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Oral Microbial and Respiratory Status of Persons with Mental Retardation/Intellectual & Developmental Disability – An Observational Cohort Study

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Abstract

Objective—The objective of this study was to determine the prevalence of select microorganisms in oral biofilms and to investigate relationships between oral and respiratory status in persons with mental retardation/intellectual and developmental disabilities (IDD).

Study Design—We conducted a 6-month-long observational cohort study with 63 persons with IDD. Oral examinations, oral sampling, and medical record reviews were performed at baseline and then monthly. Polymerase chain reaction (PCR) was used to analyze all baseline oral samples for the presence of *Streptococcus pneumoniae (S. pneumoniae), Methicillin-Resistant Staphylococcus aureus (MRSA), Prevotella melaninogenica (P. melaninogenica)* and *Candida albicans (C. albicans).* PCR analyses were also performed on participants' samples collected in the month prior to being diagnosed with a respiratory infection.

Results—All subjects had *P. melaninogenica* detected by PCR in their oral samples. Fifty-five percent (35 of 63) of participants had *S. pneumoniae, MRSA* and *C. albicans* in their oral samples at baseline. No dental decay was detected clinically, oral hygiene was fair, and dysphagia was common. During the 6 months of the study, there were 22 respiratory infections (35% of participants) – 12 pneumonias, 7 sinusitis, 1 bronchitis, and 1 upper respiratory tract infection. Participants with microorganisms in their baseline samples were significantly more likely to develop any respiratory infection and those who had poor oral status were significantly more likely to develop pneumonia. Almost 60% of participants who developed respiratory infections had the same microorganism detected in the sample collected in the month prior to infection as had been detected in their baseline sample.

Conclusion—Potentially pathogenic microorganisms in the oral cavity and poor oral status significantly increased the risk of developing respiratory infections, including pneumonia, in persons

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with IDD. The results suggest that colonization with these microorganisms may persist despite routine tooth brushing. Meticulous comprehensive oral hygiene of the oral cavity may be needed to reduce oropharyngeal microbial load.

INTRODUCTION

The association between aspiration of potentially pathogenic microorganisms from the oral cavity and pneumonia has become relatively well established over the past several years.[1] Scannapieco and colleagues first reported in 1992 that 65% of medical intensive care patients had potential respiratory pathogens in their dental plaque or on their buccal mucosa compared to 16% of preventive dental clinic patients.[2] In 2006, Azarpazhooh and Leake reviewed all published studies and reported that the literature supported fair evidence of an association between oral status and pneumonia, and good evidence that improving oral health reduces respiratory disease.[3]

Over the years, investigators have primarily studied the association between oral status and pneumonia among elderly persons residing in nursing homes[4–6] and patients in intensive care units, particularly those on mechanical ventilation.[7–9] A less studied population with similar risk factors for respiratory disease is persons with mental retardation/intellectual disability.[10] There are approximately 3.2 million persons with some type of intellectual and/or developmental disability (IDD) in the United States,[11] and almost half-million of these individuals have severe or profound disability, are medically fragile, and live in health care facilities.[12] Dysphagia is a common medical symptom in these persons that may contribute to aspiration of oral biofilms,[13] thus predisposing them to respiratory infections,[14] a major cause of morbidity and mortality in this population.[15]

Investigators have found microorganisms in oral biofilms, including dental plaque and saliva, and pulmonary secretions including *Streptococcus pneumoniae, Staphylococcus aureus, Pseudomonas sp, Klebsiella sp, Haemophilus influenzae, and Prevotella sp.*[16–20] In addition to these bacteria, *Candida albicans* colonization has been reported to impair macrophage function and facilitate *Pseudomonas aeruginosa* pneumonia in animals.[21] Colonization with *Candida* has also been reported to be a risk factor for pneumonia in humans.[22]

Although the relationship between oral status and pneumonia has been well studied, the association between microorganisms in the oral cavity and sinusitis, bronchitis, and upper respiratory tract infections is less clear. Paju and colleagues reported that identical microorganisms were recovered from inflamed maxillary sinuses as well as the oral cavity, but they did not report any temporal association.[23] Other than this one study, there is little information on the possible association of oral colonization with potentially pathogenic microorganisms and development of sinusitis, bronchitis, and upper respiratory tract infections.

