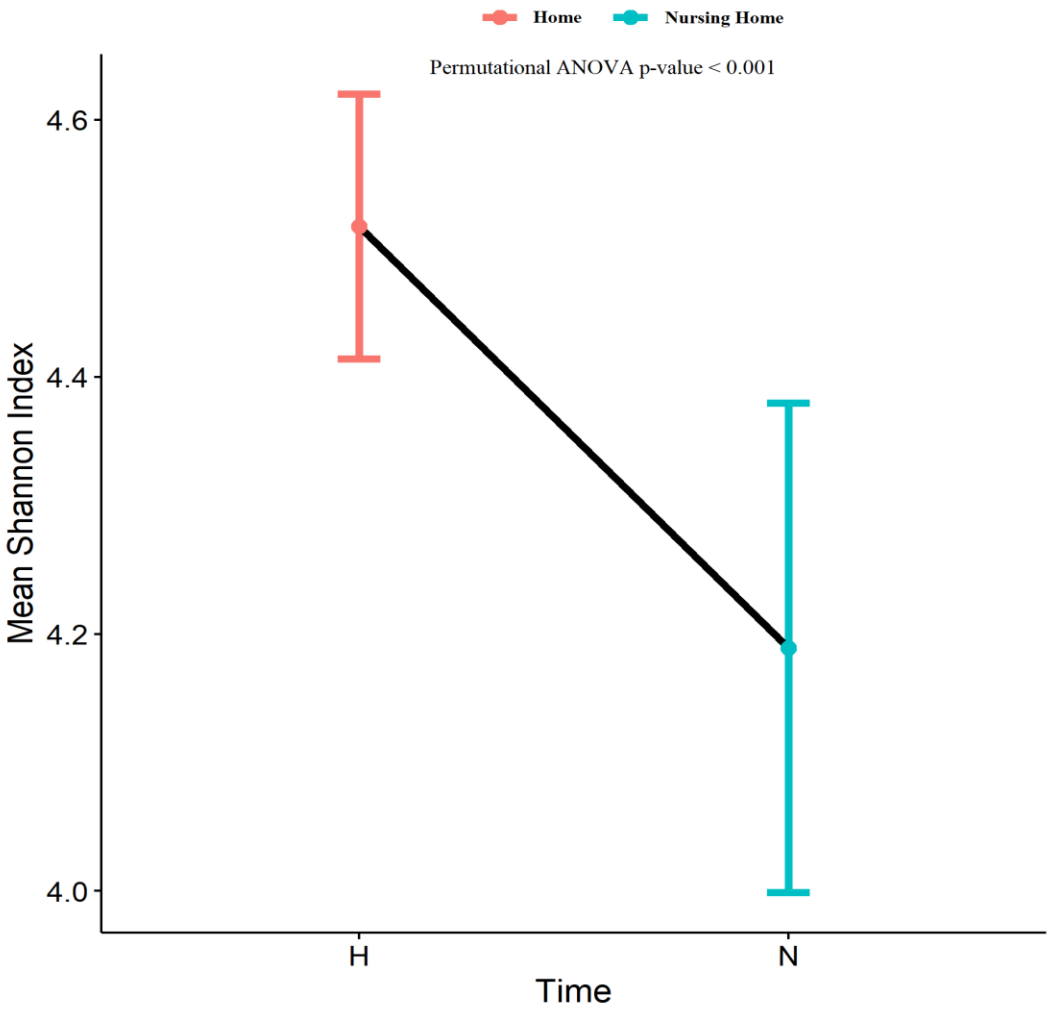

Figure 10. Baseline Mean Shannon Index Significantly Lower in Nursing Home Group vs Home Group

Beta Diversity

Bray-Curtis dissimilarity genus level mean (p=.010) and variance (p=.009) significantly differed between both groups (Figure 11). Mean Bray-Curtis dissimilarity at the genus level was higher in the nursing home compared to home group (0.60, SD=0.16 vs 0.47, SD=0.15). Additionally, Bray-Curtis dissimilarity ASV level mean (p=.003) and variance (p=.040) significantly differed between groups (Figure 12). Mean Bray-Curtis dissimilarity at the ASV level was higher in the nursing home compared to home group (0.86, SD=0.08 vs 0.79, SD=0.10).

