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Scardovia wiggsiae and *Streptococcus sobrinus* Prevalence among Orthodontic and Non-Orthodontic Patients

Melissa Trumbo[1], Namgu Kim[1], Beanca Jhanine Samiano[2], Matthew Marrujo[2], Patrick Perkins[2], Kevin Foote[2], Katherine M. Howard[3] and Karl Kingsley[3*]

[1]Department of Advanced Education in Orthodontics and Dentofacial Orthopedics, University of Nevada, Las Vegas - School of Dental Medicine, 1700 W. Charleston, Las Vegas, Nevada, USA.
[2]Department of Clinical Sciences, University of Nevada, Las Vegas - School of Dental Medicine, 1700 W. Charleston, Las Vegas, Nevada, USA.
[3]Department of Biomedical Sciences, University of Nevada, Las Vegas - School of Dental Medicine, 1001 Shadow Lane, Las Vegas, Nevada, USA.

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 MT contributed to the writing and editing of this manuscript. All authors have read and agreed to the published version of the manuscript. All authors read and approved the final manuscript.

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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.

**Corresponding author: E-mail: Karl.Kingsley@unlv.edu, karl.kingsley@unlv.nevada.edu;*

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.

Keywords: Streptococcus sobrinus (SS); Scardovia wiggsiae (SW); saliva; prevalence.

1. 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].

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.

2. METHODS

2.1 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.

2.2 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].

2.3 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 GAC TTG GCA ACT-3’, Tm=71C
Annealing temperature=lower Tm (67C) - 2C= 65C.

2.4 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.

3. 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.

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.

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.

Table 1. DNA concentration and DNA purity standards

	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=317317/1176 (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

Table 2. Study sample demographics

	Study Sample (n=187)	UNLV-SDM Patient Clinic Population	Statistical analysis
Females	n=92/187 (49.2%)	52.8%	χ2=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%	χ2=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%)		χ2=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)	χ2=2.813, d.f.=1
Pediatric (12-17 yrs)	n=107/187 (57.2%)	65.3% (Orthodontic)	P=0.0935

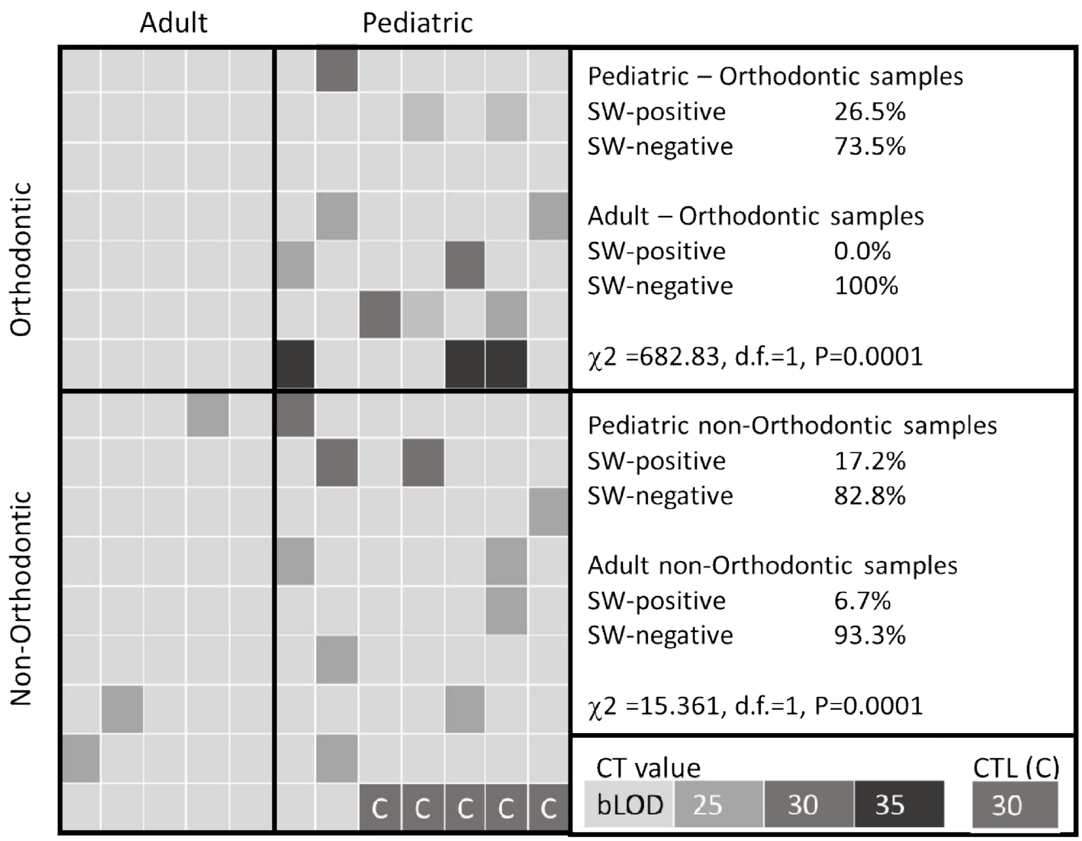

Fig. 1. qPCR screening and heat map for *Scardovia wiggsiae* (SW). Screening for SW DNA revealed the majority of samples were below the limit of detection (bLOD). Cycle threshold (CT) data revealed the highest SW prevalence among Pediatric Orthodontic samples (26.5%), fewer among Pediatric non-Orthodontic 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).