Rigorous oral hygiene can reduce oral colonization with bacteria and yeasts,[19] thus reducing pneumonia in critical care and nursing home patients.[6, 24] Persons with IDD, however, frequently resist oral hygiene procedures and have been reported to have poor oral hygiene.[25] Ferozali and colleagues investigated enhanced oral care using intermittent suction over a 90-day period. They reported that the use of a suction toothbrush decreased potentially pathogenic bacteria but they did not report any reduction in respiratory tract infections.[26]

Despite evidence that oral colonization with potential pathogenic microorganisms and poor oral status, including dysphagia, are risk factors for respiratory infections, we know little about the role of these factors in persons with mental retardation/intellectual disability. The primary objective of this study was to determine the prevalence of potential pathogenic microorganisms in oral biofilms in persons with IDD. The secondary objective was to determine if there was

an association between oral status, the presence of these microorganisms in the mouth, and respiratory infections. Specifically, we tested the hypothesis that poor oral status and oral colonization with *Streptococcus pneumoniae (S. pneumoniae), Methicillin-resistant Staphylococcus aureus (MRSA), Prevotella melaninogenica (P. melaninogenica)* and *Candida albicans (C. albicans)* increases the likelihood of subsequent development of respiratory infections such as sinusitis, upper respiratory tract infections, bronchitis, as well as pneumonia in persons with IDD.

METHODS

Study Design

The investigators designed and implemented a 6-month prospective, observational, cohort study from July 2005 through January 2006. Visual oral examinations and sample collection (as outlined below) were performed at baseline and subsequently every month for 6 months. One investigator (DH) with the assistance of a research coordinator (DW) used plastic mouth mirrors, sterile plastic implant scalers (Premier Dental, Plymouth Meeting, PA), and a mouth rest as indicated while performing the examinations and sampling. The investigator and coordinator were trained and calibrated in the use of study measures before the study commenced.

The participants' medical records were reviewed monthly to determine the incidence of respiratory infections diagnosed by the facility's physicians and for potential confounders associated with respiratory infections. Polymerase chain reaction (PCR) analysis for *S. pneumoniae*, *MRSA*, *P. melaninogenica*, and *C. albicans* was performed on all baseline samples to determine prevalence of colonization. Among participants diagnosed with respiratory infections during the study, the samples collected in the month prior to the infections were also analyzed with PCR.

Study Sample

The Human Studies Committees of the University of Louisville and the Kentucky Cabinet for Health and Human Services reviewed the study. Parents or legal guardians provided written informed consent and research authorization for the participants. Eligibility criteria were 1) mild to profound intellectual and/or developmental disability requiring institutional care; 2) dependency in two or more activities of daily living; and 3) inability to perform personal oral hygiene. Exclusion criteria included 1) existing pneumonia; 2) expected survival of < 3 months; and 3) a requirement for antibiotic prophylaxis before dental treatment.

Sample Size estimate—To estimate a sample size for this exploratory research, we used evidence from research conducted in populations with similar risk factors for potential respiratory microorganism colonization, where 30% to 60% of the dental plaque was colonized by potential respiratory pathogens.[2, 18] NCSS/PASS software (NCSS, Kayesville, Utah) indicated that a total sample of 66 participants would provide 90% power to detect a 50% difference in respiratory infection rates (i.e. 40% vs. 20%) among risk factor levels (i.e. positive PCR vs. negative PCR), alpha = 0.05.

The study sample was derived from the population of 125 residents at one state-supported intermediate care facility for mental retardation (ICF-MR) in Louisville, Kentucky. Among the 68 persons with IDD who enrolled in the study, 5 were excluded because of cardiac conditions requiring antibiotic prophylaxis before dental examination. The standard of oral care in the facility was tooth brushing performed twice a day with over-the-counter toothpaste by caregivers; antimicrobial mouth rinses such as chlorhexidine were not used.

Study Variables

Predictor Variables

Oral Status: The revised Oral Assessment Guide[27, 28] (OAG) was used to evaluate eight oral health areas (gingiva, teeth, swallow, saliva, voice, lips, mucous membranes, tongue). The OAG is a visual evaluation tool and thus can be used to collect data on the oral health status of uncooperative persons. Each area was scored from 1 = healthy to 3 = very poor status and the results totaled. Scores on the OAG ranged from a floor of 8 (good oral status) to a ceiling of 24 (very poor oral status) and the variable was thus coded as continuous.

Microbiology: Numerous organisms have been isolated from the lungs and oral cavity of persons with respiratory infections.[29] We originally planned to identify the following microorganisms: *S. pneumoniae, MRSA, Pseudomonas aeruginosa, Enterobacter, P. melaninogenica,* and *C. albicans with* polymerase chain reaction (PCR) analyses. PCR was selected as the method of analysis because the oral samples could not be transported in a timely manner to a laboratory for culture analysis. We encountered difficulties in developing and validating PCR assays for *Pseudomonas aeruginosa* and *Enterobacter* using known control bacteria, and were unable to perform PCR on study samples for these organisms. We thus used PCR assays that we were able to validate for *S. pneumoniae, MRSA, P. melaninogenica,* and *C. albicans*.

