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Comparing the Incidence and Prevalence of Oral Microbial Pathogens *Selenomonas noxia* and *Streptococcus mitis* within the UNLV-SDM Clinical Patient Population

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Authors' contributions

This work was carried out in collaboration among all authors. Authors KK and KMH were responsible for the overall project design. Authors BJS, MM, PP, KF, MT and NK were responsible for data generation and analysis. Authors KK and NK contributed to the writing and editing of this manuscript. All authors have read and agreed to the published version of the manuscript.

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ABSTRACT

Introduction: *Selenomonas noxia* (SN) is a gram-negative, anaerobic bacteria, which contributes to development and progression of periodontal disease. Some evidence now suggests *Streptococcus mitis* (SM), a gram-positive, facultative bacterium contributing to the etiology of dental caries and periodontal disease, may also influence the prevalence of SN within subgingival complexes. Based upon the overall lack of prevalence data, the objective of this study was to

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evaluate presence of SN and SM using qPCR among saliva samples taken from pediatric, adult, and orthodontic dental school clinics.
Methods: This study involved a retrospective analysis of previously collected saliva samples from an existing biologic repository. Screening for microbial presence of SN and SM was performed in duplicate using quantitative polymerase chain reaction or qPCR.
Results: From the repository containing N=1,176 samples, a total of n=196 samples were identified. Screening for SN revealed significantly higher prevalence among Pediatric Orthodontic samples (28.3%) compared with Adults (5.5%), P=0.001. No significant differences were found between Pediatric non-Orthodontic samples (16.7%) and Adult non-Orthodontic samples (12.5%), P=0.2343. Screening for SM revealed similar prevalence among Adult Orthodontic (27.8%) compared with Pediatric Orthodontic (31.7%) samples, P=0.3912. However, significant differences were observed between Pediatric non-Orthodontic (46.7%) and Adult non-Orthodontic samples (17.5%), P=0.0001.
Conclusions: This study is among the first to evaluate SN and SM co-occurrence among Pediatric and Adult Orthodontic and non-Orthodontic patient samples. The increased prevalence of both SN and SM among Pediatric patients, and Orthodontic samples more specifically, may suggest further research is needed to more fully understand the oral health risks facing these specific patients. The differential results in co-occurrence only observed among the Orthodontic patients may also suggest orthodontic therapy may be sufficient to alter oral behaviors or the oral habitat thereby altering the oral microbial constituents and possibly changing oral health and the risk for developing oral disease.

Keywords: Selenomonas noxia (SN); Streptococcus mitis (SM); saliva; qPCR screening.

1. INTRODUCTION

Selenomonas noxia (SN) is a gram-negative, anaerobic bacteria which was first discovered in the mid- to late-1980s [1-3]. This organism was subsequently identified as an active agent in the development and progression of periodontal disease [4,5]. As oral health research into this microbial constituent progressed, more evidence began to suggest the formation of specific and identifiable microbial communities and complexes associated with the presence of SN in the periodontium [6,7].

S. noxia is normally present in oral flora and can be isolated from the inflamed gingiva from patients with periodontal disease along with many other gram-negative anaerobic species, such as *F. nucleatum*, A. *actinomycetemcomitans, P. gingivalis* and *T. denticola* [8,9]. Although this organism is difficult to culture (fastidious) and no specific virulence factors for periodontal disease have been identified, evidence has strongly associated prevalence of this organism may be influenced by factors that negatively influence oral and periodontal health, such as smoking and poor oral hygiene [8,10,11]. Some evidence has also suggested that additional factors, such as orthodontic appliances and the microbes associated with these appliances may also contribute to the development and propagation of this organism [12].

As epidemiological surveys have expanded, more complex and interconnected associations between SN prevalence and systemic health have emerged, such as the potential two-way relationship between periodontal disease (and SN levels) with preterm low birth weight [13-15]. More recent evidence has suggested that microbial composition could be used to predict overweight status – as oral SN has demonstrated greater than 98% sensitivity of finding obese subjects [16]. Although the growing body of evidence strongly suggests SN has complex microbial interactions with other periodontal pathogens and may be linked with systemic disease, few studies have evaluated the link between subgingival microbial colonization by organisms, such as *Streptococcus mitis* (SM), and microbial succession and periodontal health and disease [17-19].

Some evidence now suggests that SM, a gram-positive, alpha-hemolytic, catalase negative facultative bacterium mostly found in dental plaque, allows for other bacteria to colonize the biofilm and contributes to both the etiology of dental caries and periodontal disease [20,21]. Although SM has no identified virulence factors, a recent study demonstrates a strong positive correlation between SN and SM co-occurrence, suggesting that complementary and overlapping functions may contribute to their overall prevalence within subgingival complexes [22]. As

some evidence suggests orthodontic treatment may contribute to increased levels of many highly related organisms, preliminary work from this group has attempted to evaluate the prevalence of SN, even among orthodontic patients [23-27].

However, to date no studies have evaluated the presence of these specific organisms among clinical orthodontic and non-orthodontic patients for comparison. Based upon this lack of prevalence data, the objective of this study was to evaluate the presence of SN and SM using qPCR among saliva samples taken from pediatric, adult, and orthodontic dental school clinics.