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.

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).

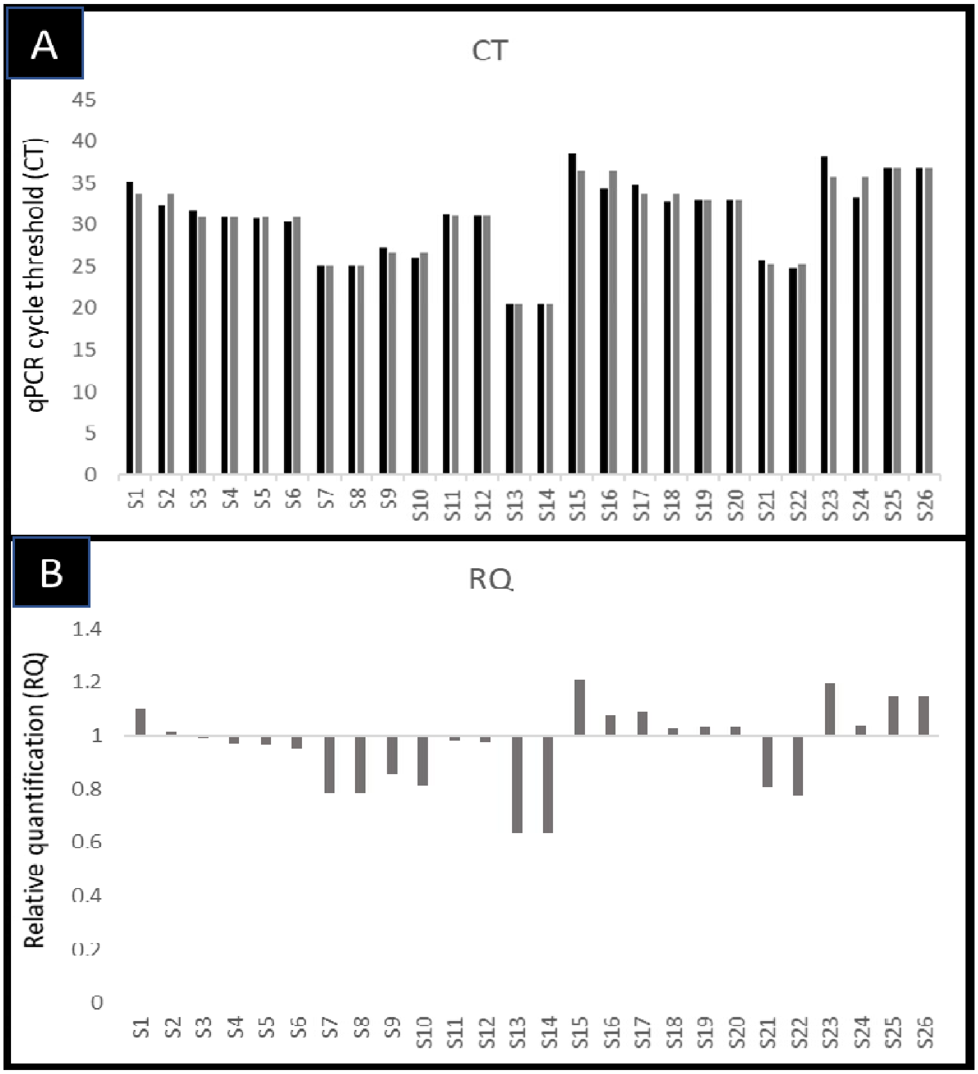

Fig. 2. Cycle threshold (CT) and relative quantification (RQ) of SW qPCR data. A) Analysis of SW-positive samples revealed CT ranging between 20.4 and 38.6 (CT ave.=30.76), which was not significantly different from the SW-positive controls (CT ave.=31.9), P=0.881. B) Comparison of these data with SW-positive controls revealed RQ between 0.63 and 1.2 (RQ ave.=0.97), which was not significantly different (P=0.781)

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).