Dentate participants' microbiological samples were collected using a sterile implant scaler. Samples were collected from 6 six indicator teeth (upper right first molar, upper right central incisor, upper left first bicuspid, lower left first molar, lower left central incisor, lower right first bicuspid, or closest adjacent tooth, if specific indicator teeth were missing); however, all teeth were evaluated when only six were present. The scaler was sequentially placed sub-gingival and drawn toward the occlusal in the inter-proximal areas of the 6 indicator teeth and the samples were combined in one vial for DNA analysis. Samples from dentate participants most likely contained oral fluids, plaque, saliva, and crevicular fluid. The edentulous participants did not wear dentures; their samples were collected by drawing the scaler over the mandibular alveolar ridge 3 times, and placing the biofilm, most likely primarily containing saliva and other oral fluids, in one vial for analysis. Samples were stored in sterile tubes containing 150 μL of TE buffer (TE; 10 mM Tris-HCl, 1 mM EDTA, pH 7.6) [30], which were initially kept on ice and then stored at −70° C until analyzed.

Polymerase Chain Reaction processing: The samples were thawed and centrifuged at 5,000 times gravity for 15 minutes, and the pellet was suspended in sterile distilled water in a sterile hood. Genomic DNA was isolated using the QIAamp® DNA Mini Kit (Qiagen, Valencia, CA) as described by the manufacturer. Isolation of DNA from each sample was confirmed by measuring absorbance with a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). The samples were then frozen pending further analysis.

Quantitative Real-Time PCR was performed on an Applied Biosystems Incorporated 7900 instrument and used primers and probes for detection of the DNA of the microorganisms. The *S. pneumoniae* assays contained primers specific to genes encoding pneumolysin, one of the most important virulence factors of *S. pneumoniae*, in custom-made assays published previously[31, 32] and designed by Applied Biosystems, Inc. (Foster City, CA). The forward primer was AGCGATAGCTTTCTCCAAGTGG, the reverse primer was CTTAGCCAACAAATCGTTTACCG, and the internal probe sequence was ACCCCAGCAATTCAAGTGTTCGCG. Assays for *MRSA, P. melaninogenica,* and *C. albicans* were similarly designed and manufactured by Applied Biosystems, Inc. (Foster City, CA). The forward primer for *MRSA* was GGTGTTGGTGAAGATATACCAAGTGA, the reverse primer was GGTTAATCAGTATTTCACCTTGTCCGT, and the internal probe was

AACCTGAATCAGCTAATAATATTTCATTAT. For *P. melaninogenica*, the forward primer was CCAGCCAAGTAGCGTGCA, the reverse primer was TGGACCTTCCGTATTACCGC, and the internal probe was AATAAGGACCGGCTAATTCCGTGCC. For *C. albicans*, the forward primer was GAGGGCAAGTCTGGTG, the reverse primer was CTGCTTTGAACACTCTAA, and the internal probe was TTT TGA TGC GTA CTG GAC CC (T GT).

A 20-μL sample was used for PCR analysis and consisted of 10 μl of TaqMan® Universal PCR Master Mix (containing the thermal stable DNA polymerase AmpliTaq Gold® enzyme to cleave the probe) (Applied Biosystems, Foster City, CA), 5 μL nuclease-free water, 1 μL 20X assay, and 4 μL of specimen. PCR cycling conditions comprised an initial denaturation step of 95°C for 10 minutes, followed by 40 cycles of denaturation of 95°C for 15 seconds, and primer annealing at 60°C for 1 minute.

A standard curve was constructed for each PCR plate by extracting DNA from known cultures of *S. pneumonaie, MRSA, P. melaninogenica,* and *C. albicans*. The amount of known organism DNA was serially diluted from concentrations of 4 nanograms of DNA per microliter to 0.004 nanograms of DNA per microliter and subsequently placed in wells on the PCR plate with the study samples to determine the amount of specific nucleic acid in each study sample. Each participant's sample was run in triplicate and the mean value used for analysis. We included negative preparation control samples (without template) with each plate of samples being analyzed as quality control.

Outcome Variables: ***PCR Results:*** Colonization was operationally defined as either present or absent based on the results of the quantitative real-time polymerase chain reaction (PCR). Specifically, we considered the microorganism present if PCR detected at least 0.004 nanograms of DNA per microliter in participant's samples – the lowest dilution of known microorganism DNA included in the PCR standard curve.

Respiratory Infections: Sinusitis, bronchitis, upper respiratory tract infection, and pneumonia were diagnosed clinically by the facility's physicians and were coded as 1 = present or 0 = not present. Jackson's Operational Definition was used for the clinical diagnosis of pneumonia by the facility's physicians:[33] (Pneumonia = A5 below plus one or more of A1 to A4; B alone or with other signs or symptoms [A1–A5].)

A. Clinical Signs and Symptoms:

1. Cough;
2. Pleuritic chest pain;
3. Fever ≥ 100 degrees Fahrenheit;
4. Purulent sputum;
5. Clinical findings of pneumonia (eg. Rales, rhonchi, dullness to percussion).

B. Radiographic Findings - Radiologic evidence of pneumonia as read by a radiologist.

Co-Morbidities: We reviewed medical records to determine if participants had any of the following conditions that have been associated with the development of respiratory infections including pneumonia: enteral tube feeding, dysphasia, asthma, diabetes mellitus, cardiovascular disease, stroke, present medications, history of antimicrobial use in the past 6 months, history of overnight hospitalization for any reason in the past 6 months, history of vaccination against pneumococcal influenza in the past year, and pneumococcal vaccination ever.