2. METHODS

2.1 Study Inclusion

This study involved a retrospective analysis of previously collected saliva samples from an existing biologic repository.

2.2 Sample Collection

From the original study, patients (or parents/legal guardians) were asked for their voluntary participation in a research study regarding microbial screening of human saliva. Inclusion criteria included all current patients of record at UNLV-SDM. Exclusion criteria included any patient (or parent/guardian) who declined to participate and any subject who was not a patient of record at UNLV-SDM.

In brief, each patient was given a sterile collection tube and asked to produce no more than 5 mL of unstimulated saliva prior to the beginning of their dental appointment. Each sample was transferred to a biomedical laboratory for storage and analysis and was given a randomly-generated, non-duplicated number to avoid any specific or identifiable patient information associated with a given sample. Only basic demographic information was noted, such as the age and sex of the patient and race or ethnicity if the participant chose to provide this information.

2.3 DNA Isolation

All samples were stored at -80°C in a locked biomedical laboratory freezer until processed. In brief, samples were thawed and DNA isolated using the phenol: chloroform extraction method using TRIzol reagent, which has been specifically designed to isolate high-quality DNA or nucleic acids from tissues and fluids, as previously described [28-30]. Concentrations and purity of DNA isolated from these samples was facilitated by a NanoDrop spectrophotometer using readings of absorbance at A260 and A280 nm. Samples with A260:A280 ratios above 1.65 were deemed acceptable for qPCR analysis and screening, with a minimum requirement of at least 10 ng of DNA.

2.4 qPCR Screening

Screening for microbial presence was performed in duplicate using quantitative polymerase chain reaction or qPCR with a 25 uL reaction mixture consisting of 12.5 uL Fast SYBR green Master Mix (Applied Biosystems), 1 uL of the DNA sample diluted to a standard concentration of 10 ng/uL, 8 uL of sterile, nuclease-free water (Promega), and 1.75 uL of both Forward and Reverse primers at 10 uM specific for 16S rRNA (positive control), *S. noxia* (SN) and *S. mitis* (SM) from Eurofins MWG Operon. Relative quantification was accomplished using the ddCT method using the 16S rRNA as a reference and the following specifications: initial incubation for two minutes at 50°C, denaturation for 10 minutes at 95°C and 35 cycles including denaturation for fifteen seconds at 95°C and annealing at the following primer-specific temperatures (nt=nucleotide; Tm=melting temperature):

2.5 Bacterial 16S rRNA

Forward 16S rRNA universal primer, 5′-ACGCGTCGACAGAGTTTGATCCTGGCT-3′
27 nt, 56% GC, Tm 76°C.

Reverse 16S rRNA universal primer, 5′-GGGACTACCAGGGTATCTAAT-3′
21 nt, 48% GC, Tm 62°C

2.6 *Selenomonas noxia* (SN) Primer

Forward primer- SN-F1, 5'-TCTGGGCTACACACGTACTACAATG-3'
25 nt, 48% GC, Tm: 68°C.

Reverse primer- SN-R1, 5'-GCCTGCAATCCGAACTGAGA-3'
20 nt, 55% GC, Tm: 68°C

2.7 *Streptococcus mitis* (SM) Primer

Forward primer - SM-F1, 5'-AGGATAAGGAACTGCACATTGGTC-3'
24 nt, 46% GC, Tm: 67°C.

Reverse primer = SM-R1, 5'-TGCATTACTTGGTGATCTCTCACC-3'
24 nt, 46% GC, Tm: 66°C.

2.8 Statistical Analysis

Parametric statistics for continuous variables, such as DNA concentration and purity, were evaluated using descriptive statistics (mean and standard deviation) and comparisons among groups were analyzed using Student's t-tests. Multiple comparisons of two-way t-tests were confirmed using analysis of variance (ANOVA). Non-parametric statistics for categorical variables, such as demographic characteristics, were evaluated using descriptive statistics (percentages) and comparison among groups were analyzed using Chi square analysis.

3. RESULTS

From the repository containing N=1,176 samples, a total of n=196 samples were identified for inclusion in this retrospective study (Table 1). Analysis of these samples revealed n=36 samples were derived from Adult Orthodontic patients, n=60 samples from Pediatric Orthodontic patients, n=40 samples from Adult non-Orthodontic patients and n=60 samples from Pediatric non-Orthodontic patients (Table 1). Analysis of sex from each subgroup revealed a nearly equal percentage of Females (overall 53.1%) and Males (46.9%), which closely resembled the overall clinic patient population (52.8%, 47.2%, respectively), P=0.6886. Analysis of Race/Ethnicity revealed approximately one-third (35.7%) of samples were derived from non-Minority (White) patients, which was significantly higher than the overall percentage from the patient clinic population (24.7%), P=0.0012. The majority of the Minority patient samples were from Hispanics (50.5%).

More detailed analysis of these samples was performed to determine DNA concentration and purity (Table 2). Overall average DNA concentration (approximately 386.1 ng/uL) was similar among all isolates subgroups, which ranged between 363.2 and 399 ng/uL, P=0.433. No significant differences in DNA purity measured by the A260:A280 nm ratio, which averaged 1.82, P=0.771.