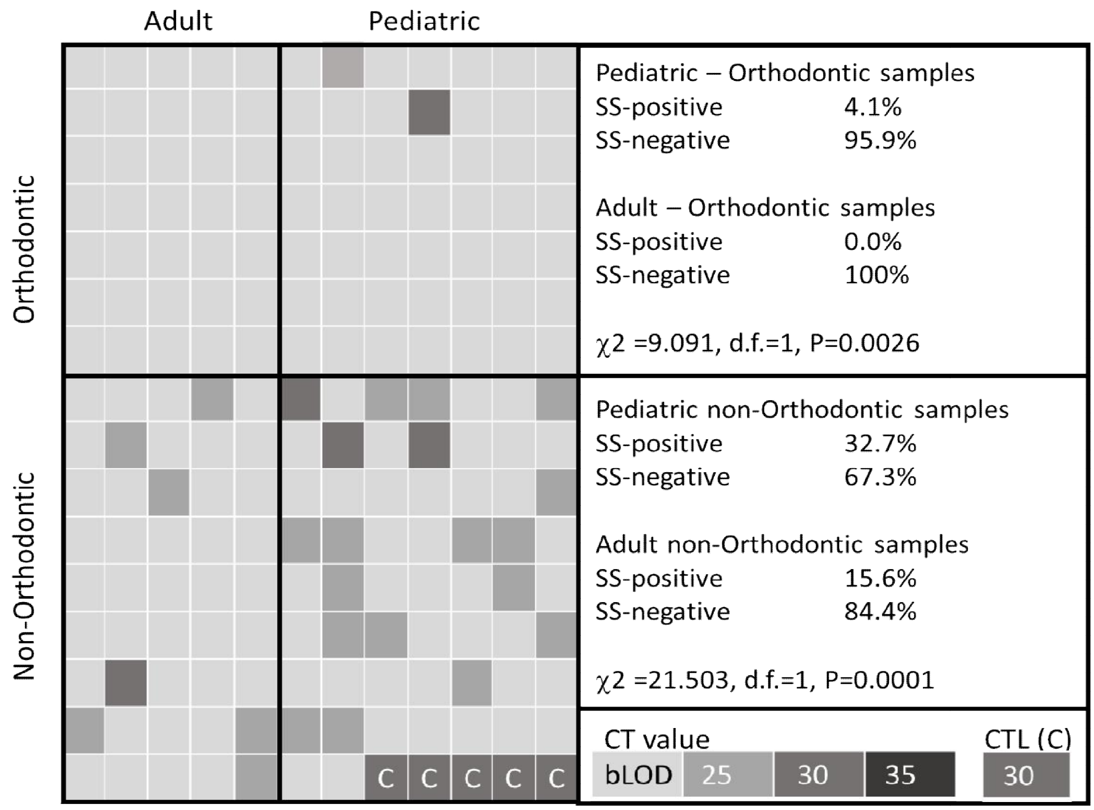

Fig. 3. qPCR screening and heat map for *Streptococcus sobrinus* (SS). Screening for SS DNA revealed the majority of samples were below the limit of detection (bLOD). Cycle threshold (CT) data revealed the highest SS prevalence among Pediatric non-Orthodontic samples (32.7%), fewer among Adult non-Orthodontic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

4. 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].