Data Management and Analysis

Data Management—Data were entered into a relational data management system using data entry screens that were identical to the data collection forms. Edit checks included range and logical consistency verification. Hard copies of all data entered were produced, verified, and stored in identical patient files. The database had several levels of password protection and data was exported for analysis using statistical software.

Statistical Analysis—Bivariate analyses included correlations, chi-square tests, and independent *t*- tests for all respiratory infections. Factors that have been associated with respiratory infections in other populations were first analyzed with bivariate techniques to investigate how they relate to oral status and potential respiratory microorganism oral colonization in this study sample. These factors included age, dysphagia, enteral/gastric tube feeding, antibiotic usage, immune status, history of asthma, diabetes mellitus, cardiovascular disease, chronic obstructive pulmonary disease, stroke (and/or neurological motor deficit), present medications, history of vaccination against influenza in the past year, and pneumococcal ever. Logistic regression analysis was used to explore which factors were associated with participants that developed respiratory infections during the study. $P < 0.05$ was considered statistically significant. Data analysis was conducted with SPSS 17 (SPSS, Chicago, IL).

RESULTS

Sixty-three institutionalized persons with IDD aged 48 ± 11 years old participated in the study and their demographic characteristics, oral health status, and medical conditions are summarized in Table I. None of the dentate participants had any clinically obvious decayed or fractured teeth. The 19 participants who were edentulous did not wear dentures and ate a pureed/soft mechanical diet or were fed via a gastric tube (6 of 19). The mean revised Oral Assessment Guide (OAG) score was 14.9 ± 1.8 with a range of 11 to 19. None of the participants had a score of 8 which would indicate excellent oral status. There were no significant differences in oral health or respiratory tract infections between participants with moderate, severe or profound intellectually/developmental disabilities or between those who were chair bound, partially mobile or fully mobile.

Respiratory Infections

During the 6 months of the study from July through January, there were 22 respiratory infections among the 63 participants. The facility physicians diagnosed seven participants with sinusitis, 1 with bronchitis, 2 with upper respiratory tract infections, and 12 with pneumonia (Table II). More participants who developed respiratory infections were enterally fed ($p = 0.01$), had microorganisms detected in baseline samples by PCR ($p < 0.01$), including *S. pneumonaie* in their baseline samples ($p < 0.01$) than those who did not develop any respiratory infections. More participants diagnosed with pneumonia during the 6 months of the study had worse OAG scores ($p = 0.01$), were older ($p = 0.04$), were enterally fed ($p = 0.01$) and had microorganisms in their baseline samples ($p = 0.03$) than those who were not diagnosed with pneumonia.

PCR Analyses

All participants had the anaerobic organism *P. melaninogenica* detected in their baseline samples. There was a significant correlation between number of teeth and amount of *P. melaninogenica* DNA detected, with more *P. melaninogenica* DNA found in participants with more teeth ($r = 0.357$, $p = 0.004$). The 19 edentulous participants had significantly less *P. melaninogenica* DNA (0.40 nanograms per microliter) than dentate participants (16.64 nanograms per microliter) in their samples ($p < 0.001$). Although both dentate and edentulous

participants who developed respiratory infections during the study tended to have larger amounts of *P. melaninogenica* than those who did not develop respiratory infections, the differences were not significant. We thus did not include *P. melaninogenica* in further analyses.

S. pneumoniae, MRSA, and C. albicans were detected by PCR analyses in 55% (35 of 63) of study participants' baseline oral samples. Fourteen participants had polymicrobial colonization and 21 participants had one species detected. *S. pneumoniae* was the most common organism found in 33% (21 of 63) participants, either alone or with *MRSA* and/or *C. albicans*. We detected *S. pneumoniae, MRSA, and C. albicans* DNA in baseline oral biofilms in 86% (6 of 7) of participants later diagnosed with sinusitis, 100% (2 of 2) with upper respiratory tract infections, the one participant diagnosed with bronchitis, and 83% (10 of 12) participants who were diagnosed with pneumonia, compared with 39% (16 of 41) who were not diagnosed with respiratory infections during the study ($p < 0.01$).

The same microorganisms found in baseline samples were also found in samples collected in the month prior to a respiratory infection in 59% (13 of 22) of participants. In 27% (6 of 22) of participants who developed respiratory infections, the microorganisms detected in baseline samples were not detected in the samples collected in the month prior to infection. Three participants without baseline positive PCR results later developed infections and only one of these three participants had *C. albicans* in the sample collected in the month prior developing pneumonia. (Table III)

Thirty percent (19 of 63) of the participants were edentulous and they were more likely to have *S. pneumonaie, MRSA*, and/or *C. albicans* (74% [14/19]) detected in their baseline oral samples than dentate participants (48% [21/41]), and this difference approached statistical significance (χ^2 (1) = 3.62, $p = 0.057$). Edentulous participants (37% [7/19]) were almost as likely to have any respiratory infection during the study as dentate participants (34% [15/44]), but less likely to have positive PCR results in the samples collected in the month prior to infection (3 of 7) than dentate participants (11 of 15). Edentulous participants were almost as likely to develop pneumonia (16% [3/19]) as were dentate participants (21% [9/44]).

