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Scardovia Wiggsiae and Streptococcus Sobrinus Prevalence Among Orthodontic and Non-Orthodontic Patients

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SCARDOVIA WIGGSIAE AND STREPTOCOCCUS SOBRINUS PREVALENCE AMONG ORTHODONTIC AND NON-ORTHODONTIC PATIENTS

By

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Abstract

SCARDOVIA WIGGSIAE AND STREPTOCOCCUS SOBRINUS PREVALENCE AMONG ORTHODONTIC AND NON-ORTHODONTIC PATIENTS

By

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Background: Dental cavities or caries have been identified as among the most prevalent of preventable oral conditions. However, studies are discovering new information regarding the incidence and prevalence of several cariogenic organisms, including *Streptococcus mutans (SM),* the recently discovered *Scardovia wiggsiae (SW),* as well as *Streptococcus sobrinus (SS).* These studies have revealed varying prevalence among different populations, such as those undergoing orthodontic treatment. Based upon this information, the main goal of the current study was to assess the prevalence of specific cariogenic organisms *(SS and SW)* within saliva samples originally obtained from a dental school-based clinic.

Methods: The protocol for this retrospective study of DNA isolated from previously collected saliva samples was reviewed and approved by the Institutional Review Board (IRB) as exempt research. In brief, clinical DNA samples were screened for *SS* and *SW* using quantitative

polymerase chain reaction (qPCR). Demographic and subgroup (Orthodontic, non-Orthodontic) analysis was also performed.

Results: This study found that pediatric (12-17 year old patient) samples were much more likely to harbor either *SW* or *SS* compared with adult (>18 year old patient) samples. In addition, this study found many more *SW*-positive samples among pediatric orthodontic patients compared with either adult or pediatric non-Orthodontic patients, which may suggest this population may be at higher risk for *SW*-related caries or other negative oral health outcomes. Finally, this study found these microbial populations to be strongly linked within the same patient samples.

Conclusions: This study has demonstrated that prevalence of *SW* and *SS* may be more highly associated with specific population subgroups, including *SS* observed in non-orthodontic patients and *SW* found among pediatric orthodontic patients. These results also differ from previous evidence, which found only minor and partially overlapping prevalence of these and other oral microbes. The results of this current study may suggest that *SS* and *SW* may be more strongly correlated within similar oral microbial communities and their presence may be directly or indirectly linked through one or more behavioral, microbial or other factors – although more research will be needed to determine these mechanisms.

Key words: *Streptococcus sobrinus (SS),* Scardovia wiggsiae (*SW*), saliva, prevalence

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Chapter 1

Background and Significance

Nearly four billion people are affected by oral conditions, including oral caries – which is among the most prevalent of all childhood diseases [1,2]. Although epidemiologic studies across many countries vary widely, most estimates suggest prevalence of dental caries among primary teeth in children at nearly 50% [3,4]. In addition, new evidence suggests that these estimates mirror those of permanent teeth, with many studies suggesting that prevalence may, in fact, exceed 55% [5-7].

More specifically, dental caries is the most prevalent, noncommunicable, preventable disease – although much remains to be discovered about the prevalence of the most important cariogenic organisms, including *Streptococcus mutans (SM)* and *Streptococcus sobrinus (SS)* among different populations, such as those in orthodontic treatment [8-10]. For example, many studies have begun to evaluate the role of fixed orthodontic appliances with changes to the oral ecosystem and cariogenic risk including these organisms [11,12]. In addition, some evidence has even evaluated these changes in cariogenic risk and microbial prevalence associated with lingual versus buccal orthodontics and even thermoplastic aligners versus fixed appliances [13,14].

Despite these advances in oral and orthodontic research, recent evidence has revealed another cariogenic pathogen *Scardovia wiggsiae (SW)* in the oral flora of dental patients with or without the presence of SM [15,16]. This organism has been confirmed to be aciduric and predictive of caries development in the presence or absence of other acid-producing microbes, such as *SM* or *Lactobacillus* species [17,18]. In addition, some evidence has suggested that *SW* may also play a

much more significant role in the development of caries lesions among orthodontic patients - although much remains to be discovered [16,19,20].

To further this area of research, some studies from this group have evaluated the presence of *SW* among pediatric and adult patients [21,22]. Further research has attempted to determine prevalence among orthodontic and non-orthodontic patients, including pediatric and adult populations [23-25]. In addition, a few of these studies have now attempted to survey the microbial ecology to determine the additional microbial constituents that may be important to the development of *SW* prevalence, such as *SM* [26-29].

However, few studies to date have examined the corresponding prevalence of both *SW* and *SS* within the same patient samples - and none among orthodontic patients [30,31]. Based upon the limited amount of information regarding *SW* prevalence and the potential association with *SS*, the main objective of this study is to evaluate the presence of these cariogenic organisms within clinical saliva samples obtained from a dental school-based setting.

Research Question

1. What is the prevalence of *S. wiggsiae* among the pediatric and adult orthodontic and non-orthodontic patient populations at UNLV-SDM?

    a. Null hypothesis (H_0): Prevalence of *S. wiggsiae* is the same among these populations

    b. Alternative hypothesis (H_A): Prevalence of *S. wiggsiae* is different among these populations

2. What is the prevalence of *S. sobrinus* among the pediatric and adult orthodontic and non-orthodontic patient populations at UNLV-SDM?

    a. Null hypothesis (H_0): Prevalence of *S. sobrinus* is the same among these populations

b. Alternative hypothesis (H_A): Prevalence of *S. sobrinus* is different among these populations

Approval

This retrospective study of previously collected saliva samples was reviewed and approved as Exempt by the Institutional Review Board (IRB) and Office for the Protection of Research Subjects (OPRS) at the University of Nevada, Las Vegas (UNLV). The original protocol for the collection of these samples and creation of the saliva repository was reviewed and approved under protocol OPRS#1305-4466M, which was titled "The Prevalence of Oral Microbes in Saliva from the UNLV School of Dental Medicine Pediatric and Adult Clinical Population".

Under the original study protocols, patients 18 years of age and older (Adults) who agreed to participate provided written Informed Consent. Patients under the age of 18 (12 – 17 years of age in this study) who agreed to participate provided written Pediatric Assent - with the additional requirement of an adult parent or guardian providing written Informed Consent. Participation was voluntary and no remuneration was given or offered to any parent, patient or guardian. 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 asked to provide an unstimulated saliva sample of up to 5 mL in a sterile collection tube at the time of their regularly scheduled clinical visit and each tube was marked with a randomly generated, non-duplicated number to prevent the identification of any patient- or medical record-specific information from entering the salivary repository. Only basic demographic information, such as the age, sex and race or ethnicity of the participant was noted.