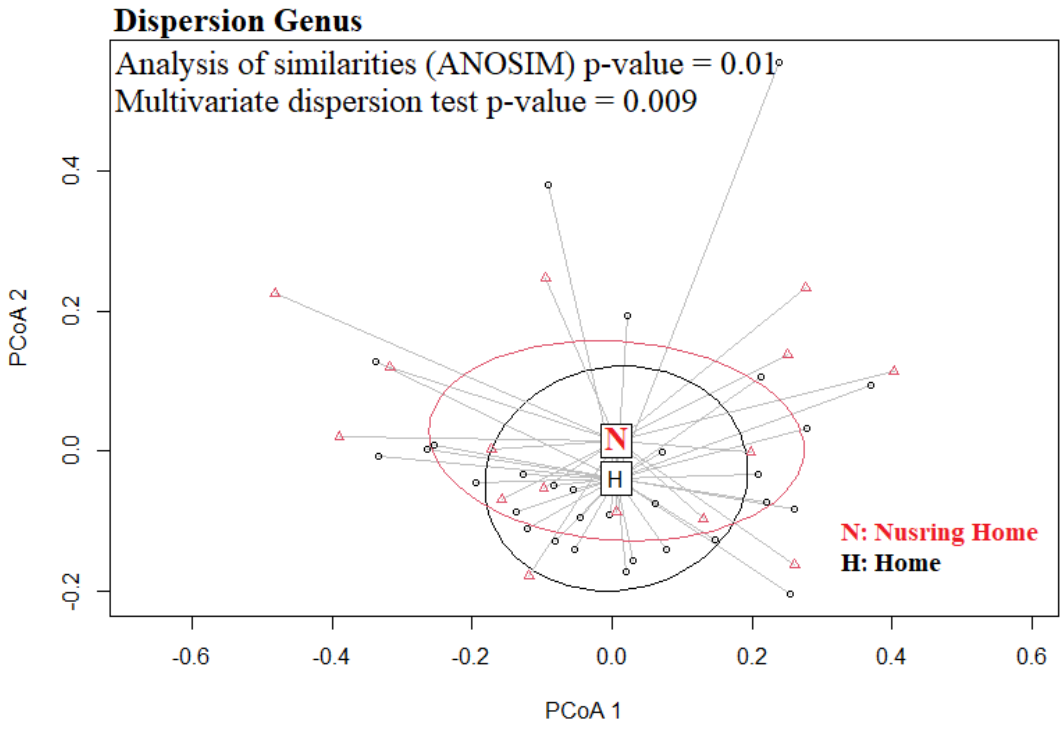

Figure 11. Bray-Curtis Dissimilarity Mean and Variance Significantly Different Between Groups and Higher in Nursing Home Group at Genus Level

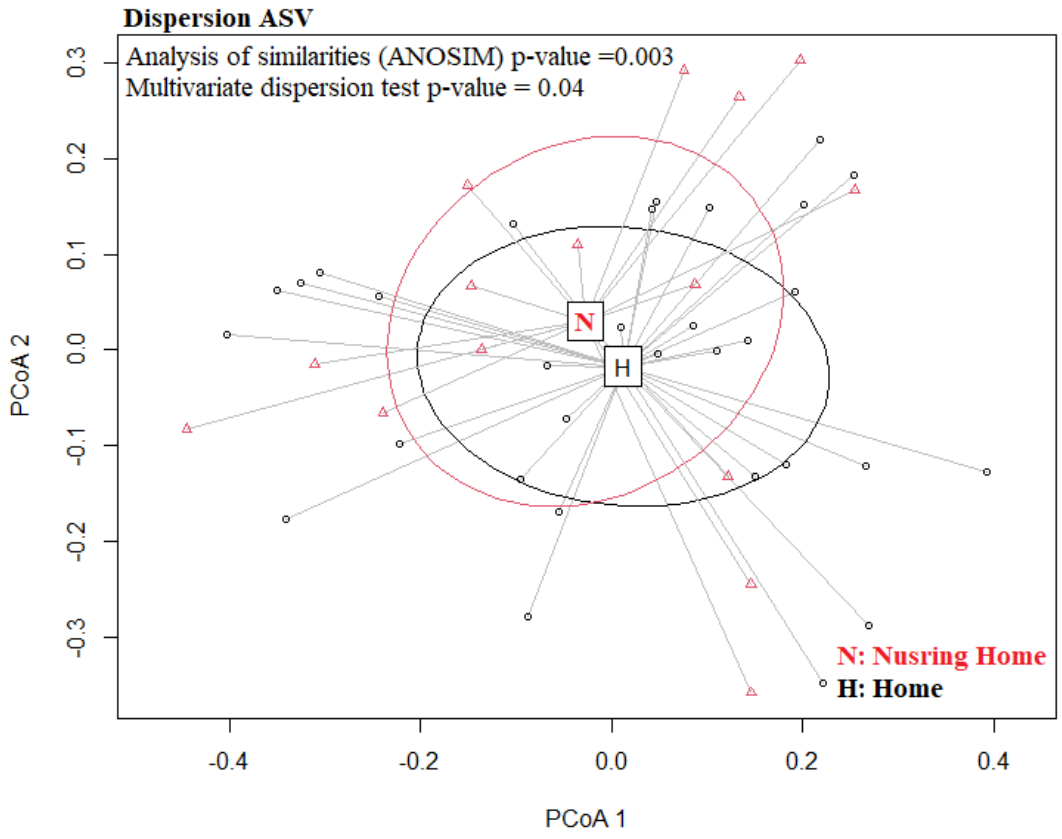

Figure 12. Bray-Curtis Dissimilarity Mean and Variance Significantly Different Between Groups and Higher in Nursing Home Group at ASV (Species) Level