Multivariate Analyses—We conducted bivariate analyses prior to building a logistic regression model of independent predictors for developing a respiratory infection (sinusitis, bronchitis, upper respiratory tract infection, and pneumonia). Chi-Square and independent *t*-tests revealed no statistically significant differences in the distribution of any of the study variables between participants who had positive or negative baseline PCR results for *S. pneumonaie, MRSA* or *C. albicans*. Differences between those who had a respiratory infection (n = 22) and those who did not develop respiratory infections (n = 41) were: 1) positive baseline PCR *S. pneumonaie, MRSA, C. albicans* results – nineteen of the 22 (86%) who developed respiratory infections had baseline positive PCR results compared to 16 of 41 (39%) who did not develop respiratory infections ($p \leq 0.001$), 2) Enteral feeding – eleven of 22 (50%) participants who developed respiratory infections were enterally fed compared to 8 of 41(19%) who ate a soft or pureed diet ($p = 0.012$) [see Table II]. Other factors that were marginally significant ($0.05 < p < 0.10$) included age, edentulous, OAG score, and the swallow sub-scale of the OAG. OAG scores for participants who had respiratory infections tended to have worse scores (15.44 ± 1.7) compared to those who did not have infections (14.6 ± 1.7, $p = 0.08$).

Logistic regression was used to explore factors associated with the development of a respiratory infection and factors with $p < 0.10$ in the bivariate analysis were considered for the model. Forward stepwise likelihood ratio selection method was used to build the model and two-way and when possible, some three-way interactions were also included, but this was limited given the size of the study. With respiratory infection as the outcome only two independent factors were retained in the model as significant using 0.05 as the selection criteria: a positive PCR

result at baseline and enteral feeding. The odds ratio for positive PCR result at baseline indicated that when controlling for the other variables in the model, participants with a positive PCR result at baseline had 9 times the odds of developing any respiratory infection (95% CI 2.3–38.8, $p = 0.002$) and those who were enterally fed had almost 4 times the odds of developing respiratory infections (95% CI 1.1–13.8, $p = 0.041$).

Additionally, we examined pneumonia as an outcome of it's own since it is the respiratory infection associated with the greatest morbidity. In the bivariate analysis, age, OAG score, enteral feeding, and positive PCR at baseline were significant (Table II). Finally, we conducted logistic regression analyses for only pneumonia to identify the best independent predictors of pneumonia. As before, any factor that was significant at the $p = 0.10$ level in the bivariate analysis was considered for entry into the logistic model. Once again, we used a forward stepwise likelihood ratio selection regression method. Factors for consideration included age, OAG score, poor OAG, several of the OAG sub-scales (gingiva, tongue, swallow), enteral feeding, and a positive baseline PCR result. Many two-way and some three-way interactions were also included for entry, but surprisingly only one factor, OAG score, was selected and retained in the model. Many of these factors were mildly correlated which most likely explains why, although several factors were significant in the bivariate analyses, only OAG score was selected as an independent predictor of pneumonia in the multivariate logistic regression model. The odds ratio for OAG score was 1.6 (95% CI 1.1–2.5, $p = 0.019$), indicating that a one unit increase in the OAG score would correspond to a 1.6 factor increase in the odds of developing pneumonia.

DISCUSSION

The objective of this study was to determine the prevalence of select microorganisms in oral biofilms and to investigate relationships between oral and respiratory status in persons with mental retardation/intellectual and developmental disabilities (IDD). We tested the hypothesis that poor oral status and oral colonization with *S. pneumoniae, MRSA, P. melaninogenica,* and *C. albicans* increased the likelihood of subsequent development of respiratory tract infections in persons with IDD.

The results of this study confirm the hypothesis that poor oral status and the presence of potentially pathogenic microorganisms in oral biofilms increases the likelihood of developing respiratory infections in persons with IDD. Using PCR analyses, we detected *S. pneumonaie, MRSA, and C. albicans* DNA in almost 60% of study participants and the anaerobic bacteria *P. melaninogenica* in all participants' oral biofilms. We found that participants with positive PCR results for *S. pneumonaie, MRSA, and C. albicans* in baseline samples were almost 9 times as likely to later be diagnosed with sinusitis, bronchitis, upper respiratory tract infection, and pneumonia than those negative PCR results.