Molecular screening of each sample was accomplished using primers specific for SN and were performed in duplicate (Fig. 1). These data revealed differential results within each sample subgroup. For example, a small subset of the Adult Orthodontic samples harbored SN (n=2/36 or 5.5%) compared with a significantly larger percentage among the Pediatric samples (n=17/60 or 28.3%), P=0.001. Comparisons between the non-Orthodontic samples revealed similar prevalence among Pediatric (n=10/60 or 16.7%) and Adult (5/40 or 12.5%) samples, P=0.2343.

Microbial screening of each sample was then performed to assess prevalence of SM (Fig. 2). These data also revealed differential results within each sample subgroup. More specifically, no significant differences were observed in the prevalence of SM between Adult Orthodontic samples (n=10/36 or 27.8%) compared with the Pediatric Orthodontic samples (n=19/60 or 31.7%), P=0.3912. However, comparisons between the non-Orthodontic samples revealed significant differences between the prevalence among Pediatric (n=28/60 or 46.7%) and Adult (7/40 or 12.5%) samples, P=0.0001.

More detailed analysis of the SN and SM-positive samples revealed cycle threshold (CT) counts, where the fluorescence of the qPCR product can be detected above background levels, that ranged from 25 to 39.6 (Fig. 3). The vast majority of SN samples (91.1%) exhibited CT counts below the SN-positive control standard (diluted to 10 ng/uL) at CT=28.4 (Fig. 3A), while the majority of SM-positive samples exhibited CT counts above the SM-positive control standard (diluted to 10 ng/uL) at CT=30.4 (81.3%) (Fig. 3B).

To graphically display the correlation and prevalence of these organisms within the sample categories, a modified Venn diagram was created (Fig. 4). This graphic display revealed that within the Adult Orthodontic samples most of the SN-positive samples were also SM-positive - although only a small proportion of SM-positive samples also harbored SN (SN:SM ratio 0.2). However, a small proportion of the SN-positive samples were found among SM-negative samples. Similarly, within the Pediatric Orthodontic samples, most of the SN-positive samples also harbored SM - although not all of the SM-positive samples harbored SN (SN:SM ratio 0.80). Similar to the Adult Orthodontic samples, a small proportion of the SN-positive samples were found among the SM-negative samples. In contrast, within the Pediatric and Adult non-Orthodontic samples, all of the SN-positive samples were found within the SM-positive sample subgroups.

Table 1. Demographic analysis of study sample

	Adult orthodontic	Adult non-ortho	Pediatric ortho	Pediatric non-ortho	UNLV SDM	Statistical analysis
			Sex			
Females	n=19/36(53.8%)	n=22/40(55%)	n=32/60(53.3%)	n=31/60(51.7%)	52.8%	χ2=0.161 d.f.=1
Males	n=17/36(47.2%)	n=18/40(45%)	n=28/60(46.7%)	n=29/60(48.3%)	47.2%	P=0.6886
			Ethnicity			
White	n=14/36(38.9%)	n=15/40(37.5%)	n=22/60(36.7%)	n=19/60(31.7%)	24.7%	χ2=10.453 d.f.=1
Minority	n=22/36(61.1%)	n=25/40(62.5%)	n=38/60(63.3%)	n=41/60(68.3%)	75.3%	P=0.0012
Hispanic	n=19/36(52.8%)	n=20/40(50%)	n=31/60(51.6%)	n=29/60(48.3%)	52.1%	
Asian/Black	n=3/36(8.3%)	n=5/40(12.5%)	n=7/60(11.7%)	12/60(20%)	23.2%	

Table 2. DNA analysis

	Adult orthodontic	Adult non-ortho	Pediatric ortho	Pediatric non-ortho	Statistical analysis
DNA average concentration	X= 399 ng/uL STD 96.15	X=363.2 ng/uL STD 94.19	X=395.6 ng/uL STD 98.4	X=386.2 ng/uL STD 82.16	Two-tailed T-test P=0.433
DNA Average purity	A260:A80 X=1.79 STD=0.062	A260:A280 X=1.82 STD=0.071	A260:A280 X=1.84 STD=0.077	A260:A280 X=1.81 STD=0.81	Two-tailed T-test P=0.771