Explore Relationship Between Oral Microbiome and NV-HAP Development

Two patients out of 46 developed probable NV-HAP (4%). The first patient was admitted to the hospital from home and developed probable NV-HAP on day 5 of hospitalization. During hospital days 1 and 3, *Streptococcus* was the most common oral bacteria genus colonized. On hospital day 5 when the patient developed probable NV-HAP, *Neisseria* became the most predominant oral bacteria genus (21%). *Neisseria* species detected on day 5 comprised of *Neisseria macacae-mucosa-sicca* (*N. macacae-mucosa*) (15%) and *Neisseria bacilliformis* (*N. bacilliformis*) (6%). *Neisseria* was not detected on hospital day 1 and was only detected in small quantity on hospital day 3 (0.6%). *Neisseria* was still largely present on hospital day 6; however, *Actinomyces* became the most common oral bacteria genus. Shannon Index values for the patient ranged from 4.6, 5.1, 5.0, and 5.3 across hospitalization.

The second patient was admitted to the hospital from a nursing home and developed probable NV-HAP on day 3 of hospitalization. Upon study enrollment (hospital day 2), *Veillonella* accounted for majority of oral bacteria genus colonization (59%), with the most common *Veillonella* species being *Veillonella parvula* (*V. parvula*) (47%). *Streptococcus* became more frequent on hospital day 3 (32%), which was when probable NV-HAP occurred, followed again by *Veillonella* on hospital day 5. The most frequently detected *Streptococcus* species detected on day 3 was *Streptococcus mitis-oralis-sanguinis* (*S. mitis-oralis-sanguinis*) (22%). *Granulicatella* comprised most of the oral bacteria genera colonization on hospital day 7 (58%). Shannon Index values for the patient ranged from 3.6, 4.3, 3.5, and 3.2 across hospitalization. Refer to Table 12 for additional clinical characteristics and Table 13 for most common relative abundance percentages of oral bacteria genera across hospitalization from patients who developed probable NV-HAP.

Table 12. Characteristics of Patients Who Developed NV-HAP

Variable	Patient Number One				Patient Number Two			
Time Point	1 (Day 1)	2 (Day 3)	3 (Day 5)	4 (Day 6)	1 (Day 2)	2 (Day 3)	3 (Day 5)	4 (Day 7)
Pre-Hospital Residence	Home				Nursing home			
Admitting Diagnosis	Atrial fibrillation; hyponatremia				Altered mental status			
Dental Device	Yes (dental bridges)				Yes (dentures)			
NV-HAP Diagnosis	No	No	Yes	Yes	No	Yes	Yes	Yes
GCS Score	15	15	15	15	14	14	14	14
OHAT Score	4	5	7	5	13	11	11	13
Oral Care Frequency/Day, mean	1	0	0	1	1	0	0	0
Chlorhexidine Gluconate	No	No	No	No	No	No	No	No
Oxygen	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Antibiotics	No	No	Yes	Yes	Yes	Yes	No	No
Antacids	No	No	No	No	No	No	No	No
Hospital LOS days, mean	6.2				6.1			
30-Day Post-Study Re-Admit	No				Yes (Re-admit diagnoses: Acute encephalopathy; acute on chronic respiratory failure; a fib; CHF)			

CHF, congestive heart failure; *GCS*, Glasgow Coma Scale; *LOS*, length of stay; *NV-HAP*, non-ventilator hospital acquired pneumonia, *OHAT*, Oral Health Assessment Tool

Table 13. Longitudinal Oral Bacteria Genera Relative Abundance Percentage Changes During Hospitalization in Patients who Developed Probable NV-HAP

