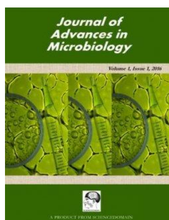

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Screening a Saliva Repository for *Scardovia wiggsiae* and *Streptococcus mutans*: A Pilot Study

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AT, KK and KMH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JM and SM managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Many studies have evaluated the prevalence of cariogenic pathogens among dental school patients, most notably the Gram-positive organism *Streptococcus mutans* (SM). Recent evidence has suggested another cariogenic pathogen *Scardovia wiggsiae* (SW) may also be present in the oral flora of a smaller subset of dental patients.
Objective: Few studies to date have examined the corresponding prevalence of both SM and SW within the same patient samples, therefore the main objective of this study was to evaluate the presence of these cariogenic organisms within a dental school-based setting.
Experimental Methods: Screening was facilitated using DNA extracted from a pre-existing patient saliva repository and processed using qPCR. SW-positive (n=27) and SW-negative (n=15) samples

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were subsequently screened for the presence of SM. The samples were nearly evenly divided between males and females (45%, 55%, respectively) and were mostly Hispanic minorities (n=22/42 or 52%).
Results: This analysis revealed that 45% of samples (n=19/42) also harbored SM. More detailed analysis revealed that the vast majority of SM-positive samples (n=15/19 or 79%) were derived from SW-positive samples, while only a small percentage of SM-positive samples (n=4/19 or 21%) were derived from SW-negative samples.
Conclusions: The limited numbers of studies available regarding SW prevalence have suggested that SW and SM may inhabit similar and overlapping niches within the oral microbiome. In fact, some work has suggested the potential for competition and interactive inhibition between these organisms within the oral cavity. The preliminary data from this pilot study suggest SM and SW may, in fact, be present in the same patients and may not therefore be exclusively competitive – at least in this cross sectional study. However, due to the large differences observed among these samples, further research will be needed to further elucidate and validate these findings.

Keywords: Scardovia wiggsiae; Streptococcus mutans; saliva screening; microbial prevalence.

ABBREVIATIONS

SW : Scardovia wiggsiae
SM : Streptococcus mutans
qPCR : Quantitative polymerase chain reaction
DNA : Deoxyribonucleic acid
IRB : Institutional Review Board
OPRS : Office for the Protection of Human Subjects
UNLV : University of Nevada Las Vegas
SDM : School of Dental Medicine

1. INTRODUCTION

The formation of dental caries (cavities) is a complex, multi-dimensional process that necessarily involves many risk factors – including the acquisition and colonization of cariogenic oral bacteria [1,2]. The most frequently associated oral pathogens are the acid-producing and acid-tolerant oral streptococcus species, such as *Streptococcus mutans* (*S. mutans*) [3,4]. Many studies have established and confirmed the critical role of the formation of biofilm in the virulence of *S. mutans*, and the critical role this may play in determining the balance of the oral microbiome towards health or disease [5-7].

More recent efforts have discovered a novel cariogenic pathogen, *Scardovia wiggsiae* (*S. wiggsiae*) among the oral bacteria of children with severe early childhood caries [8-10]. This pathogen has also been confirmed among the oral microbiota among patients with increased caries risk, such as orthodontic patients [11,12]. Despite these efforts, much remains unknown about the prevalence of this organism and the potential interactions with other cariogenic bacteria that might influence oral health or disease [13-15].

Studies from this group have surveyed the prevalence of oral microbial pathogens, such as *S. mutans* among pediatric and orthodontic populations [16,17]. In addition, pilot studies to evaluate the presence of *S. wiggsiae* among this patient population have also emerged [12,18,19]. However, to date few (if any) of these studies have performed simultaneous screenings of both *S. mutans* and *S. wiggsiae* to determine if the presence of either organism might be associated with differences in the prevalence of the other. The primary goal of this study was to determine if this type of association may exist among the oral microbiota of patient samples obtained from an existing saliva repository.

2. METHODOLOGY

2.1 Study Design

This was a retrospective study to evaluate the presence of both *S. wiggsiae* and *S. mutans* among patient samples from an existing saliva repository. Approval for this study was granted from the Institutional Review Board (IRB) under Protocol #1502-5068M - The Prevalence of Oral Microbes in Saliva from the University of Nevada Las Vegas (UNLV) School of Dental Medicine (SDM) pediatric and adult clinical population. Samples were originally collected between 2010 and 2016. In brief, each sample was given a unique, non-duplicated randomly generated identification number to protect patient anonymity – with only basic demographic information (age, sex, race/ethnicity) noted at the time of saliva collection.

2.2 Sample Selection

Patient saliva samples that were previously identified as harboring *S. wiggsiae* DNA were selected for screening using qPCR (n=27). Selected samples that were previously identified as not harboring *S. wiggsiae* DNA were also identified from patient collections during the same time period (n=15), which would reduce the potential for time-dependent degradation of samples to influence the outcome and final results. DNA was then isolated from each clinical sample using the Amersham Biosciences GenomicPrep DNA isolation kit and the manufacturer recommended protocol, as previously described [20-22].