Research Design

Screening will be facilitated by using DNA extracted from a pre-existing patient saliva repository and processed using qPCR to identify *SW* and *SS* in the samples.

Scardovia wiggsiae

SW forward primer, GTGGACTTTATGAATAAGC

SW reverse primer, CTACCGTTAAGCAGTAAG

Streptococcus sobrinus

SS forward primer, TTCAAAGCCANGACCAAGCTAGT

SS reverse primer, CCAGCCTGAGATTCAGCTTGT

Independent (predictor) variables include:

- No brackets versus brackets (Orthodontic, versus non-Orthodontic)
- Age
- Sex

- Dependent (outcome) variable
  - *SW*- or *SS*-positive qPCR screening result

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Chapter 2

Scardovia wiggsiae and Streptococcus sobrinus prevalence among orthodontic and non-orthodontic patients

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Role of Authors:
KK and KMH were responsible for the overall project design. BJS, MM, PP, KF, MT, and NK were responsible for data generation and analysis. KK and MT contributed to the writing and editing of this manuscript. All authors have read and agreed to the published version of the manuscript.

Abstract

Background: Dental cavities or caries have been identified as among the most prevalent of preventable oral conditions. However, studies are discovering new information regarding the incidence and prevalence of several cariogenic organisms, including *Streptococcus mutans (SM),* the recently discovered *Scardovia wiggsiae (SW),* as well as *Streptococcus sobrinus (SS).* These studies have revealed varying prevalence among different populations, such as those undergoing orthodontic treatment. Based upon this information, the main goal of the current study was to assess the prevalence of specific cariogenic organisms (*SS* and *SW*) within saliva samples originally obtained from a dental school-based clinic.

Methods: The protocol for this retrospective study of DNA isolated from previously collected saliva samples was reviewed and approved by the Institutional Review Board (IRB) as exempt research. In brief, clinical DNA samples were screened for *SS* and *SW* using quantitative

polymerase chain reaction (qPCR). Demographic and subgroup (Orthodontic, non-Orthodontic) analysis was also performed.

Results: This study found that pediatric (12-17 year old patient) samples were much more likely to harbor either *SW* or *SS* compared with adult (>18 year old patient) samples. In addition, this study found many more *SW*-positive samples among pediatric orthodontic patients compared with either adult or pediatric non-Orthodontic patients, which may suggest this population may be at higher risk for *SW*-related caries or other negative oral health outcomes. Finally, this study found these microbial populations to be strongly linked within the same patient samples.

Conclusions: This study has demonstrated that prevalence of *SW* and *SS* may be more highly associated with specific population subgroups, including *SS* observed in non-orthodontic patients and *SW* found among pediatric orthodontic patients. These results also differ from previous evidence, which found only minor and partially overlapping prevalence of these and other oral microbes. The results of this current study may suggest that *SS* and *SW* may be more strongly correlated within similar oral microbial communities and their presence may be directly or indirectly linked through one or more behavioral, microbial or other factors – although more research will be needed to determine these mechanisms.

Key words: *Streptococcus sobrinus (SS), Scardovia wiggsiae (SW),* saliva, prevalence

Introduction

Nearly four billion people are affected by oral conditions, including oral caries – which is among the most prevalent of all childhood diseases [1,2]. Although epidemiologic studies across many countries vary widely, most estimates suggest prevalence of dental caries among primary teeth in children at nearly 50% [3,4]. In addition, new evidence suggests that these estimates mirror those of permanent teeth, with many studies suggesting that prevalence may, in fact, exceed 55% [5-7].

More specifically, dental caries is the most prevalent, noncommunicable, preventable disease – although much remains to be discovered about the prevalence of the most important cariogenic organisms, including *Streptococcus mutans (SM*) and *Streptococcus sobrinus (SS)* among different populations, such as those in orthodontic treatment [8-10]. For example, many studies have begun to evaluate the role of fixed orthodontic appliances with changes to the oral ecosystem and cariogenic risk including these organisms [11,12]. In addition, some evidence has even evaluated these changes in cariogenic risk and microbial prevalence associated with lingual versus buccal orthodontics and even thermoplastic aligners versus fixed appliances [13,14].

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To further this area of research, some studies from this group have evaluated the presence of *SW* among pediatric and adult patients [21,22]. Further research has attempted to determine prevalence among orthodontic and non-orthodontic patients, including pediatric and adult populations [23-25]. In addition, a few of these studies have now attempted to survey the microbial ecology to determine the additional microbial constituents that may be important to the development of *SW* prevalence, such as *SM* [26-29].

However, few studies to date have examined the corresponding prevalence of both *SW* and *SS* within the same patient samples - and none among orthodontic patients [30,31]. Based upon the limited amount of information regarding *SW* prevalence and the potential association with *SS*, the main objective of this study is to evaluate the presence of these cariogenic organisms within clinical saliva samples obtained from a dental school-based setting.

Methodology

Human subject approval

This retrospective study of previously collected saliva samples was reviewed and approved as Exempt by the Institutional Review Board (IRB) and Office for the Protection of Research Subjects (OPRS) at the University of Nevada, Las Vegas (UNLV). The original protocol for the collection of these samples and creation of the saliva repository was reviewed and approved under protocol OPRS#1305-4466M, which was titled "The Prevalence of Oral Microbes in Saliva from the UNLV School of Dental Medicine Pediatric and Adult Clinical Population".

Under the original study protocols, patients 18 years of age and older (Adults) who agreed to participate provided written Informed Consent. Patients under the age of 18 (12 – 17 years of age

in this study) who agreed to participate provided written Pediatric Assent - with the additional requirement of an adult parent or guardian providing written Informed Consent. Participation was voluntary and no remuneration was given or offered to any parent, patient or guardian. 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 asked to provide an unstimulated saliva sample of up to 5 mL in a sterile collection tube at the time of their regularly scheduled clinical visit and each tube was marked with a randomly generated, non-duplicated number to prevent the identification of any patient- or medical record-specific information from entering the salivary repository. Only basic demographic information, such as the age, sex and race or ethnicity of the participant was noted.