Among those diagnosed with respiratory infections during the study, almost two-thirds had the same microorganism detected in the sample collected in the month prior to the infection as had been detected in the baseline sample. Based on the results of this study, the use of routine tooth brushing does not appear to be effective in eliminating potentially pathogenic microorganisms in the oral cavity of persons with moderate to profound IDD.

S. pneumonaie was the most common microorganism detected in baseline oral biofilms, either alone or with *MRSA* and *C. albicans,* in both dentate and edentulous participants. It was associated with subsequent diagnosis of not only pneumonia, but also sinusitis, upper respiratory tract infections, and bronchitis. Participants subsequently diagnosed with sinusitis, bronchitis, and upper respiratory infections most frequently had only *S. pneumonaie* detected in their samples collected in the month prior to infection. Participants diagnosed with

pneumonia during the study frequently had polymicrobial PCR results in the samples collected in the month prior to pneumonia.

C. albicans is not generally considered a major cause of respiratory infections but it is frequently found in mechanically ventilated[34] and nursing home patients.[35] Recent evidence, however, has suggested that *Candida* species may impair macrophage function[21] and may facilitate *Pseudomonas* ventilator-associated pneumonia.[22] Although we were not able to perform PCR analysis for *Pseudomonas* in our study, it is possible that the participants who had *C. albicans* detected in their samples may have also had *Pseudomonas* colonization that contributed to respiratory infections.

The results of this study are consistent with other studies published on the association between aspiration and respiratory infections. Almost all participants in this study had dysphagia and 65% had epilepsy or seizure disorders, known risk factors for aspiration of oropharyngeal secretions. Morton and colleagues investigated the contribution of neurodisability to respiratory tract infections in children. They found that children with greater disability and who experienced direct and reflux aspiration were more likely to have severe respiratory tract infections than those who did not have aspiration.[36]

Our PCR results suggest that the entire oropharyngeal region, not just teeth and dental plaque, serves as a reservoir for multiple potentially pathogenic organisms. We collected samples from dentate participants that likely contained plaque, saliva, and crevicular fluid; edentulous participant's samples likely contained saliva and biofilms. A study reported by Bahrani-Mougeot and colleagues provided support for this concept with an investigation using molecular analysis of tongue and lung samples in 16 patients diagnosed with ventilator-associated pneumonia. They reported a wide diversity of bacteria, found the same species in both tongue and lung samples in 14 of 16 (88%) of patients, and polymicrobial colonization.[37] In contrast with our study, Bahrani-Mougeot *et al*. enrolled subjects and collected samples when they already had suspected pneumonia and so could not determine if bacterial colonization of the oropharynx preceded possible aspiration and pneumonia. The results of our study suggest that colonization precedes respiratory infection and the colonization may persist.

El-Solh and colleagues investigated the aerobic and anaerobic microbiology of severe aspiration pneumonia in elderly persons residing in long-term care facilities.[20] They used quantitative protected broncho-alveolar lavage sampling of 95 residents of long-term care facilities admitted to their intensive care unit over a 2-year period. They found that *Prevotella* spp. was the most common anaerobic bacteria and that 22% of patients had polymicrobial infection. In our study, all participants had *P. melaninogenica* in their baseline samples and the only difference we detected among study variables was that dentate participants had greater quantities of *P. melaninogenica* DNA in their samples than did edentulous participants. Additional research using respiratory samples (ie. Broncheo-alveolar lavage sampling for a diagnosis of pneumonia) as well as oral samples would provide more information on the role of anaerobic bacteria in respiratory infections.

The observed 59% prevalence of potentially pathogenic microorganisms is consistent with reports in other at-risk populations. Scannapieco and colleagues reported 65% prevalence in medical intensive care unit patients and Sumi and colleagues examined the dental plaque of 138 dependent elderly persons with culture techniques and found 9 species of microorganisms in the dental plaque of 64% of the elderly.

Our results suggest that the oral cavity of persons with IDD serves as a reservoir of bacteria that may be aspirated into the lungs, especially in persons with swallowing disorders. We observed that participants who were diagnosed with pneumonia during the study had significantly worse scores on the OAG tongue sub-scale indicating coating of the tongue.

Participants diagnosed with pneumonia also had poorer OAG swallow sub-scale scores indicating that they did not have normal swallow reflexes. Caregivers who provide oral hygiene for persons with IDD should pay particular attention to tongue cleaning and should be aware of the person's swallow status. Suctioning during oral hygiene procedures may assist in reducing aspiration of dislodged biofilms.

The results of this study are relevant to the specialty of Oral Medicine because they add to the knowledge base of the relationship between oral microbial status and systemic disease. The presence of potentially pathogenic microorganisms in oral biofilms despite routine tooth brushing suggests additional measures may be required to reduce the oral bacterial load in persons with IDD. Because our study was observational, we cannot determine whether improving oral status and reducing oral colonization with potentially pathogenic microorganisms of persons with IDD will decrease respiratory infections in this population. However, our results would suggest that a randomized interventional trial may be warranted.