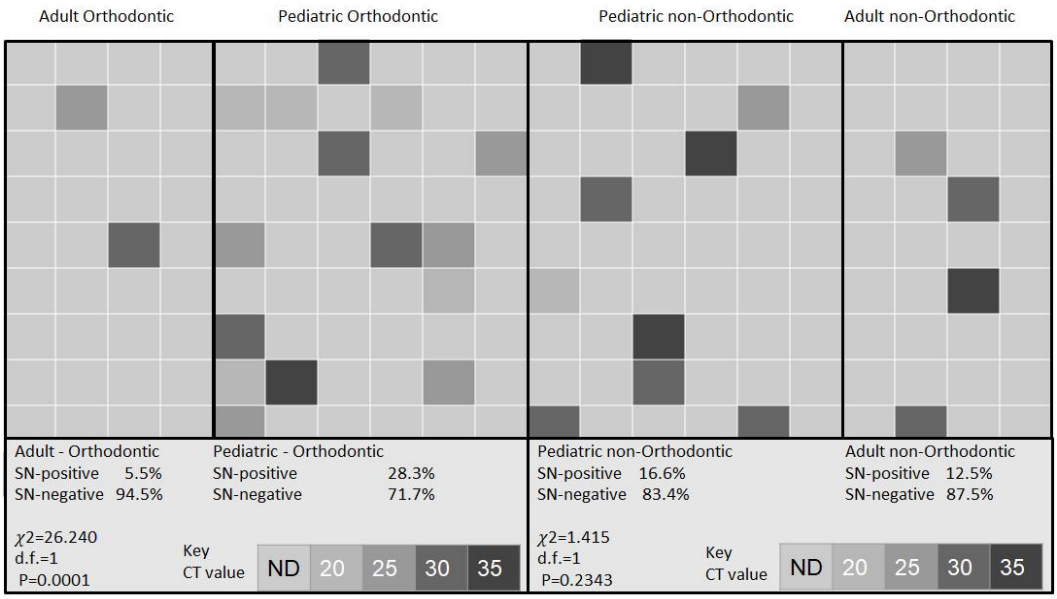

Fig. 1. qPCR screening for *Selenomonas noxia* (SN). Screening for SN revealed the majority of samples were not detectable (ND) above the background fluorescence. Analysis of cycle threshold (CT) data revealed significantly higher prevalence among orthodontic samples among Pediatric patients (28.3%) compared with adults (5.5%), P=0.001. No significant differences were found between pediatric non-orthodontic samples (16.7%) and adult non-orthodontic samples (12.5%), P=0.2343

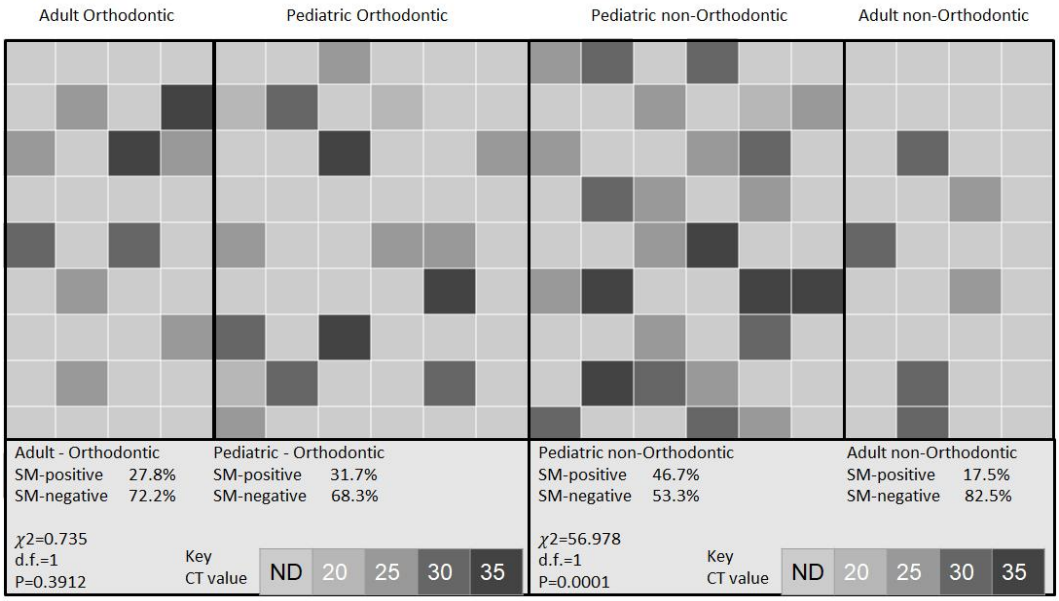

Fig. 2. qPCR screening for *Streptococcus mitis* (SM). Screening for SM revealed the majority of samples were not detectable (ND) above the background fluorescence. Analysis of cycle threshold (CT) data revealed similar prevalence among Orthodontic samples from adult patients (27.8%) compared with pediatric patients (31.7%), P=0.3912. Significant differences were observed between pediatric non-orthodontic samples (46.7%) and adult non-orthodontic samples (17.5%), P=0.0001