DNA isolation

Samples were previously transferred to a biomedical laboratory for storage at -80C and subsequent processing. In brief, each sample was thawed and DNA was immediately isolated from each sample using the phenol:chloroform extraction method. Quantitative assessment of DNA was determined using a spectrophotometer measuring absorbance at 260 and 280 nm. The ratio of A260:A280 is used to determine DNA quality, with minimum qPCR screening quality at or above a ratio of 1.65. DNA quantification was performed using absorbance at A260, calculated using an average extinction coefficient of 0.020 for double-stranded DNA (ug/mL)/cm [32].

qPCR screening

Screening for *SW* and *SS* was performed in duplicate using reactions containing 15 uL Fast SYBR green, fluorescent master mix, 10 uL nuclease-free distilled water, 2.0 uL of sample DNA diluted to a standard concentration of 10 ug/mL and 1.5 uL of forward and reverse primers specific for each respective organism. Quantification of qPCR results was performed using the ddCT method with 16S rRNA as the reference standard for positive control reactions. Reaction parameters included incubation at 50C for two minutes, denaturation at 95C for ten minutes and 40 cycles of denaturation for 15 seconds at 95 with annealing at the designated temperatures indicated (nt=nucleotide; melting temperature=Tm) for each primer set:

Positive control, bacterial 16S rRNA

Forward 16S rRNA universal primer, 5′-ACG CGT CGA CAG AGT TTG ATC CTG GCT-3′, Tm=76C

Reverse 16S rRNA universal primer, 5′-GGG ACT ACC AGG GTA TCT AAT-3′, Tm=62C

Annealing temperature=lower Tm (62C) - 2C= 60C.

Scardovia wiggsiae (SW)

Forward primer, 5’-GTG GAC TTT ATG AAT AAG C-3’, Tm=55C

Reverse primer, 5’-CTA CCG TTA AGC AGT AAG-3’, Tm=56C

Annealing temperature=lower Tm (55C) - 2C= 53C.

Streptococcus sobrinus (SS)

Forward primer, 5’-GAT GAT TTG GCT CAG GAT CAA TCC TC-3’, Tm=67C

Reverse primer, 5'-ACT GAG CCA GTA GTA GAC TTG GCA ACT-3', Tm=71C

Annealing temperature=lower Tm (67C) - 2C= 65C.

Statistical analysis

Demographic characteristics (age, sex, race/ethnicity) are reported using simple descriptive statistics and comparisons between categorical variables were analyzed using Chi square analysis, which is appropriate for non-parametric data. Quantitative data (including DNA concentrations) are represented using descriptive statistics and comparisons between continuous variables were analyzed using Student's t-tests, which are appropriate for parametric data.

Results

Screening of the overall repository consisting of N=1,176 existing samples revealed many samples that met either the minimum criteria for DNA concentration or DNA purity (Table 1). In brief, a total of n=317 or 26.9% met the minimum concentration requirements for qPCR screening and analysis. Analysis of absorbances revealed a total of n=276 or 23.5% met the minimum purity requirement for qPCR screening and analysis. Reconciliation of these samples revealed a final sample size of n=187 or 15.9% that met both the DNA concentration and DNA purity standards for inclusion in this study.

Table 1. Analysis of DNA samples.

	Samples meeting minimum DNA concentration >[5 ng]	Samples meeting minimum DNA purity A260:A280: >1.65	Samples meeting DNA concentration and purity Combined total
Sample size	N=317 317/1176 (26.9%)	N=276 276/1176 (23.5%)	N=187 187/1176 (15.9%)
Average [DNA]	461.58 ng/uL STD=54.19	337.1 ng/uL STD=41.52	315.47 ng/uL STD=82.25
Average A260:A280	2.02 STD=0.36	1.89 STD=0.14	1.81 STD=0.075

Of the samples that met the minimum criteria for qPCR quality or quantity, demographic analysis of these samples revealed that nearly equal numbers of samples from females and males were present, which closely matched the percentages from the overall clinic population, P=0.4229 (Table 2). However, analysis of the race and ethnicity of the study sample revealed significantly higher percentages of non-minority (White) patients among the study samples (40.6%) than the overall clinical population (24.7%), which was statistically significant, P=0.0002.

In addition, slightly less than half of the samples identified in this study were from orthodontic patients versus non-orthodontic patients – which closely approximated the overall objectives of the study, P=0.4237. More in depth analysis of the patient samples revealed less than half of these patients were derived from adults (42.8%), which also approximates the distribution of patients among the Orthodontic clinic (34.7%), P=0.0013.

Table 2. Study sample demographics.

	Study Sample (n=187)	UNLV-SDM Patient Clinic Population	Statistical analysis
Females	n=92/187 (49.2%)	52.8%	$\chi 2$=0.642, d.f.=1
Males	n=95/187 (50.8%)	47.2%	P=0.4229
White (non-minority)	n=76/187 (40.6%)	24.7%	$\chi 2$=15.655, d.f.=4
Minority	n=111/187 (59.4%)	75.3%	P=0.0013
Hispanic	n=63/187 (33.7%)	52.1%	
Black	n=26/187 (13.9%)	11.8%	
Asian/Other	n=22/187 (11.8%)	11.4%	
Orthodontic	n=86/187 (45.9%)		$\chi 2$=0.640, d.f.=1
Non-Orthodontic	n=101/187 (54.1%)		P=0.4237
Adult (>18 years)	n=80/187 (42.8%)	34.7% (Orthodontic)	$\chi 2$=2.813, d.f.=1
Pediatric (12-17 yrs)	n=107/187 (57.2%)	65.3% (Orthodontic)	P=0.0935

Each sample was then screened in duplicate using qPCR primers specific for *SW* (Fig. 1). These data revealed that none of the Adult Orthodontic samples (0%) and few of the Adult non-Orthodontic samples (6.7%) harbored *SW*. However, this organism was significantly more prevalent among Pediatric non-orthodontic samples (17.2%) and among the Pediatric Orthodontic samples (26.5%), P=0.0001.