2.3 qPCR Screening

DNA screening was accomplished using primers specific for each organism [17,23]. The probes for *S. wiggsiae* (SwP)and *S. mutans* (SmP) were each labeled with 6-carboxyfluorescein (FAM) at the 5'end and with tetramethyl-6-carboxyrhodamine (TAMRA) on the 3-end, as specified:

Forward primer-SW, GTGGACTTTATGAATAAGC (19 bp)
Reverse primer-SW, CTACCGTTAAGCAGTAAG(18 bp)
SwP[6 ~ FAM] 5'-AGCGTTGTCCGGATTTATT-3'G [TAMRA]

Forward primer-SM, GCCTACAGC TCAGAGATGCTATTCT (26 bp)
Reverse primer-SM, GCCATACACCACTCATGAATTGA (23 bp)
SmP [6 ~ FAM] 5'-GAAACCAACCCAACTTTAGCTTGGAT-3'G [TAMRA]

All qPCR reactions were performed using TaqMan universal PCR master mix with the probe concentration at 0.2 uM and a minimum of 5.0 uL of target (sample) DNA. All reactions were performed in duplicate using incubation at 50C (2 min), denaturation at 95C (10 min), 40 cycles at 95C (15 sec) and 60C (1 min).

2.4 Statistics

Demographic information was summarized and presented as simple, descriptive statistics (both number and percentage). The composition of the study sample was compared with the overall clinic composition from which it was originally collected and these data were analyzed using Chi Square ($\chi 2$) software from GraphPad (San Diego, CA) [24]. This analysis was also used to analyze the qPCR results.

3. RESULTS

The demographic analysis revealed that the study samples (n=42) were derived from nearly equal numbers of females and males (Table 1). However, the racial and ethnic composition of the study sample had a much higher proportion from minority patients than the overall clinic population from which that sample was collected (p=0.0005). The study sample was comprised of both pediatric and adults, which ranged in age from 12 – 41 years.

DNA was subsequently isolated from the pre-selected samples, which was within the range specified by the manufacturer (Table 2). Successful isolation was accomplished and the quality assessed using absorbance readings at 260 and 280 nm (A260:A280 nm ratio). Quantity of DNA was also determined to be sufficient for qPCR screening of all samples.

Table 1. Study sample demographics

	Study sample (n=42)	Clinic population	Statistical analysis
Sex			
Male	45.2% (n=19)	49.1%	$\chi 2$=0.640, d.f.=1
Female	54.8% (n=23)	50.9%	p=0.4236
Ethnicity / Race			
White	23.8% (n=10)	41.4%	$\chi 2$=11.947, d.f.=1
Minority	76.2% (n=32)	58.6%	p=0.0005
Hispanic	52.4% (n=22)	35.9%	
African American	16.7% (n=7)	13.1%	
Other	7.1% (n=3)	4.2%	
Age range	12-41 yrs.	2 – 91 yrs.	

Table 2. DNA isolation and analysis

	Study sample (n=42)
DNA recovery	n=42/42 (100%)
	Range (manufacturer estimate) 95-100%
DNA purity	A260:A280 range: 1.51 – 2.00 Ave: 1.71
	Acceptable range (manufacturer) 1.65 – 2.00
DNA concentration	[316.2 ng/uL] range: 91.2 – 873.4
	Manufacturer range: 100 – 1000 ng/uL