Time Point 1 (Baseline; Within 48 Hours)		Time Point 2 (Hospital Day 3)		Time Point 3 (Hospital Day 5)		Time Point 4 (Hospital Day 6 or 7)	
Patient Number One with Probable NV-HAP							
Streptococcus	36.6%	*Streptococcus*	29.0%	*Neisseria*	21.4%	*Actinomyces*	17.0%
Actinomyces	17.5%	*Rothia*	12.6%	*Streptococcus*	14.6%	*Streptococcus*	15.0%
Prevotella	12.7%	*Prevotella*	9.7%	*Rothia*	12.8%	*Neisseria*	14.1%
Patient Number Two with Probable NV-HAP							
Veillonella	59.0%	*Streptococcus*	32.3%	*Veillonella*	33.3%	*Granulicatella*	57.5%
Streptococcus	11.4%	*Veillonella*	12.7%	*Granulicatella*	31.9%	*Veillonella*	19.6%
Granulicatella	10.8%	*Bifidobacterium*	12.3%	*Rothia*	10.4%	*Enterococcus*	6.2%

Discussion

Oral Microbiome Across Hospitalization

In our study, S*treptococcus*, *Rothia*, *Prevotella*, *Veillonella*, *Neisseria*, and *Actinomyces* were oral bacteria genera that had the highest relative abundance throughout hospitalization, all of which are common bacterial genera present in the human oral cavity. [34] Over the course of hospitalization the most frequent oral bacteria genera remained relatively stable with the exception of an increase in *Neisseria* on hospital days 5 and 7 instead of *Actinomyces* and *Veillonella*, respectively. Microbes in the oral cavity have a symbiotic relationship and generally only become pathogenic when commensal microbes can no longer maintain their protective barrier. [34] Though the bacterial genera noted are common to the oral cavity, they can also cause disease and/or infection given the right circumstances. For instance, *Rothia* and *Prevotella* are part of normal oral bacteria genera; however, they can cause local and systemic disease depending on fluctuations in clinical conditions (e.g., translocation to lower airway via microaspiration). [35,36]

Alpha diversity reflects the variety and abundance of organisms within a sample/ community. [37] Shannon Index equally incorporates both richness (number of different taxa within a community without considering frequencies) and evenness (equitability of taxa frequencies within a community); [37] higher values indicate greater taxonomic diversity. Although mean Shannon Index values showed statistical differences over time (likely due to the large sample size from longitudinal sampling, thus having large power to detect small changes in alpha diversity), values stayed relatively consistent over hospitalization, showing little clinical relevance. This is comparable to similar short-term hospital findings that found no differences in the salivary microbiome between baseline and 72-hour follow-up specimens. [29] Notably, in our

study, mean Shannon Index values were significantly different between groups over time, suggesting that clinical differences between groups (such as pre-hospital residence) may play an important role in oral microbiome changes during hospitalization.

Clinical Practice Takeaways

Literature supports oral care completed at least twice daily to significantly reduce the incidence of NV-HAP. [5] Although oral care was not completed nearly as frequently in our study as what is expected for patient care to prevent NV-HAP, mean oral care completion for the home group was higher than the nursing home group (1.0 vs 0.3 times/day), emphasizing the important of oral care versus no oral care. Oral care is not limited to just a nursing intervention and should be an interdisciplinary approach to care (e.g., respiratory, dental, etc.). The role of dental services within the hospital setting may be useful and considered. [38]

Importance of Pre-Hospital Residence/Environment

Although the baseline oral bacterial genera with highest relative abundance were similar between groups, significant differences were noted between several baseline oral bacterial genera with lower relative abundance percentages, mean Shannon Index values, and Bray-Curtis dissimilarity. A study comparing salivary microbiome differences between nursing home and community dwelling older adults found similar common oral bacteria genera to ours in the nursing home group including *Actinomyces, Streptococcus, Veillonella,* and *Haemophilus*. [39] The study also found that *Prevotella* and *Veillonella* were more prevalent in patients with periodontitis. [39] In our study, most of the oral bacterial genera found significantly more frequently in the nursing home group hold negative clinical implications. For example, *Anaeroglobus* and *Pseudoramibacter* may play a role in periodontitis. [40,41] *Bifidobacterium* is a caries-associated bacterium and *Propionibacterium* is found in endodontic infections. [42,43] The

higher prevalence of more pathogenic bacteria genera may have contributed to the higher mean baseline OHAT score in the nursing home group (10.6 vs 4.6), indicating poor oral health. [44]

Mean baseline Shannon Index was significantly lower in the nursing home compared to home group, indicating at baseline there was a less diverse oral taxonomic profile in the nursing home group. Beta diversity reflects the microbial similarity between samples. [37] Bray-Curtis dissimilarity is a commonly used index of beta diversity that measures the compositional dissimilarity between samples/groups; higher values indicate greater dissimilarity. [45] Our study found that Bray-Curtis dissimilarity was significantly higher in the nursing home group, indicating greater oral microbial dissimilarity among nursing home patients. This could potentially be attributed to environmental causes, as nursing home environments vary from one another reflecting constant changes in people/patients, crowded setting, and clinical fluctuations when compared to a more stable and consistent home environment. It is also possible that oral care in nursing home patients may be lacking or dissimilar from one another (e.g., combination of toothbrushing, oral swabs, oral products, etc.) as compared to individuals who live at home and generally complete their own oral care which may be more consistent and standardized (generally toothbrushing and toothpaste). Other additional differences between groups, such as oral diet and the number of times they eat each day, could contribute to oral microbial dissimilarity. [46]