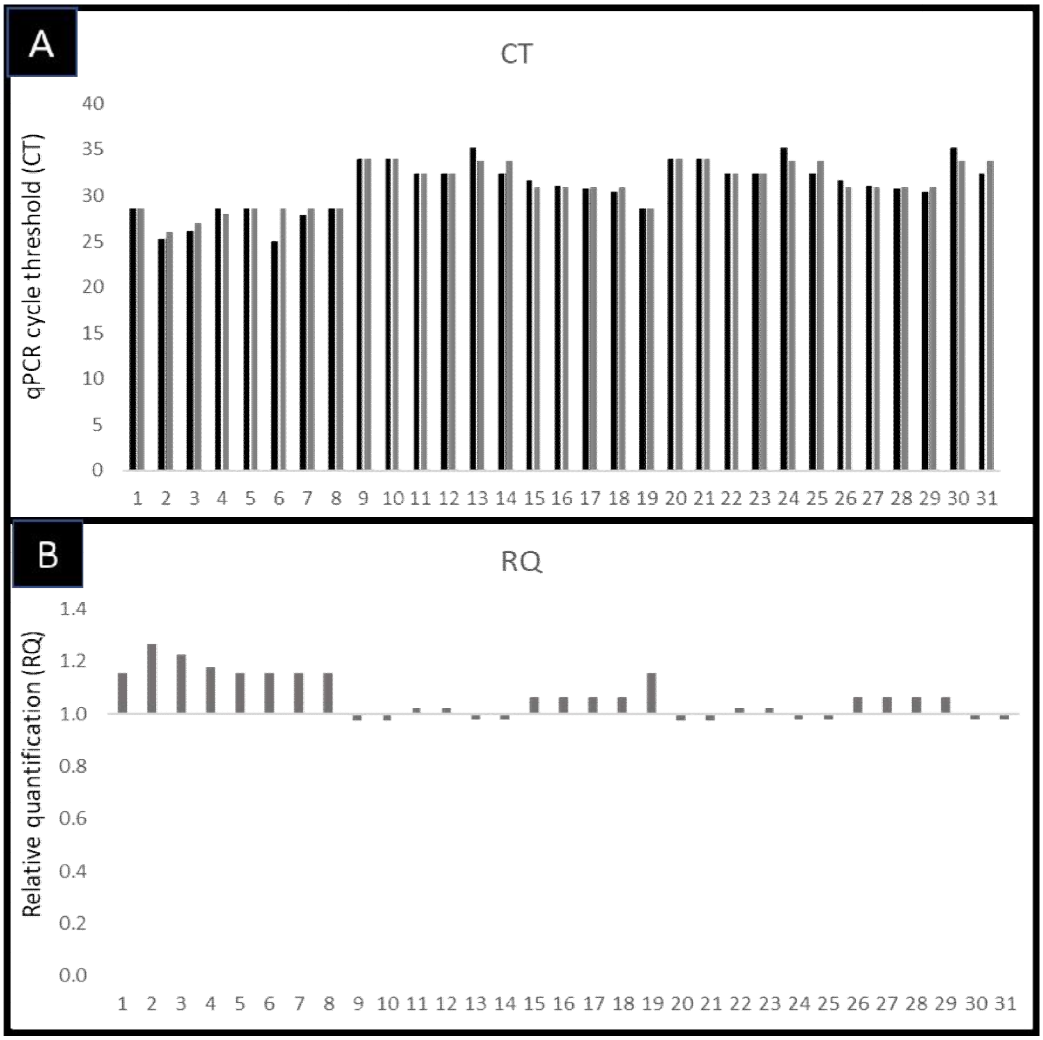

Fig. 4. Cycle threshold (CT) and relative quantification (RQ) of SS qPCR data. A) Analysis of SS-positive samples revealed CT ranging between 25.1 and 35.2 (CT ave.=31.13), which was not significantly different from the SS-positive controls (CT ave.=30.3), P=0.682. B) Comparison of these data with SS-positive controls revealed RQ between 0.97 and 1.27 (RQ ave.=1.03), which was not significantly different (P=0.892)

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.

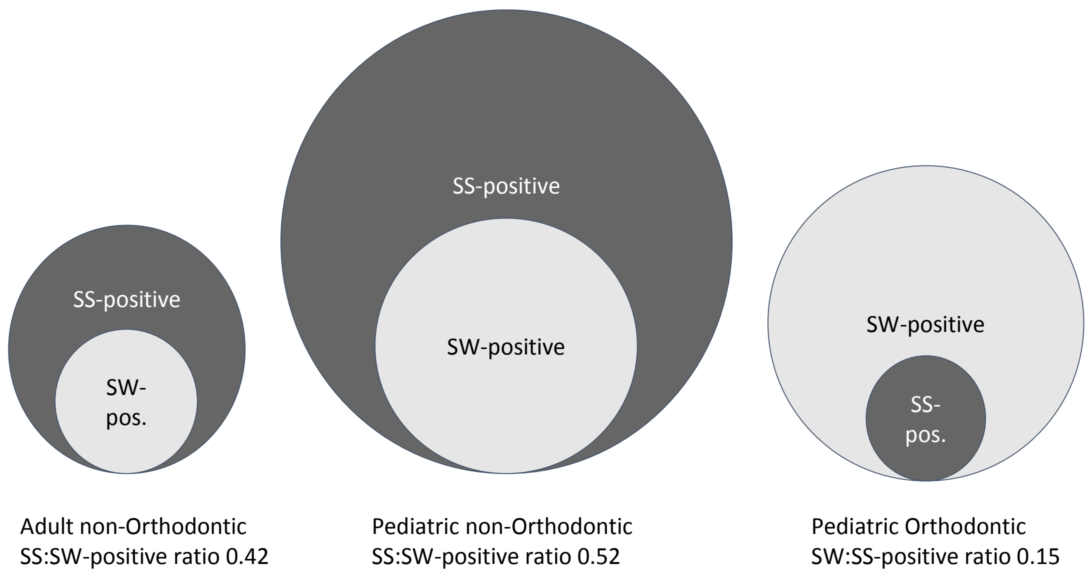

Fig. 5. Graphic analysis of microbial prevalence among positive samples. Plotting SS- and SW-positives samples revealed all SW-positive samples and non-Orthodontic patients were within the SS-positive subgroups. Pediatric Orthodontic samples harbored more SW-positive samples, which harbored a smaller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

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.

5. 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 authors.

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

Authors have declared that no competing interests exist.

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