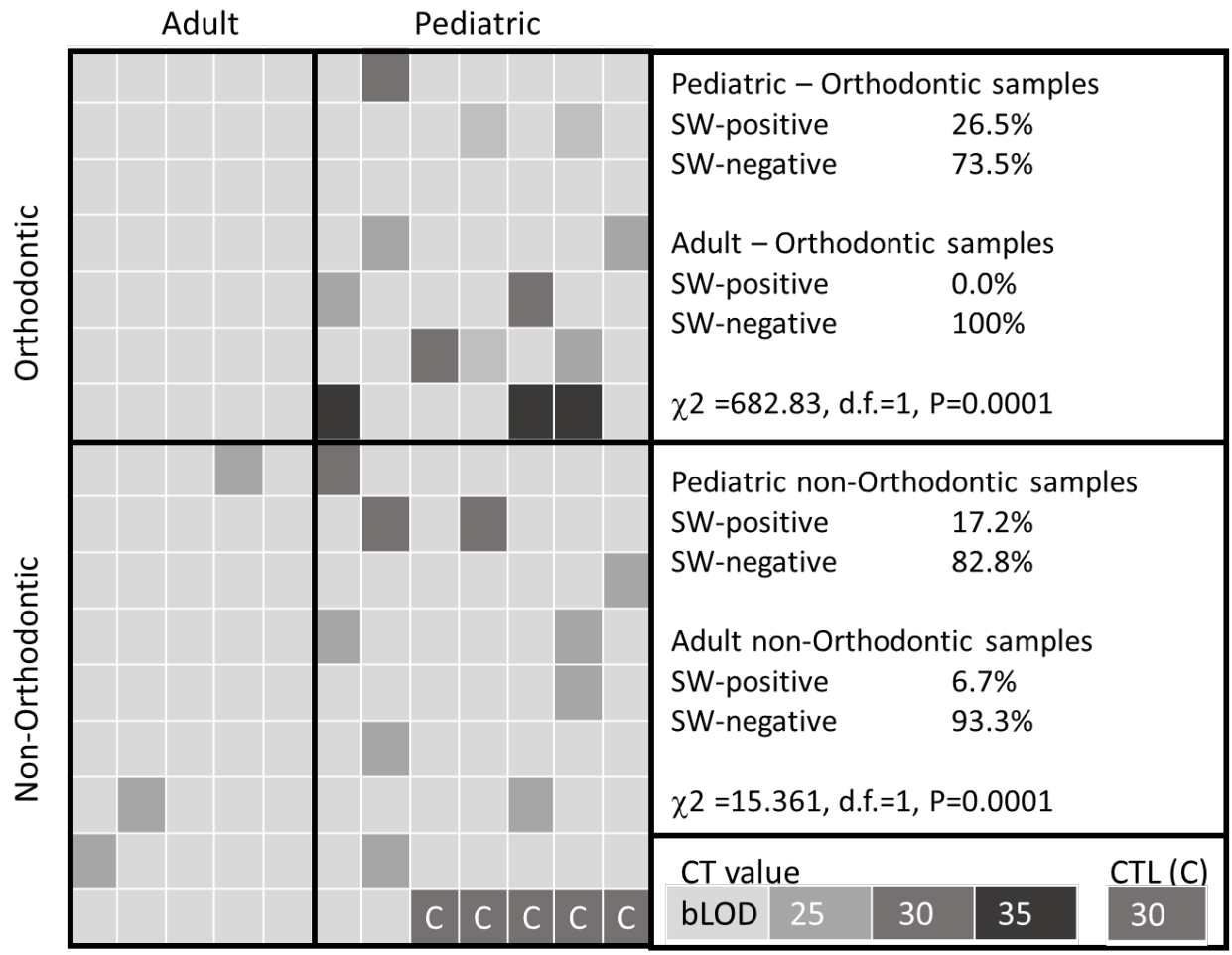

Figure 1. qPCR screening and heat map for *Scardovia wiggsiae (SW).* Screening for *SW* DNA revealed the majority of samples were below the limit of detection (bLOD). Cycle threshold (CT) data revealed the highest *SW* prevalence among Pediatric Orthodontic samples (26.5%), fewer among Pediatric non-Orthodontic samples (17.2%) and Adult non-Orthodontic samples (6.7%) and none among Adult Orthodontic samples.

More detailed analysis of the *SW*-positive samples revealed cycle threshold (CT) counts, where the fluorescence of the qPCR product can be detected above background levels, that ranged from 20.4 to 38.6 (Fig. 2). Less than half (44.4%) exhibited CT counts below cycle 30, while the

majority of *SW*-positive samples exhibited CT counts above 30 (55.6%), ranging from 30.9 to 38.6 (CT ave.=30.76) (Fig. 2A). To further evaluate and quantify these results, relative quantification (RQ) was evaluated compared to prepared standards of salivary samples from *SW*-positive patients diluted to 10 ng/uL (CT ave. 31.9) (Fig. 2B). These data revealed RQ for the *SW*-positive samples ranged between 0.63 and 1.2 (RQ ave. =0.97).

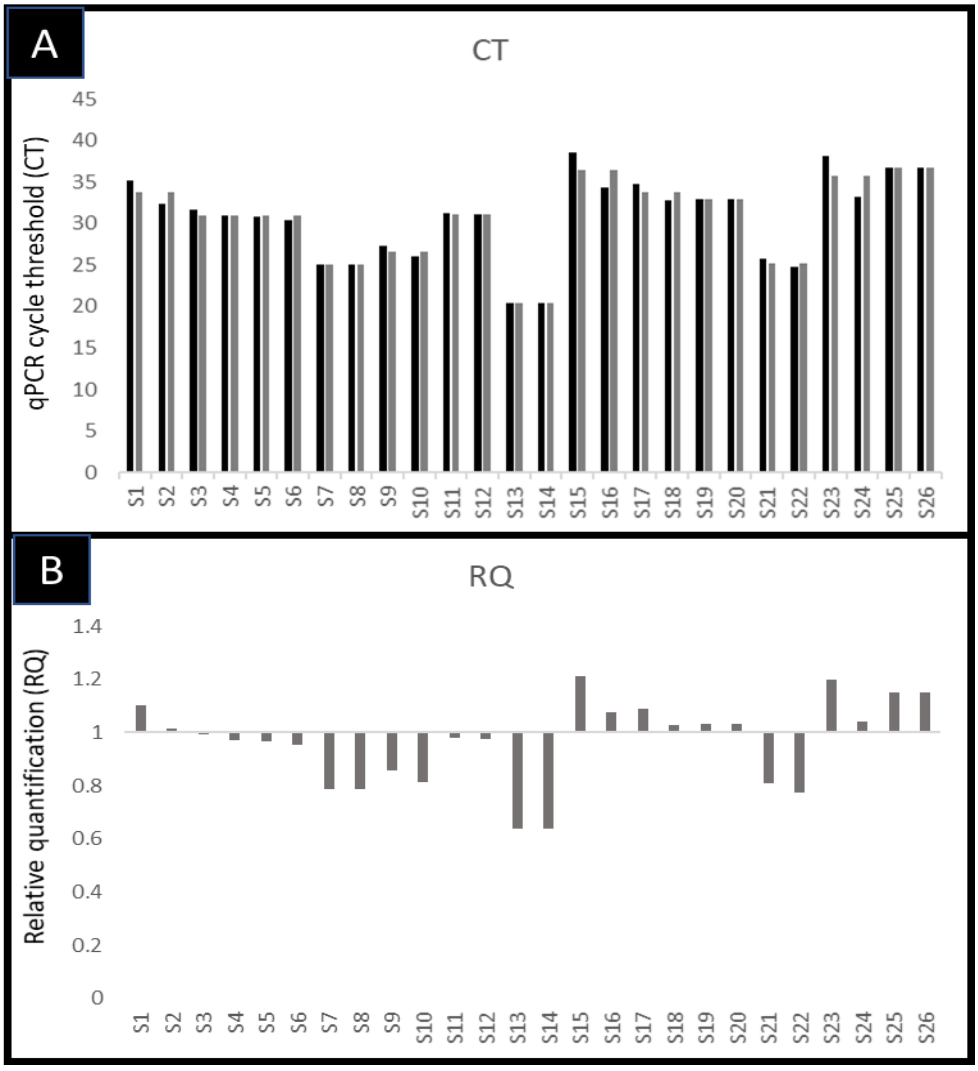

Figure 2. Cycle threshold (CT) and relative quantification (RQ) of *SW* qPCR data. A) Analysis of *SW*-positive samples revealed CT ranging between 20.4 and 38.6 (CT ave.=30.76), which was not

significantly different from the *SW*-positive controls (CT ave.=31.9), P=0.881. B) Comparison of these data with *SW*-positive controls revealed RQ between 0.63 and 1.2 (RQ ave.=0.97), which was not significantly different (P=0.781).