Clinical Practice Takeaways

Though oral care remains important for all hospitalized patients, nursing home patients require special attention to oral care starting at hospital admission. Nursing home patients comprise a “high risk” group for NV-HAP, as they are admitted to the hospital already having: (1) a higher baseline mean OHAT score (indicating poor oral health); (2) greater baseline oral

colonization with bacterial genera associated with oral disease (e.g., periodontitis, dental caries, and endodontic infection); (3) less baseline oral microbial diversity; and (4) greater baseline Bray-Curtis dissimilarity compared to one another. This subgroup also had significantly higher rates of cognitive impairment, delirium, and frailty, meaning they will likely need assistance from staff members to complete oral care.

Combining a Clinical Picture of the Oral Microbiome, Clinical Variables, and Probable NV-HAP Development

Our study had a 4% rate of probable NV-HAP (2/46 patients), which is slightly higher than the current national average of around 1%.[3] Patients in our study who developed probable NV-HAP had oral care completed only a few times across hospitalization (total mean 0.5 and 0.3 times/day), which is a primary modifiable risk factor for NV-HAP development.[5] The first patient who developed NV-HAP was admitted from home and had a lower mean baseline OHAT score of 4, but did not have any oral care on hospital day 3 prior to developing probable NV-HAP. The patient also stated to the PI that no staff members helped assist her out of bed throughout hospitalization until day 7, which was after the patient had already been diagnosed with probable NV-HAP and put on IV antibiotics. The second patient who developed NV-HAP was admitted from a nursing home and had a higher average baseline OHAT score of 13, similar to other patients in our study admitted from nursing homes. Oral care was completed once by a staff member upon hospital admission, but the patient received no oral care the rest of hospitalization by staff and the patient was not able to complete self-care due to altered mental status. The pandemic visitation restrictions may also have contributed to the patient having limited visitors available to assist in completing oral care or potentially the patient may not have had any family.

The first patient who developed probable NV-HAP had no *Neisseria* detected at baseline and very little colonization on day 3 (0.6%), but *Neisseria* (species *N. macacae-mucosa* and *N. bacilliformis*) comprised 21% of oral colonization on day 5, which was the same day of probable NV-HAP development. *Neisseria* is a gram-negative bacteria including both pathogenic and commensal organisms found within the upper respiratory tract of humans.[47] *N. macacae-mucosa* has been found within the upper respiratory tract in neutropenic patients.[48] *N. bacilliformis* can cause opportunistic infections related to the oral cavity and respiratory tract, as well as sepsis in patients with greater risk factors (e.g., immune suppression).[49] Several factors can contribute to alterations of *Neisseria* colonization within the upper respiratory tract such as sex hormone fluctuations, certain disease states (e.g., inflammatory bowel disease and pancreatic cancer), ethanol use, and propionic acid used as a growth substrate (found in food preservatives and propionic acid-producing bacteria are common in the oral cavity such as *Corynbacterium* and *Actinomyces*).[47] *Actinomyces* was the patient's second most common oral bacterial genus colonized at baseline and more specifically, *Actinomyces odontolyticus* (*A. odontolyticus*) was the most frequent *Actinomyces* species found at baseline (16%). *A. odontolyticus* is a gram-positive bacteria associated with dental caries, oral infections surrounding teeth implants, and pulmonary infections, including cases of pneumonia.[50] The patient had a dental device (dental bridges).

The second patient who developed probable NV-HAP had oral colonization largely with *Veillonella* (most common species *V. parvula*) upon hospital admission, but had an increase in *Streptococcus* (most common species *S. mitis-oralis-sanguinis*) on day 3 (same day of probable NV-HAP development). Several *Veillonella* species are among the most prevalent gram-negative bacteria found in the oral cavity.[51] *V. parvula* is commonly associated with oral disease,

including dental caries, periodontitis, and endodontic infections.[51] The patient also had a dental device (dentures), which could have been a risk factor for certain baseline oral bacteria species colonization. The patient also had cognitive impairment, increasing the likelihood of dependency on staff members to remove/clean their dentures. The length of time that oral devices remain in place, potentially without adequate cleansing, could contribute to development of NV-HAP. *Streptococcus* is a gram-positive bacteria that commonly resides in the oral cavity of healthy individuals.[52] However, *Streptococcus* does have the ability to cause disease such as infective endocarditis and odontofacial infections.[52] The *S. mitis* group, including *S. mitis, S. oralis,* and *S. sanguinis,* can also exacerbate influenza infection (especially in immunocompromised patients).[52]