The results of Ferozali and colleagues pilot study to address the effectiveness of intermittent suctioning to reduce potentially pathogenic bacteria in persons with IDD are promising.[26] The authors reported that tooth brushing twice a day with a traditional or a single-use suction toothbrush combined with sodium bicarbonate and 1.5% hydrogen peroxide resulted in a significant decrease in bacteria from baseline to post-intervention compared to the control group that received traditional tooth brushing with toothpaste twice a day. Similarly, Yoneyama and colleagues also demonstrated that meticulous oral hygiene and weekly professional oral care reduced pneumonia in both dentate and edentulous nursing home residents.[6] Although there is evidence that oral chlorhexidine application in concentrations ranging from 0.12% to 2.0% may be effective in reducing oropharyngeal bacterial colonization in intensive care patients,[7, 38] the long-term use of chlorhexidine has not been evaluated in persons with IDD and may or may not be advisable.

Potential limitations need to be considered when interpreting the results of this study. We performed PCR with a limited number of assays for select microorganisms and thus may well have underestimated the type and quantity of other microorganisms associated with respiratory infections. It is likely that using additional assays and/or culture techniques would have detected higher prevalence rates. We evaluated residents of a single facility in Kentucky, the majority of whom had profound IDD with swallowing disorders. Our results thus may not be generalizable to persons with different levels of IDD in other facilities or those living in community settings who may receive different or more comprehensive oral hygiene therapies. We were not able to confirm the adequacy or frequency of oral hygiene provision by caregivers but overall the oral hygiene appeared to be adequate as by the acceptable scores on the teeth sub-scale of the OAG scale (a proxy assessment of oral hygiene). Finally, we relied on clinical diagnoses by the facility's physicians and thus could not determine if the respiratory infections were bacterial, viral, mycobacterial, or fungal in origin. Investigators conducting studies in the future should endeavor to include a laboratory diagnosis as well as a clinical diagnosis to determine the specific etiology of respiratory infections.

In spite of these limitations, we believe there are several important strengths of the study. We followed the participants for 6 months which was twice as long as the Ferozali pilot study, the only other similar study we could locate in the literature.[26] We identified oral colonization with potentially pathogenic microorganisms as an additional risk factor for sinusitis, bronchitis, upper respiratory tract infections as well as pneumonia in persons with IDD. We included dentate and edentulous persons and demonstrated that edentulous individuals have similar risk factors for respiratory infections. Finally, we used a validated PCR assays for the microorganisms including an assay for detection of oral colonization with *S. pneumoniae* with

primers specific to genes encoding pneumolysin – one of the most important virulence factors of *S. pneumonaie* and associated with the most serious respiratory tract infections.

Conclusion

The results of this study provide additional support for the relationship between oral and systemic health, specifically respiratory infections, in a vulnerable population. The results also suggest that comprehensive meticulous oral hygiene procedures may be needed in all persons with IDD, including those who are dentate, edentulous, and/or enterally fed.

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Table 1

Characteristics of the Study Participants (n = 63)

Age (yr)	48 ± 11
Gender (M/F)	37 / 26
Race (AA/W)	6 / 57
Body Mass Index (kg/m^2)	23 ± 5
Number of Teeth (n)	15 ± 11
Edentulous (no teeth)	19 (30%)
Possessed 1–20 teeth	17 (27%)
Possessed 21 or more teeth	27 (43%)
Cooperation	
Fully cooperative	15 (24%)
Moderately cooperative	10 (16%)
Very uncooperative	38 (60%)
Ambulatory Status	
Chair Bound	44 (70%)
Partial Mobility	12 (19%)
Full Mobility	7 (11%)
Revised Oral Assessment Guide (ROAG)	14.9 ± 1.8
Fair oral status	46 (73%)
Very poor oral status	17 (27%)
Gingiva sub-score of ROAG	
(Proxy assessment of periodontal health)	1.6 ± 0.4
Good gingival health	24 (38%)
Fair gingival health	18 (29%)
Poor gingival health	21 (33%)
Teeth sub-score of ROAG	2.0 ± 0.7
(Proxy assessment of oral hygiene)	
Good oral hygiene	18 (29%)
Fair oral hygiene	19 (30%)
Poor oral hygiene	26 (41%)
Medical Conditions	
Enteral Feeding Tube	30%
Diabetes	10%
Hypertension	10%
History of pneumonia	16%
Dysphagia	97%
Epilepsy/Seizure disorder	65%
Cerebral palsy	24%
Aphagia	40%
Gastro-esophageal reflux disease	24%
Profound Intellectual/Developmental Disability	87%
Severe Intellectual/Developmental Disability	11%

Data are shown as mean ± SD or number of patients (%).