Each sample was then screened in duplicate using qPCR primers specific for *SS* (Fig. 3). These data revealed that none of the Adult Orthodontic samples (0%) harbored *SS*, however a higher percentage of the Adult non-Orthodontic samples (15.6%) did, P-0.0001. In addition, this organism was found among Pediatric Orthodontic samples (4.1%), but in significantly higher percentages among the Pediatric non-Orthodontic samples (32.7%), P=0.0026.

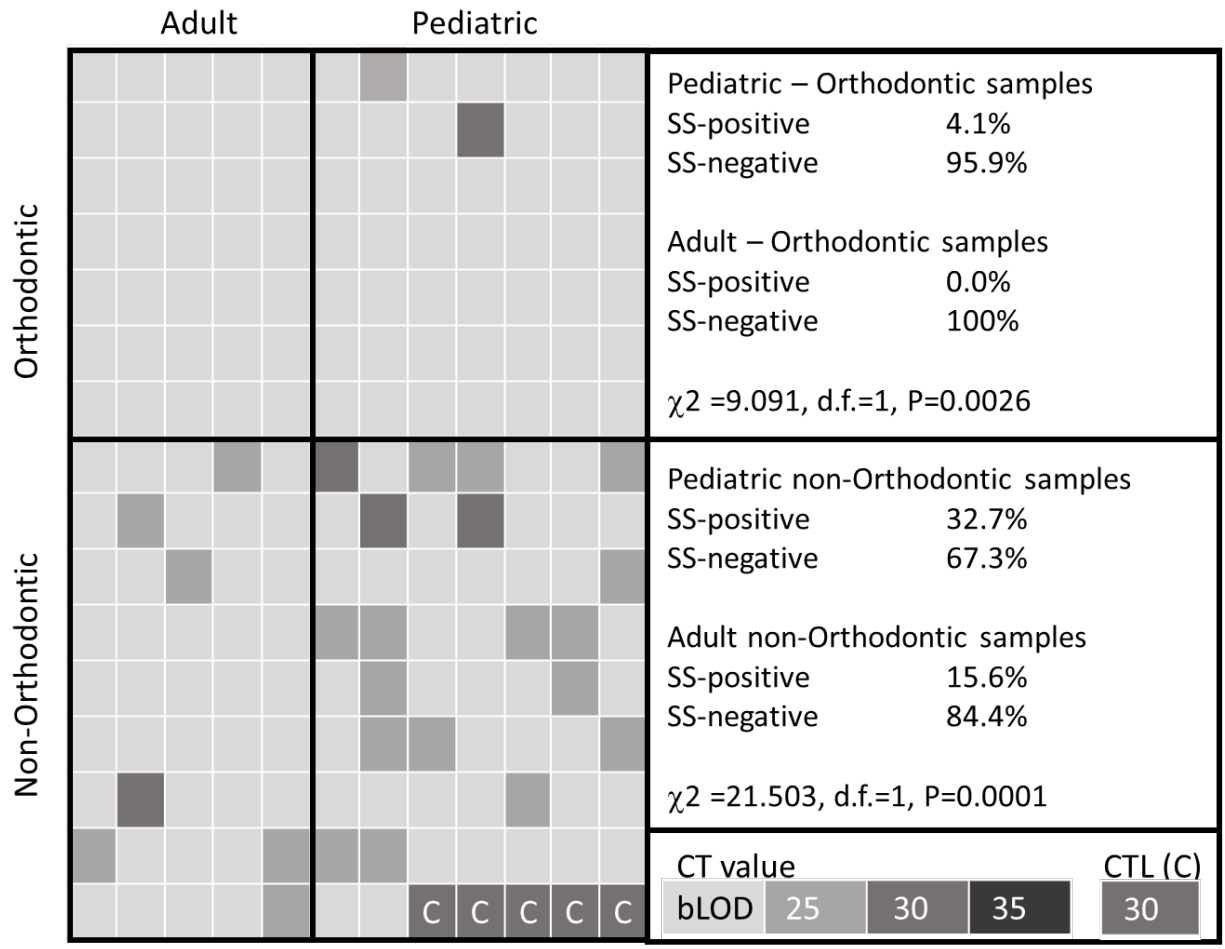

Figure 3. qPCR screening and heat map for *Streptococcus sobrinus (SS*). Screening for *SS* DNA revealed the majority of samples were below the limit of detection (bLOD). Cycle threshold (CT) data revealed the highest *SS* prevalence among Pediatric non-Orthodontic samples (32.7%), fewer among Adult non-Orthodontic samples (15.6%) and Pediatric Orthodontic samples (4.1%) and none among Adult Orthodontic samples.

More detailed analysis of the *SS*-positive samples revealed cycle threshold (CT) counts that ranged from 25.1 to 35.2 (Fig. 4). More than two-thirds (67.7%) exhibited CT counts above cycle 30, while a smaller percentage of *SS*-positive samples exhibited CT counts below 30 (32.3%), (CT

ave.=31.13) (Fig. 4A). To more quantify these results, relative quantification (RQ) was evaluated compared to prepared standards of salivary samples from *SS*-positive patients diluted to 10 ng/uL (CT ave. 30.3) (Fig. 4B). These data revealed RQ for the *SS*-positive samples ranged between 0.97 and 1.27 (RQ ave. =1.03).