Baseline oral bacterial alpha diversity (Shannon Index) differed between the two patients who developed probable NV-HAP, likely due to pre-hospital environment or potentially from the second patient already being on antibiotics. The first patient who developed probable NV-HAP had a baseline Shannon Index value of 4.6. Shannon Index values interestingly increased for the remainder of hospitalization for the patient (5.1, 5.0, 5.3), despite pneumonia development. The patient received oral care at baseline and on the final day of hospitalization, which could account for the initial and final increases in oral bacterial diversity values. The second patient who developed probable NV-HAP had a baseline Shannon Index of 3.6 and was admitted from a nursing home, which was lower than other nursing home patients in our study. The patient's baseline oral bacteria genera comprised over half (59%) with one genus, which is reflective of the lower Shannon Index value. Interestingly, the second patient's Shannon Index values increased to 4.3 on day 3 (indicating a more diverse oral microbiome), but progressively decreased across hospitalization (3.5 and 3.2 on hospital days 5 and 7, respectively). The patient

received oral care once at baseline (hospital day 2), which could explain the initial increase in oral bacterial diversity. We were unable to record whether the patient's dentures were left in the oral cavity overnight across hospitalization, which could have contributed to the decline in Shannon Index values.

In addition, the timing of antibiotics could also have been a contributing factor to oral bacterial diversity changes in both patients. The second patient was already admitted to the hospital on antibiotics due to a urinary tract infection. The patient was diagnosed with probable NV-HAP on day 3 of hospitalization and according to the medication administration record, received intravenous antibiotics from hospital admission through day 4. EMR documentation indicated that the patient received adequate antibiotic coverage for the probable pneumonia diagnosis.

Clinical Practice Takeaway

Pre-hospital variables such as pre-hospital environment, dental devices, and baseline oral health status may influence baseline oral bacterial taxonomy and thereafter clinical outcomes, including NV-HAP development. Patients in our study (including those who developed probable NV-HAP) received very little oral care and the importance of oral care does not go unnoticed. Oral care remains an important primary intervention to prevent NV-HAP that should be completed at least two times/day on hospitalized patients. [5,9] Different types of oral care serve different purposes. For example, toothbrushing physically removes dental plaque and biofilm/bacteria accumulation, which could reduce likelihood of pneumonia development. [9] Toothbrushing also serves as a patient comfort measure. Oral swabs/foam sticks may be used to clean and moisten the oral cavity to prevent mucosa breakdown. [9]

Both patients who developed probable NV-HAP also had dental devices (dental bridges/dentures), which may have influenced oral bacterial taxonomy and contributed to bacteria accumulation/biofilm formation, particularly when oral care is lacking. Hospitalized patients who have dental devices should have consistent oral care completed. [53] Lastly, hospitals and clinicians should consider the inclusion of a comprehensive assessment of NV-HAP risk (including clinical variables such as pre-hospital residence, cognitive status, oral health status, presence of a dental device, etc.) for patients upon both hospital admission and during hospital progression.

Future Research Implications

Future research studies should further explore the relationship between additional clinical variables and the oral microbiome in non-ventilated hospitalized patients. The impact of oral care on oral bacterial taxonomy should also be explored in further detail. Exploring individuals' oral microbiome pre-hospitalization to gain a better understanding of the "baseline" oral microbiome upon hospitalization and contributing factors would be useful. It will also be important for future research related to the oral microbiome, oral care, and NV-HAP to consider both the nursing home setting and nursing home patients within the hospital setting. Examining current oral health status and oral care practices, as well as exploring the oral microbiome in nursing home patients within the nursing home setting are all important future research trajectories.

In our two cases of probable NV-HAP, oral bacteria genera and species appeared to be more meaningful indicators of NV-HAP compared to oral bacterial diversity measures. When combined with a patient's entire clinical picture (e.g., pre-hospital residence, dental device, etc.), oral bacteria genera/species could provide clinically relevant information on patient's baseline and continuing risk for and protective factors against NV-HAP development over the course of

hospitalization. Future similar research should consider a larger sample size which includes a greater number of probable NV-HAP cases.

Future research studies should consider the use of 16S rRNA sequencing versus metagenomic shotgun sequencing. Metagenomic shotgun sequencing uses untargeted sequencing of all microbial genomes (rather than single genes as with 16S rRNA sequencing) to understand taxonomic composition, functional ability of microbial communities, and whole genome sequences. [54] Certain studies that require identification of fungi and/or viruses (such as with patients who are immunocompromised) would benefit from using metagenomic shotgun sequencing. Sequencing technique will likely depend on the study sample and resource availability (e.g., financial cost and lab capabilities, as the latter is more costly and labor intensive).