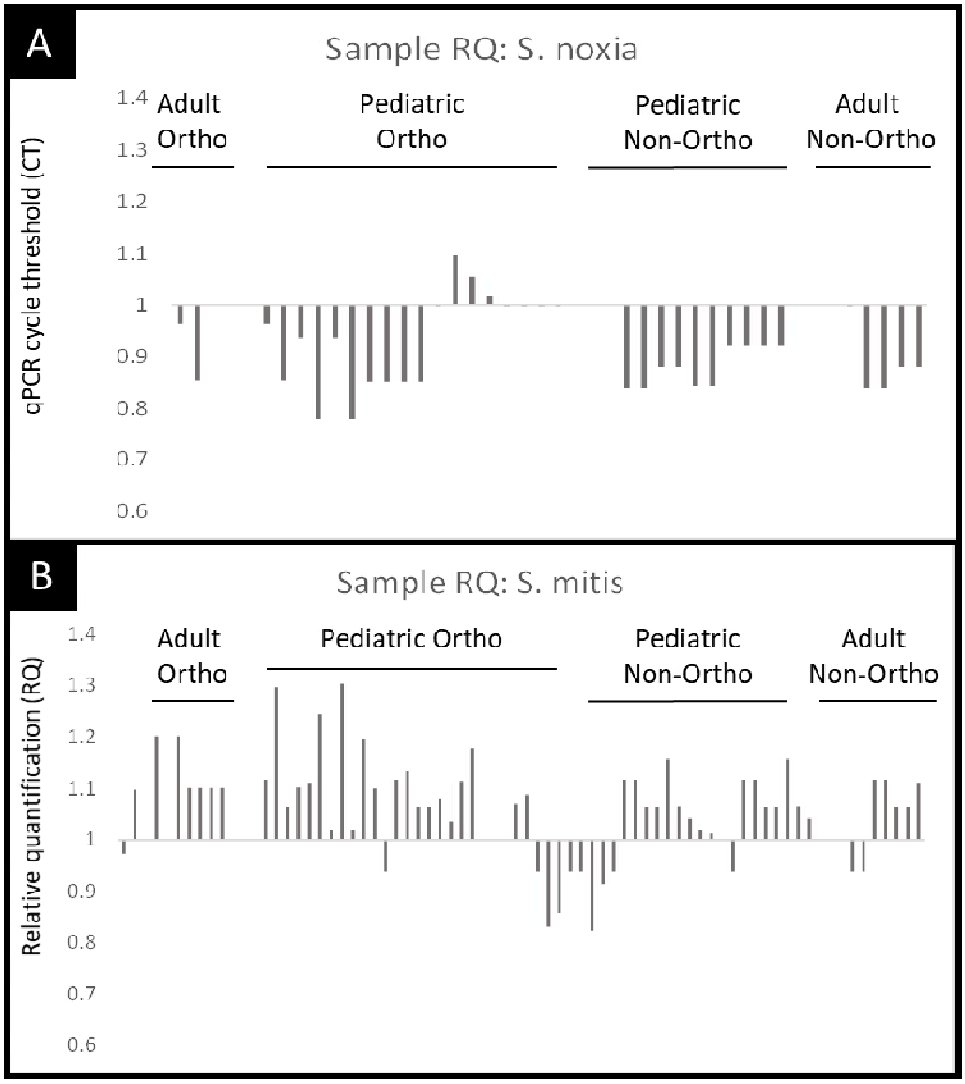

Fig. 3. Cycle threshold (CT) and relative quantification (RQ). A) Analysis of qPCR result from the SN-positive samples revealed CT ranging between 26.3 and 36.5 (CT ave.=31.53), which was significantly different from the SN-positive controls (CT ave.=28.49), P=0.031. Calculation of RQ revealed the majority of samples were below the SN positive controls (RQ ave.=0.911) B) comparison of these data with SM-positive controls revealed CT ranging between 25 and 39.6 (CT ave.=32.31), which was significantly different from the SM-positive controls (CT ave.=30.41), P=0.0281. Calculation of revealed the majority of samples were above the SM RQ average (RQ ave.=1.26)

4. DISCUSSION

Based upon this lack of prevalence data for these specific organisms among clinical Orthodontic and non-Orthodontic patients, the objective of this study was to evaluate the presence of SN and SM using qPCR among saliva samples taken from Pediatric, Adult, and Orthodontic dental school clinics. The results of this study demonstrated several important findings. First, these data suggest that SN is not found among the majority of samples but may be more specifically concentration among specific subgroups including pediatric orthodontic patients, which may suggest that new studies involving periodontal pathogens may be needed to understand how the prevalence of these organisms may be changing – particularly among minority and underserved populations undergoing orthodontic therapy [31,32].

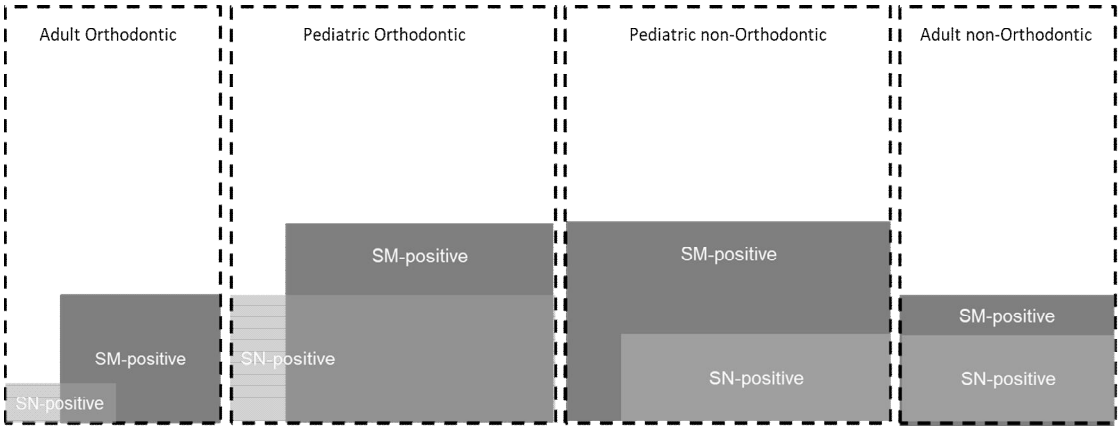

Fig. 4. Graphic analysis of microbial prevalence among patient samples. Within the pediatric and adult non-orthodontic samples, all of the SN-positive samples were found within the SM-positive sample subgroups. Analysis of the adult and pediatric orthodontic samples revealed most of the SN-positive samples were also SM-positive - although a smaller proportion of SM-positive samples also harbored SN. However, a small proportion of the SN-positive samples were found among SM-negative samples