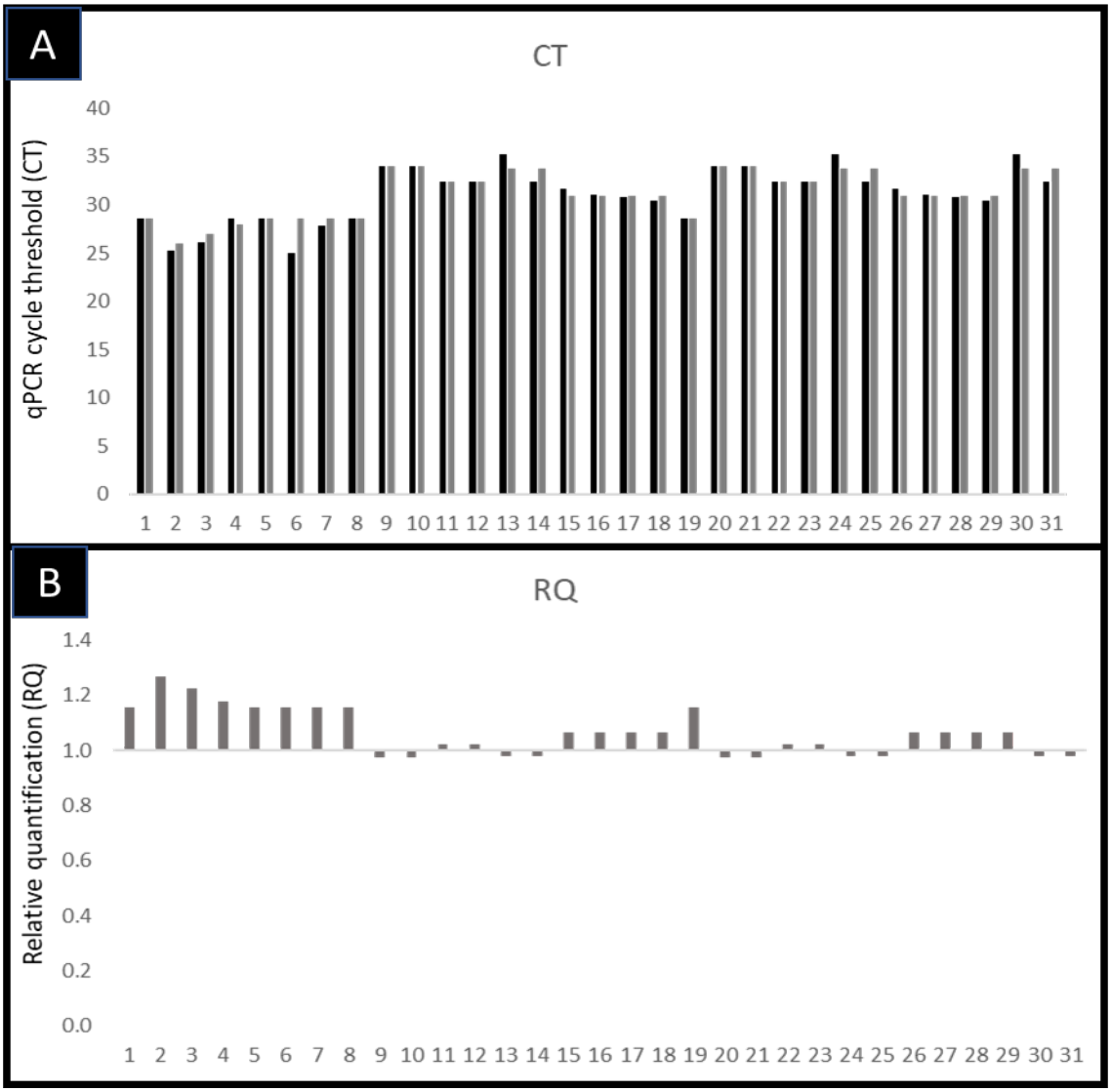

Figure 4. Cycle threshold (CT) and relative quantification (RQ) of *SS* qPCR data. A) Analysis of *SS*-positive samples revealed CT ranging between 25.1 and 35.2 (CT ave.=31.13), which was not

significantly different from the *SS*-positive controls (CT ave.=30.3), P=0.682. B) Comparison of these data with *SS*-positive controls revealed RQ between 0.97 and 1.27 (RQ ave.=1.03), which was not significantly different (P=0.892).

To visualize these results and to determine the correlation and prevalence of these organisms within the samples, a logic (Venn) diagram was created (Fig. 5). This graphic display revealed that within the Adult non-Orthodontic samples all of the *SW*-positive samples were also *SS*-positive - although not all *SS*-positive samples harbored *SW* (*SS:SW* ratio 0.42). Similarly, within the Pediatric non-Orthodontic samples all of *SW*-positive samples also harbored *SS* - although not all of the *SS*-positive samples harbored *SW* (*SS:SW* ratio 0.52). In both Adult and Pediatric non-Orthodontic samples, a greater proportion of these samples harbored *SS* and all of the *SW*-positive samples were found within the *SS*-positive sample subgroups. Although none of the Adult Orthodontic samples were positive, the results from the Pediatric Orthodontic samples revealed a striking difference with all of the *SS*-positive samples found within the *SW*-positive samples - and a much greater number of samples testing *SW*-positive overall (*SW:SS* ratio 0.15).

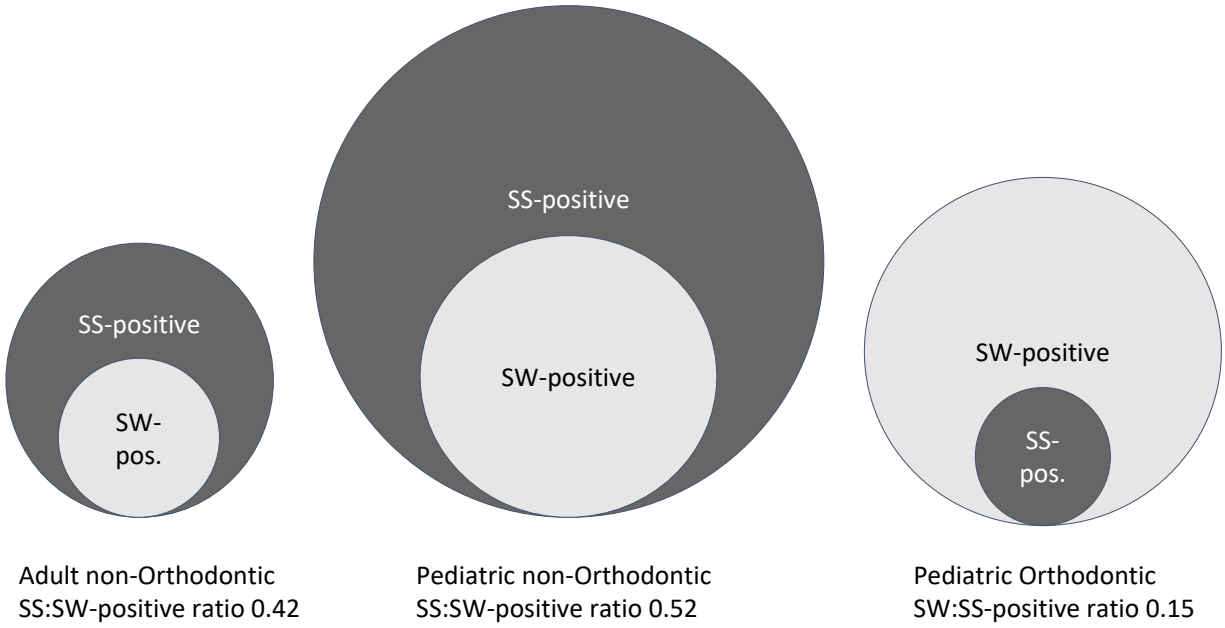

Figure 5. Graphic analysis of microbial prevalence among positive samples. Plotting *SS*- and *SW*-positives samples revealed all *SW*-positive samples and non-Orthodontic patients were within the *SS*-positive subgroups. Pediatric Orthodontic samples harbored more *SW*-positive samples, which harbored a smaller proportion of the *SS*-positive samples.