Table 2

Associations between all respiratory infections* and pneumonia and study variables

	No Respiratory Infections (n = 41)	Respiratory Infections* (n = 22)	*P* value	No Pneumonia (n = 51)	Pneumonia (n = 12)	*P* value
Gender[a] - M / F	24 / 17	13 / 9	0.97	29 / 22	8 / 4	0.53
Age[b] – yrs	46.1 ± 9.0	50.8 ± 12.6	0.09	46.4 ± 10.3	53.1 ± 10.5	0.04
Edentulous[a] – N (%)	12 (29)	7 (32)	0.09	16 (31)	3 (25)	0.66
Number of teeth[b]	15.0 ± 11.4	14.6 ± 11.1	0.88	14.9 ± 11.5	14.9 ±10.1	0.98
OAG Score[b] (range 8–24)	14.6 ± 1.7	15.4 ± 1.7	0.09	14.6 ± 1.7	16.0 ± 1.7	0.01
Poor Oral Health – [a] N (%) (OAG ≥ 16)	9 (22)	8 (34)	0.22	11 (22)	6 (50)	0.05
OAG Sub-scales[b] (range 1 to 3)						
Gingiva	1.6 ± 0.4	1.7 ± 0.4	0.45	1.6 ± 0.4	1.8 ± 0.3	0.09
Teeth	1.9 ± 0.7	2.0 ± 0.7	0.79	1.9 ± 0.7	2.2 ± 0.6	0.23
Mucous Membranes	1.3 ± 0.3	1.4 ± 0.3	0.49	1.3 ± 0.3	1.4 ± 0.2	0.16
Saliva	1.4 ± 0.3	1.4 ± 0.4	0.90	1.4 ± .36	1.5 ± 0.4	0.38
Voice	2.6 ± 0.7	2.8 ± 0.5	0.15	2.7 ± 0.6	2.8 ± 0.6	0.42
Lips	1.5 ± 0.3	1.5 ± 0.2	0.75	1.6 ± 0.3	1.5 ± 0.2	0.64
Tongue	1.8 ± 0.3	1.8 ± 0.2	0.48	1.8 ± 0.3	2.0 ± 0.3	0.03
Swallow	2.4 ± 0.8	2.7 ± 0.5	0.08	2.5 ± 0.8	2.8 ± 0.4	0.02
Dysphagia[a] – N (%)	39 (95)	22 (100)	0.29	49 (96)	12 (100)	0.48
Seizure Disorder[a] – N (%)	26 (63)	15 (68)	0.70	34 (66)	7 (58)	0.58
Enteral Feeding[a] – N (%)	8 (19)	11 (50)	0.01	12 (23)	7 (58)	0.01
Microorganisms Detected in Baseline Oral Biofluids[a] - N (%)	16 (39)	19 (86)	<0.01	25 (49)	10 (83)	0.03
***S. Pneumonaie* Detected in Baseline Oral Boifluids[a] - N (%)**	11 (27)	16 (73)	<0.01	19 (37)	8 (67)	0.06

* - Respiratory infections include sinusitis, bronchitis, upper respiratory tract infections, and pneumonia Data reported as N (%) or mean ± Standard deviation

OAG = Oral Assessment Guide

[a] - Chi-Square test

[b] = Independent samples *t* – test,

Table 3

Microorganisms Detected by PCR at Baseline and Month prior to Respiratory Infections

	Participants who developed respiratory Infection N (%)	Month of Respiratory infection	Microorganisms detected in theMonth Prior to Respiratory Infection	Respiratory Infection	Dentate or Edentulous
Microorganism in Baseline Samples (N)					
SP (N = 14)	7 (50%)	September November November December December December December	*SP, MRSA, CA* None None *SP, MRSA* *SP* *SP* None	Pneumonia Pneumonia Sinusitis Pneumonia Upper RTI* Sinusitis Upper RTI*	Dentate Edentulous Edentulous Dentate Edentulous Dentate Edentulous
SP + *MRSA* (N = 4)	2 (50%)	October December	None *SP*	Pneumonia Bronchitis	Edentulous Edentulous
SP + *CA* (N = 7)	5 (71%)	September October November November December	*SP, CA* *CA* *SP* *SP, CA* *SP*	Pneumonia Pneumonia Pneumonia Pneumonia Sinusitis	Dentate Dentate Edentulous Dentate Dentate
SP + *MRSA* + *CA* (N = 2)	2 (100%)	December January	None *SP*	Pneumonia Sinusitis	Dentate Dentate
MRSA + *CA* (N = 1)	1 (100%)	December	No Sample	Sinusitis	Dentate
MRSA (N = 1)	0 (0%)	-	-	-	Edentulous
CA (N = 6)	2 (33%)	September December	*SP, MRSA, CA* *SP*	Pneumonia Pneumonia	Dentate Dentate
None (N = 28)	3 (11%)	September September December	*CA* No Sample None	Pneumonia Sinusitis Pneumonia	Dentate Dentate Dentate

*RTI = Respiratory Tract Infection

SP = Streptococcus pneumonaie

SA = Staphylococcus aureus

MRSA = Methicilin-resistant Staphylococcus aureus

CA = Candida albicans