In addition, this study found no significant differences in SN prevalence between pediatric and adult non-orthodontic populations but a much larger percentage of pediatric orthodontic patients harbored this organism. More specifically, these results may suggest that changes in the oral cavity associated with orthodontic treatment among non-adult patients may foster an environment conducive to this organism, which may have significant implications for oral and system health among these patient populations [33-35]. However, a major limitation of this study remains the lack of any temporal information to determine if placement and/or removal of orthodontic brackets is sufficient to subsequently modulate SN prevalence to match those observed among the non-Orthodontic patients.

The results of this study also confirmed other previous observations about SM, an organism that facilitates colonization of oral biofilms and may contribute to the etiology of both dental caries and periodontal disease [20,21]. The current study observed a much higher prevalence of SM than SN among all patient samples, with much higher percentages observed among the pediatric non-orthodontic patients. The prevalence of this organism among these patient samples, which are mainly low-income and minority – may suggest these observations could be associated with supragingival biofilm formation and caries risk in these patients rather than subgingival plaque and periodontal pathogens, such as SN [36,37]. More research will be needed to confirm and more fully understand the associations and patterns observed in this study.

Finally, the graphic analysis of microbial prevalence among positive samples revealed all of the SN-positive samples were found within the SM-positive sample subgroups among both of the non-Orthodontic groups (Adult and Pediatric), which seems to confirm the co-occurrence and positive correlations observed in previous studies [20-22]. However, although most of the SN-positive samples among Orthodontic samples were also SM-positive - a small proportion of the SN-positive samples were found among SM-negative samples, which was observed in both Adults and Pediatric patients. These data may suggest that changes to microbial constituents or biofilm composition may be directly or indirectly linked with orthodontic therapy and more research will be needed to understand the potential implications of these observations and findings.

Although this study provides novel evidence regarding the differential co-occurrence of these microbial constituents between Orthodontic and non-Orthodontic patient samples, it is important to understand the limitations particular to this study design. For example, the cross-sectional (one-time sampling) nature of the original collection protocol does not allow for any temporal analysis of the associations or correlations between microbial prevalence and these population subgroups. In addition, the

retrospective nature of this study also prevented the inclusion of any clinical information regarding the overall oral status of these patients for analysis. For example, no information regarding patient oral hygiene status, the presence of gingivitis or periodontitis, smoking status or other confounding variables was collected in the previous study protocols. Based upon the lack of this clinical information, future prospective studies might collect decayed, missing, and filled teeth (DMFT) scores for caries risk assessment or periodontal pocket depth (PPD) for periodontal risk assessment, as well as other relevant clinical assessments, which might greatly enhance the clinical relevance of similar studies regarding microbial prevalence. Finally, longitudinal assessments (pre-, during, post-treatment) should be conducted to determine if Orthodontic treatment is sufficient to change the microbial prevalence as reported in this study.

5. CONCLUSIONS

This study is among the first to evaluate SN and SM co-occurrence among Pediatric and Adult Orthodontic and non-Orthodontic patient samples. The increased prevalence of both SN and SM among the Pediatric patients, and the Pediatric Orthodontic population more specifically, may suggest further research is needed to more fully understand the oral health risks facing these specific patients. The differential results in co-occurrence only observed among the Orthodontic patients may also suggest orthodontic therapy may be sufficient to alter oral behaviors or the oral habitat thereby altering the oral microbial constituents and possibly changing oral health and the risk for developing oral disease.

CONSENT

Informed Consent was obtained for all patients over the age of 18 years who agreed to voluntary participation and Pediatric Assent was obtained from all patients under the age of 18 years with the addition of Informed Consent from their parent or legal guardian.

ETHICAL APPROVAL

The original protocol for the collection of these samples was reviewed and approved by the Office for the Protection of Human Subjects (OPRS) and Institutional Review Board (IRB) at the University of Nevada, Las Vegas (UNLV) under OPRS#1305-4466M “The Prevalence of Oral Microbes in Saliva from the UNLV School of Dental Medicine Pediatric and Adult Clinical Population” in May 2013. The protocol for the current study was reviewed and approved as Exempt under protocol #1502-506M.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tanner A, Bouldin HD, Maiden MF. Newly delineated periodontal pathogens with special reference to selenomonas species. Infection. 1989;17(3):182-7.
   DOI: 10.1007/BF01644027
   PMID: 2661440.
2. Kolenbrander PE, Andersen RN, Moore LV. Coaggregation of *Fusobacterium nucleatum*, *Selenomonas flueggei, Selenomonas infelix, Selenomonas noxia,* and Selenomonas sputigena with strains from 11 genera of oral bacteria. Infect Immun. 1989;57(10):3194-203.
   DOI: 10.1128/IAI.57.10.3194-3203.1989
   PMID: 2777378; PMCID: PMC260789.
3. Maiden MF, Tanner A, Moore WE. Identification of *Selenomonas* species by whole-genomic DNA probes, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, biochemical tests and cellular fatty acid analysis. Oral Microbiol Immunol. 1992;7(1):7-13.
   DOI: 10.1111/j.1399-302x.1992.tb00012.x
   PMID: 1528628.
4. Tanner A, Kent R, Maiden MF, Taubman MA. Clinical, microbiological and immunological profile of healthy, gingivitis and putative active periodontal subjects. J Periodontal Res. 1996;31(3):195-204.
   DOI: 10.1111/j.1600-0765.1996.tb00484.x
   PMID: 8814590.