Discussion

Based upon the limited amount of information regarding *SW* prevalence and the association with *SS*, the main objective of this study was to evaluate the presence of these cariogenic microorganisms within clinical saliva samples obtained from a dental school-based setting. These results demonstrated several novel findings that will require further study to understand the clinical implications and potential guidelines and recommendations that might need to be modified.

First, this study found that pediatric (mainly teenage) samples were much more likely to harbor

either *SW* or *SS* compared with adult samples. This may be related to two separate inter-related factors. First, there is some evidence to suggest that adults over the age of 18 years seeking orthodontic treatment may be more highly motivated to maintain high standards of hygiene during orthodontic treatment compared with pediatric patients that might be under treatment at the request of parents or guardians [33,34]. In addition, there may also be some evidence to suggest that adults may be more highly motivated to maintain higher standards of oral hygiene to control halitosis, which may be related to the ability to work and comply with workplace standards of hygiene that may be more stringent than found among middle or high school classroom environments [35]. Finally, the differences between these two populations may also be related to the expression of hormones among the younger teenage population – a key modulating influence of the oral microbiome that may influence and mediate the prevalence of these organisms [36].

Second, the analysis of prevalence from this study found many more *SW*-positive samples among pediatric orthodontic patients compared with either adult or pediatric non-Orthodontic patients, which may suggest this population may be at higher risk for *SW*-related caries or other negative oral health outcomes [37]. Whether this is related to hygiene or other influences related to orthodontic treatment is not within the scope of this study but are factors that should be further explored in future studies to determine why this observation has been made in several studies of this nature [22-29,38]. In addition, the observation that all the *SW*- and *SS*-positive samples were present in mutual overlapping samples may suggest that some microbial interactions may be associated with their propagation or other commensal mechanisms may influence their mutual growth within the same environments. However, the striking shift from *SS*-positive to *SW*-positive samples between pediatric non-Orthodontic and pediatric Orthodontic patients does suggest that

some effect of orthodontic therapy may influence the relative proportion of these organisms within the same microbial environment.

As with all retrospective studies, there are several limitations that should be considered when evaluating these results. First, there was no clinical information regarding the hygiene status, caries risk or caries experience (decayed, missing, filled teeth or DMFT) recorded with these samples during the original collection - limiting the clinical inferences that can be made between these various population subgroups. In addition, no information regarding the length of orthodontic treatment was obtained during the original sample collection, which might provide valuable information regarding the timing and strength of influence this variable might have on the results observed. Finally, the original sample collection was a cross-sectional study - with only one sample collected from each patient at one time point from one specific dental school-based clinical population. Therefore, no pre- and post-analysis of microbial prevalence was possible, which could mean that differences in pre-existing microbial populations and the particular patients within this specific clinical population may have also influenced the outcomes observed in this study. To address these limitations, future research studies might include more detailed clinical information (such as DMFT scores), as well as pre- and post-treatment analysis from additional patients derived from other clinics to validate the results observed from this study.

Conclusions

The results of this study provide some evidence that *SW* and *SS* microbial prevalence may be associated with specific population subgroups, such as *SS* within non-orthodontic patients and SW within pediatric orthodontic patients. Unlike previous studies, which demonstrated partially overlapping prevalence of oral microbes - these observations suggest that *SS* and *SW* may be

strongly associated within oral microbial communities and their presence may be directly or indirectly linked through one or more factors yet to be determined. Future research will be needed to more fully understand these complex and interdependent relationships.

Consent

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

Ethical Approval

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Acknowledgements

This study is part of a Masters in Oral Biology thesis by MT. In addition, preliminary data from this study have been submitted for presentation to the American Association for Dental Research (AADR) 2021 conference.

Competing Interests

The authors declare no conflict of interest.

Author Contributions

KK and KMH were responsible for the overall project design. BJS, MM, PP, KF, MT, and NK were responsible for data generation and analysis. KK and MT 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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Chapter 3

Summary and Conclusions:

Based upon the limited amount of information regarding *SW* prevalence and the association with *SS*, the main objective of this study was to evaluate the presence of these cariogenic microorganisms within clinical saliva samples obtained from a dental school-based setting. These results demonstrated several novel findings that will require further study to understand the clinical implications and potential guidelines and recommendations that might need to be modified.

First, this study found that pediatric (mainly teenage) samples were much more likely to harbor either *SW* or *SS* compared with adult samples. This may be related to two separate inter-related factors. First, there is some evidence to suggest that adults over the age of 18 years seeking orthodontic treatment may be more highly motivated to maintain high standards of hygiene during orthodontic treatment compared with pediatric patients that might be under treatment at the request of parents or guardians [32,33]. In addition, there may also be some evidence to suggest that adults may be more highly motivated to maintain higher standards of oral hygiene to control halitosis, which may be related to the ability to work and comply with workplace standards of hygiene that may be more stringent than found among middle or high school classroom environments [34]. Finally, the differences between these two populations may also be related to the expression of hormones among the younger teenage population – a key modulating influence of the oral microbiome that may influence and mediate the prevalence of these organisms [35].

Second, the analysis of prevalence from this study found many more *SW*-positive samples among pediatric orthodontic patients compared with either adult or pediatric non-Orthodontic patients,

which may suggest this population may be at higher risk for *SW*-related caries or other negative oral health outcomes [36]. Whether this is related to hygiene or other influences related to orthodontic treatment is not within the scope of this study but are factors that should be further explored in future studies to determine why this observation has been made in several studies of this nature [22-29, 37]. In addition, the observation that all the *SW*- and *SS*-positive samples were present in mutual overlapping samples may suggest that some microbial interactions may be associated with their propagation or other commensal mechanisms may influence their mutual growth within the same environments. However, the striking shift from *SS*-positive to *SW*-positive samples between pediatric non-Orthodontic and pediatric Orthodontic patients does suggest that some effect of orthodontic therapy may influence the relative proportion of these organisms within the same microbial environment.