Lastly, it would be beneficial to explore the impact of oral microbial collection method (saliva vs. oral swab) on oral bacterial taxonomy and oral bacterial diversity. Several patients allowed us to use both data collection methods (saliva and oral swab) to compare oral bacterial findings at the same time point, which will be important for our future sub-analyses. Towards the end of data collection (particularly days 5 and 7), several patients expressed less interest in study participation due to fatigue. Some patients were also unable to provide saliva samples due to a variety of reasons (e.g., xerostomia, confusion, etc.), so an alternative, quick sampling method that yields similar, high-quality data on the oral microbiome would be useful for future studies.

Limitations

Although the target sample size was 58 study completers, there were recruitment challenges due to the COVID-19 pandemic and difficulties enrolling patients from the nursing

home setting. Given the anticipated large effect size, a smaller sample was recruited and the target sample size for patients from the home setting was achieved.

Our nursing home group was also smaller than our home group (15 patients compared to 31 patients, respectively). There were fewer nursing home patients admitted to our study units and they were more challenging to recruit, which is important to note for studies involving recruitment of nursing home patients.[15] Despite the smaller sample size in the nursing home group, we still detected significant differences between groups.

Next, several patients were unable to provide saliva samples and instead oral swabs were collected (six patients: two patients had oral swabs collected at one time point; one patient had oral swabs collected at two time points; one patient had oral swabs collected at three time points; and two patients had oral swabs collected at all four time points), which could have affected study findings for this subgroup. Oral swabs were collected consistently by the PI and from the same site in the oral cavity to reinforce consistency.

Additionally, we used 16S rRNA sequencing in our study to analyze oral salivary specimens, which did not allow us to detect fungi or viruses. This could be an important piece in understanding patients' oral microbiome and should be considered in studies depending on the study sample and resource availability. Finally, we lacked dental expertise in our study, as we did not have the resources able to include such expertise. We suggest future studies consider including a dental perspective to allow for a more comprehensive overview of the oral microbiome.

Conclusions

Although oral bacteria genera remained consistent, oral bacterial diversity changed across hospitalization and over time between nursing home and home groups in non-ventilated older

adults. The oral microbiome, including several oral bacteria genera and oral bacterial diversity, differed between patient populations depending on pre-hospital environment, emphasizing the importance of a tailored approach to oral care based on patient's individualized clinical factors. Additionally, specific oral bacteria genera may be meaningful indicators of NV-HAP development and warrant further research. Future studies should also continue exploring the relationship between clinical variables and the oral microbiome in non-ventilated patients during both pre-hospitalization (including the nursing home setting) and across hospitalization.

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CHAPTER FIVE: NARRATIVE SUMMARY

Reflections and Summary of Research

Conducting research within the clinical setting, particularly amid the COVID-19 pandemic, was rewarding yet unpredictable, emphasizing the importance of both study researcher and protocol adaptability. Recruitment of non-ventilated hospitalized older adults from home was successful, while recruitment of hospitalized older adults from nursing homes presented greater challenges. Nursing home patients often were admitted to the hospital having community-acquired pneumonia (part of our exclusion criteria) or had cognitive impairment; therefore, requiring a proxy to consent on their behalf, which presented its own set of challenges. Overall study strengths included using an interdisciplinary approach to pair clinical data with bioinformatics; short enrollment window; longitudinal data collection; and coupling of oral microbiome with clinical data including pre-hospital residence, oral care, and oral health status.

Longitudinal findings showed that oral bacterial taxonomy remained relatively consistent in non-ventilated older adults across hospitalization. However, oral alpha diversity (Shannon Index values) significantly changed both over time and over time by group during hospitalization. Nursing home patients comprised a subgroup that had unique clinical and oral microbial differences compared to patients admitted from home, making them a "high-risk" group for NV-HAP. Compared to patients from home, patients admitted from a nursing home had: (1) higher mean baseline Oral Health Assessment Tool scores (indicating worse oral health); (2) greater baseline oral colonization with bacteria genera associated with oral disease; (3) less oral alpha diversity (lower Shannon index values) at baseline that worsened across hospitalization; (4) greater baseline Bray-Curtis dissimilarity compared to one another; and (5)

less frequent oral care across hospitalization. Clinical variables, including pre-hospital environment, may account for differences between groups and warrant further exploration.

Our study found a probable NV-HAP rate of 4% (2 out of 46 patients), which is higher than the current national average rate.[1] NV-HAP development may be associated with specific oral microbial changes and different clinical variables. Findings emphasize the importance of identifying "high-risk" patients for NV-HAP and a tailored approach to oral care.