5. Tanner A, Maiden MF, Macuch PJ, Murray LL, Kent RL Jr. Microbiota of health, gingivitis, and initial periodontitis. J Clin Periodontol. 1998;25(2):85-98.
DOI: 10.1111/j.1600-051x.1998.tb02414.x
PMID: 9495607.

6. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25(2):134-44.
DOI: 10.1111/j.1600-051x.1998.tb02419.x
PMID: 9495612.

7. Haffajee AD, Cugini MA, Tanner A, Pollack RP, Smith C, Kent RL Jr, Socransky SS. *Subgingival microbiota* in healthy, well-maintained elder and periodontitis subjects. J Clin Periodontol. 1998;25(5):346-53.
DOI: 10.1111/j.1600-051x.1998.tb02454.x
PMID: 9650869.

8. Boström L, Bergström J, Dahlén G, Linder LE. Smoking and *Subgingival microflora* in periodontal disease. J Clin Periodontol. 2001;28(3):212-9.
DOI: 10.1034/j.1600-051x.2001.028003212.x
PMID: 11284533.

9. Craig RG, Boylan R, Yip J, Bamgboye P, Koutsoukos J, Mijares D, et al. Prevalence and risk indicators for destructive periodontal diseases in 3 urban American minority populations. J Clin Periodontol. 2001;28(6):524-35.
DOI: 10.1034/j.1600-051x.2001.028006524.x
PMID: 11350519.

10. Torresyap G, Haffajee AD, Uzel NG, Socransky SS. Relationship between periodontal pocket sulfide levels and subgingival species. J Clin Periodontol. 2003;30(11):1003-10.
DOI: 10.1034/j.1600-051x.2003.00377.x
PMID: 14761124.

11. Natto S, Baljoon M, Dahlén G, Bergström J. Tobacco smoking and periodontal microflora in a Saudi Arabian population. J Clin Periodontol. 2005;32(6): 549-55.
DOI: 10.1111/j.1600-051X.2005.00710.x
PMID: 15882210.

12. Anhoury P, Nathanson D, Hughes CV, Socransky S, Feres M, Chou LL. Microbial profile on metallic and ceramic bracket materials. Angle Orthod. 2002;72(4):338-43.
DOI: 10.1043/0003-3219(2002)072<0338:MPOMAC>2.0.CO;2
PMID: 12169034.

13. Buduneli N, Baylas H, Buduneli E, Türkoğlu O, Köse T, Dahlen G. Periodontal infections and pre-term low birth weight: A case-control study. J Clin Periodontol. 2005;32(2):174-81.
DOI: 10.1111/j.1600-051X.2005.00670.x
PMID: 15691348.

14. Adriaens LM, Alessandri R, Spörri S, Lang NP, Persson GR. Does pregnancy have an impact on the subgingival microbiota? J Periodontol. 2009;80(1):72-81.
DOI: 10.1902/jop.2009.080012
PMID: 19228092.

15. Bieri RA, Adriaens L, Spörri S, Lang NP, Persson GR. Gingival fluid cytokine expression and subgingival bacterial counts during pregnancy and postpartum: a case series. Clin Oral Investig. 2013;17(1):19-28.
DOI: 10.1007/s00784-012-0674-8
Epub 2012 Jan 17.
PMID: 22249562.

16. Goodson JM, Groppo D, Halem S, Carpino E. Is obesity an oral bacterial disease? J Dent Res. 2009;88(6):519-23.
DOI: 10.1177/0022034509338353
PMID: 19587155;
PMCID: PMC2744897.

17. Kamma JJ, Diamanti-Kipioti A, Nakou M, Mitsis FJ. Profile of subgingival microbiota in children with mixed dentition. Oral Microbiol Immunol. 2000;15(2):103-11.
DOI: 10.1034/j.1399-302x.2000.150206.x
PMID: 11155173.

18. Mager DL, Ximenez-Fyvie LA, Haffajee AD, Socransky SS. Distribution of selected bacterial species on intraoral surfaces. J Clin Periodontol. 2003;30(7):644-54.
DOI: 10.1034/j.1600-051x.2003.00376.x
PMID: 12834503.

19. Teles FR, Teles RP, Uzel NG, Song XQ, Torresyap G, Socransky SS, Haffajee AD. Early microbial succession in redeveloping dental biofilms in periodontal health and disease. J Periodontal Res. 2012;47(1):95-104.
DOI: 10.1111/j.1600-0765.2011.01409.x
Epub 2011 Sep 5.