Based on the findings presented throughout this document, the null hypotheses can be rejected and the alternative hypotheses can be accepted for research questions 1 and 2.

1. What is the prevalence of *S. wiggsiae* among the pediatric and adult orthodontic and non-orthodontic patient populations at UNLV-SDM?
    a. Alternative hypothesis (H_A): Yes, the prevalence of *S. wiggsiae* is different among these populations
2. What is the prevalence of *S. sobrinus* among the pediatric and adult orthodontic and non-orthodontic patient populations at UNLV-SDM?
    a. Alternative hypothesis (H_A): Yes, the prevalence of *S. sobrinus* is different among these populations

Additional findings: Are *SW* and *SS* found among the same patients or different patients? The results of this study provide evidence to suggest that *SS* and *SW* may be strongly associated within

the same patients (oral environments) and more research will be needed to determine the strength and relationships between these microbes.

Limitations and Recommendations:

As with all retrospective studies, there are several limitations that should be considered when evaluating these results. First, there was no clinical information regarding the hygiene status, caries risk or caries experience (decayed, missing, filled teeth or DMFT) recorded with these samples during the original collection - limiting the clinical inferences that can be made between these various population subgroups. In addition, no information regarding the length of orthodontic treatment was obtained during the original sample collection, which might provide valuable information regarding the timing and strength of influence this variable might have on the results observed. Finally, the original sample collection was a cross-sectional study - with only one sample collected from each patient at one time point from one specific dental school-based clinical population. Therefore, no pre- and post-analysis of microbial prevalence was possible, which could mean that differences in pre-existing microbial populations and the particular patients within this specific clinical population may have also influenced the outcomes observed in this study. To address these limitations, future research studies might include more detailed clinical information (such as DMFT scores), as well as pre- and post-treatment analysis from additional patients derived from other clinics to validate the results observed from this study.

The results of this study provide some evidence that *SW* and *SS* microbial prevalence may be associated with specific population subgroups, such as *SS* within non-orthodontic patients and *SW* within pediatric orthodontic patients. Unlike previous studies, which demonstrated partially

overlapping prevalence of oral microbes - these observations suggest that *SS* and *SW* may be strongly associated within oral microbial communities and their presence may be directly or indirectly linked through one or more factors yet to be determined. Future research will be needed to more fully understand these complex and interdependent relationships.

Appendices

Appendix A – IRB Permissions

UNLV

Biomedical IRB
Notice of Excluded Activity

DATE: February 6, 2015

TO: **Dr. Karl Kingsley,** School of Dental Medicine

FROM: Office of Research Integrity – Human Subjects

RE: Notification of IRB Action
Protocol Title: **The Prevalence of Oral Microbes in Saliva from the UNLV School of Dental Medicine Pediatric and Adult Clinical Population**
Protocol# 1502-5068M

This memorandum is notification that the project referenced above has been reviewed as indicated in Federal regulatory statutes 45CFR46.

The protocol has been reviewed and deemed excluded from IRB review. It is not in need of further review or approval by the IRB.

Any changes to the excluded activity may cause this project to require a different level of IRB review. Should any changes need to be made, please submit a Modification Form.

If you have questions or require any assistance, please contact the Office of Research Integrity – Human Subjects at IRB@unlv.edu or call 702-895-2794.

Office of Research Integrity – Human Subjects
4505 Maryland Parkway • Box 451047 • Las Vegas, Nevada 89154-1047
(702) 895-2794 • FAX: (702) 895-0805 • IRB@unlv.edu

Appendix B – Updated IRB Permissions

UNLV

UNLV Biomedical IRB - Administrative Review
Notice of Excluded Activity

DATE:	March 3, 2021
TO:	Karl Kingsley, PhD, MPH
FROM:	UNLV Biomedical IRB
PROTOCOL TITLE:	[1717625-1] Retrospective analysis of microbial prevalence from DNA isolated from saliva samples originally obtained from the University of Nevada, Las Vegas (UNLV) School of Dental Medicine (SDM) pediatric and clinical population
SUBMISSION TYPE:	New Project
ACTION:	EXCLUDED - NOT HUMAN SUBJECTS RESEARCH
REVIEW DATE:	March 3, 2021
REVIEW TYPE:	Administrative Review

Thank you for your submission of New Project materials for this protocol. This memorandum is notification that the protocol referenced above has been reviewed as indicated in Federal regulatory statutes 45CFR46.

The UNLV Biomedical IRB has determined this protocol does not meet the definition of human subjects research under the purview of the IRB according to federal regulations. It is not in need of further review or approval by the IRB.

We will retain a copy of this correspondence with our records.

Any changes to the excluded activity may cause this protocol to require a different level of IRB review. Should any changes need to be made, please submit a Modification Form.

If you have questions, please contact the Office of Research Integrity - Human Subjects at IRB@unlv.edu or call 702-895-2794. Please include your protocol title and IRBNet ID in all correspondence.

Office of Research Integrity - Human Subjects
4505 Maryland Parkway . Box 451047 . Las Vegas, Nevada 89154-1047
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Appendix C - Permission to Use Copyrighted Material

University of Nevada, Las Vegas

I, Karl Kingsley, holder of copyrighted material entitled *Scardovia wiggsiae* and *Streptococcus sobrinus* prevalence among orthodontic and non-orthodontic patients, authored by Melissa Trumbo, and Karl Kingsley originally published in Microbiology Research Journal International, March 2021, hereby give permission for the author to use the above described material in total or in part for inclusion in a Master's thesis at the University of Nevada, Las Vegas.

I also agree that the author may execute the standard contract with ProQuest for storage and reproduction of the completed thesis, including the materials to which I hold copyright

Melissa Trumbo March 22, 2021

Signature Date

Melissa Trumbo Resident

Name (typed) Title

Curriculum Vitae

Graduate College

University of Nevada, Las Vegas

Melissa Trumbo

Email: melissa.trumbo10@gmail.com

Degrees:
Bachelor of Science – Business Administration, 2008
College of the Ozarks, Point Lookout, Missouri

Doctor of Dental Surgery, 2013
The University of Missouri-Kansas City School of Dentistry, Kansas City, Missouri

Thesis Title:
Scardovia wiggsiae and Streptococcus sobrinus prevalence among orthodontic and non-orthodontic patients

Thesis Examination Committee:
Chairperson, Karl Kingsley, Ph.D. M.P.H.
Chairperson, Katherine Howard, Ph.D.
Committee Member, Brian Chrzan, D.D.S., Ph.D.
Graduate Faculty Representative, Courtney Coughenour Ph.D.
Graduate Coordinator, Brian Chrzan, D.D.S., Ph.D.