Impact of Research

Ultimately, the primary study intent was to work towards the reduction of NV-HAP rates. Study findings contribute to the gap in literature addressing oral microbiome changes in non-ventilated patients across hospitalization, as well as with different clinical variables. Findings will also help clinicians better identify patients at high risk for NV-HAP development and emphasize the importance of individualized oral care.

Future Research Plans and Trajectories

Based on study findings, there are several related future research opportunities. First, the relationship between additional clinical variables and the oral microbiome should be further explored in non-ventilated patients both pre-hospitalization and across hospitalization. Pre-hospital variables may influence oral health and the oral microbiome, making it important to better understand "baseline" oral microbiome data. The impact of specific clinical variables, particularly oral care, on oral bacterial taxonomy should be explored in further depth.

Next, there are many important research opportunities related to the oral microbiome, oral care, and NV-HAP within the nursing home population (both in the nursing home and hospital setting), as many oral microbial and clinical differences were noted between nursing home and home groups. Examining oral health status, oral care practices, and the oral

microbiome in nursing home patients within the nursing home setting will be important future research trajectories to better understand these differences.

In our two cases of probable NV-HAP, oral bacteria genera and species appeared to be more meaningful indicators of NV-HAP compared to oral bacterial diversity measures. When combined with a patient's entire clinical picture (e.g., pre-hospital residence, dental device, etc.), oral bacteria genera/species could provide clinically relevant information on patient's baseline and continuing risk for and protective factors against NV-HAP development over the course of hospitalization. Future similar research should consider a larger sample size which includes a greater number of probable NV-HAP cases. Related research should also consider usage of 16S rRNA sequencing versus metagenomic shotgun sequencing.

Lastly, the impact of oral microbial collection method (saliva vs. oral swab) on oral bacterial taxonomy and diversity should be assessed. A subset of patients was unable to provide saliva samples due to a variety of reasons; therefore, an alternative sampling method that yields similar, high-quality data on the oral microbiome would be useful for future studies.

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APPENDIX: IRB LETTER

UNIVERSITY OF CENTRAL FLORIDA

Institutional Review Board
FWA00000351
IRB00001138, IRB00012110
Office of Research
12201 Research Parkway
Orlando, FL 32826-3246

APPROVAL

November 5, 2020

Dear Kimberly Emery:

On 11/5/2020, the IRB reviewed the following submission:

Type of Review:	Initial Study, Expedited Category 3 and 5
Title:	Exploration of the Oral Microbiome in Non-Ventilated Hospitalized Patients
Investigator:	Kimberly Emery
IRB ID:	STUDY00002404
Funding:	Name: National Institutes of Health (NIH)
Grant ID:	
IND, IDE, or HDE:	None
Documents Reviewed:	• F31 Revision Muller Letter of Support.pdf, Category: HIPAA; • F31 Revision Penoyer Letter of Support.pdf, Category: HIPAA; • Signed UCF IRB Advisor Form Sole.pdf, Category: Faculty Research Approval; • Final EHS Study Specific COVID-19 Plan KE.pdf, Category: Other; • KE Final F31 Resubmission.pdf, Category: Sponsor Attachment; • NIH Notice of Award, Category: Sponsor Attachment; • Rathbun IBC BARA Response.pdf, Category: Other; • UCF IRB Confusion Assessment Method Tool.docx, Category: Other; • UCF IRB Consent Form, Category: Consent Form; • UCF IRB Demographic and Baseline Data Collection Form Draft.docx, Category: Other; • UCF IRB Longitudinal Data Collection Form Draft.docx, Category: Other; • UCF IRB Measurement of Variables Table.docx, Category: Other; • UCF IRB Mini-Mental State Examination.docx, Category: Other; • UCF IRB Oral Health Assessment Tool.docx, Category: Other;

	• UCF IRB Protocol Facilities and Other Resources.docx, Category: Other; • UCF IRB Protocol Third Submission KE 10-29-2020.docx, Category: IRB Protocol; • UCF IRB Respective Contributions.docx, Category: Other;

The IRB approved the protocol on 10/29/2020.

In conducting this protocol, you are required to follow the requirements listed in the Investigator Manual (HRP-103), which can be found by navigating to the IRB Library within the IRB system. Guidance on submitting Modifications and a Continuing Review or Administrative Check-in are detailed in the manual. When you have completed your research, please submit a Study Closure request so that IRB records will be accurate.

If you have any questions, please contact the UCF IRB at 407-823-2901 or irb@ucf.edu. Please include your project title and IRB number in all correspondence with this office.

Sincerely,

Renea Carver
Designated Reviewer