PMID: 21895662;
PMCID: PMC3253172.

20. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25(2):134-44.
DOI: 10.1111/j.1600-051x.1998.tb02419.x
PMID: 9495612.
21. Teles FR, Teles RP, Sachdeo A, Uzel NG, Song XQ, Torresyap G, et al. Comparison of microbial changes in early redeveloping biofilms on natural teeth and dentures. J Periodontol. 2012;83(9):1139-48.
DOI: 10.1902/jop.2012.110506.
Epub 2012 Mar 23.
PMID: 22443543;
PMCID: PMC4041159.
22. Williams RJ, Howe A, Hofmockel KS. Demonstrating microbial co-occurrence pattern analyses within and between ecosystems. Front Microbiol. 2014;5:358.
DOI: 10.3389/fmicb.2014.00358
PMID: 25101065;
PMCID: PMC4102878.
23. Davidowitz R, Howard KM, Kingsley K. Salivary screening for *Selenomonas noxia* in the oral cavity of pediatric patients. Current Dental Research Journal. 2019;1(1).
24. Bui Q, Nguyen C, McDaniel J, McDaniel S, Kingsley K, Howard KM. *Selenomonas noxia* screening among pediatric patient samples: A pilot study. J Oral Heal Dent Care. Open Access. 2017;1:1009.
25. Nimrat Dhillon, Karl Kingsley, Katherine M. Howard. Prevalence of *Selenomonas noxia* among Pediatric and Adult Orthodontic Patients. International Journal of Research and Reports in Dentistry. 2019;2(1):1-7.
26. McDaniel S, McDaniel J, Tam A, Kingsley K. Howard KM. Oral microbial ecology of *Selenemonas noxia* and *Scardovia wiggsiae*. Microbiology Research Journal International. 2017;21(3):1-8.
DOI : 10.9734/MRJI/2017/36110
27. Anhoury P, Nathanson D, Hughes CV, Socransky S, Feres M, Chou LL. Microbial profile on metallic and ceramic bracket materials. Angle Orthod. 2002;72(4):338-43.
DOI:10.1043/0003-3219(2002)072<0338:MPOMAC>2.0.CO;2
PMID: 12169034.
28. Emett J, David R, McDaniel J, McDaniel S, Kingsley K. Comparison of DNA extracted from pediatric saliva, gingival crevicular fluid and site-specific biofilm samples. Methods Protoc. 20209;3(3):48.
DOI: 10.3390/mps3030048
PMID: 32660039;
PMCID: PMC7565886.
29. Tiku V, Todd CJ, Kingsley K. Assessment of oral human papillomavirus prevalence in a multi-ethnic pediatric clinic population. Compend Contin Educ Dent. 2016; 37(10):e1-e4.
PMID: 27875050.
30. Row L, Repp MR, Kingsley K. Screening of a pediatric and adult clinic population for caries pathogen *Scardovia wiggsiae.* J Clin Pediatr Dent. 2016;40(6):438-444.
DOI: 10.17796/1053-4628-40.6.438
PMID: 27805882.
31. Dahlen G, Fejerskov O, Manji F. Current concepts and an alternative perspective on periodontal disease. BMC Oral Health. 2020;20(1):235.
DOI: 10.1186/s12903-020-01221-4
PMID: 32847557;
PMCID: PMC7448340.
32. Borrell LN, Crawford ND. Socioeconomic position indicators and periodontitis: examining the evidence. Periodontol. 2000. 2012;58(1):69-83.
DOI: 10.1111/j.1600-0757.2011.00416.x
PMID: 22133367;
PMCID: PMC3233193.
33. Lamont RJ, Koo H, Hajishengallis G. The oral microbiota: Dynamic communities and host interactions. Nat Rev Microbiol. 2018;16(12):745-759.
DOI: 10.1038/s41579-018-0089-x
PMID: 30301974;
PMCID: PMC6278837.
34. Sun L, Wong HM, McGrath CPJ. The factors that influence oral health-related quality of life in 15-year-old children. Health Qual Life Outcomes. 2018;16(1):19.
DOI: 10.1186/s12955-018-0847-5
PMID: 29347943;
PMCID: PMC5774101.
35. Li LW, Wong HM, Sun L, Wen YF, McGrath CP. Anthropometric measurements and periodontal diseases in children and adolescents: A systematic

review and meta-analysis. Adv Nutr. 2015; 6(6):828-41.
DOI: 10.3945/an.115.010017
PMID: 26567204;
PMCID: PMC4642430.

36. Tsang O, Major K, Santoyo S, Kingsley K, Nguyen L. qPCR Screening of pediatric saliva samples to evaluate effects of dental sealants on cariogenic bacteria *Streptococcus mutans* and *Scardovia wiggsiae*. Enviro Dental Journal. 2021;1(2).

37. Carr G, Alexander A, Nguyen L, Kingsley K. Oral site specific sampling reveals differential location for *Scardovia wiggsiae*. Microbiology Research Journal International. 2020;30(1):47-55. Available:https://doi.org/10.9734/mrji/2020/v30i130189

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