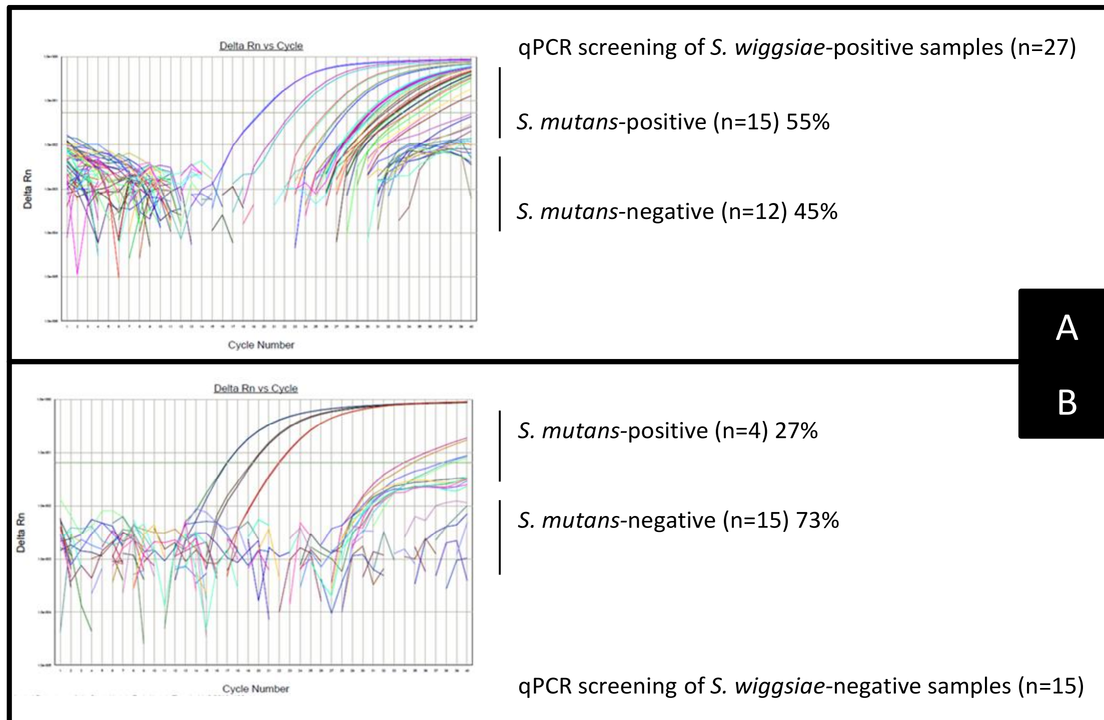


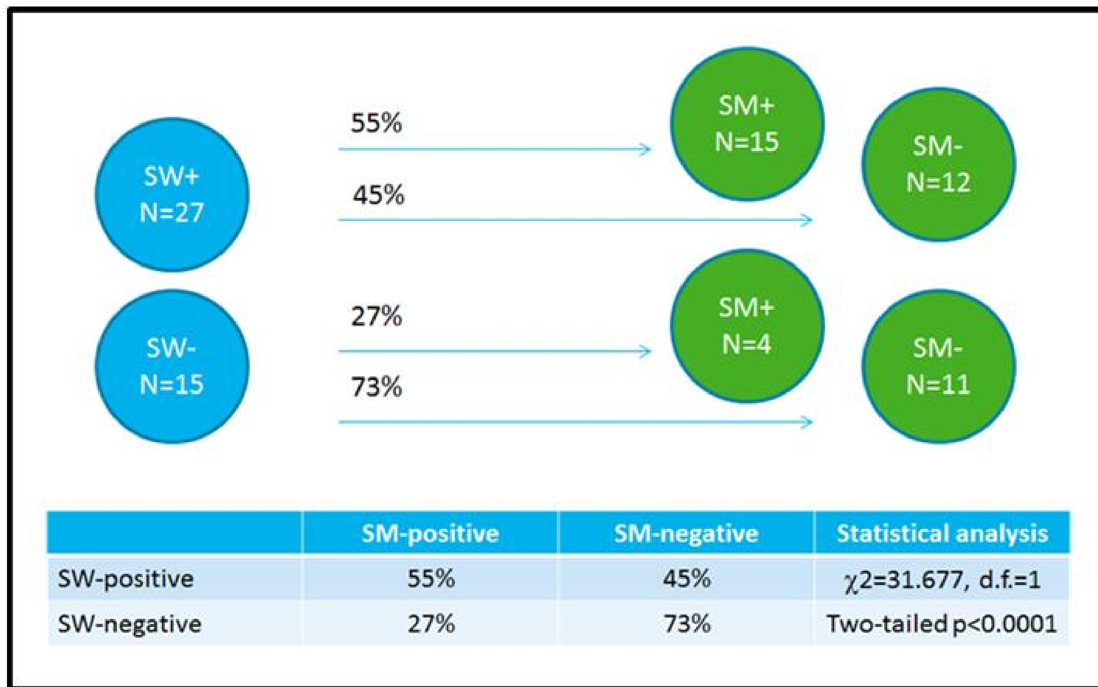
Fig. 1. Screening of study samples using qPCR. A) Screening of *Scardovia*-positive samples revealed more than half also harbor *S. mutans* DNA (55%). B) Screening of *Scardovia*-negative samples revealed relative few also contain DNA from *S. mutans* (27%)

The screening of study samples using qPCR was performed, which revealed differential results for each of the organisms evaluated (Fig. 1). More specifically, qPCR screening of the previously identified *S. wiggsiae*-positive samples confirmed n=27 samples harbored DNA from this organism. qPCR analysis confirmed the *S. wiggsiae*-negative samples (n=15) and revealed only a small fraction (27%) harbored DNA from *S. mutans* (Fig. 1B).

More detailed analysis of these results revealed that the vast majority of *S. mutans*-positive samples (79%) were derived from *S. wiggsiae*-positive samples (Fig. 2). Only a small percentage of *S. mutans* positive samples (21%) were derived from the *S. wiggsiae*-negative samples. Chi-square analysis of these results strongly suggests this distribution was unlikely due to chance (p<0.00001).

4. DISCUSSION

The primary goal of this study was to perform highly sensitive qPCR screenings of patient saliva samples for both *S. mutans* and *S. wiggsiae* to determine if the presence of either organism might be associated with differences in the prevalence of the other using an existing saliva repository. This pilot study has revealed that both cariogenic pathogens *S. mutans* and *S. wiggsiae* may, in fact, be present in the same



	SM-positive	SM-negative	Statistical analysis
SW-positive	55%	45%	χ2=31.677, d.f.=1
SW-negative	27%	73%	Two-tailed p<0.0001

Fig. 2. Statistical analysis of *S. wiggsiae* and *S. mutans* screening. Although *S. mutans* was found in both *Scardovia*-positive and –*negative* samples, detailed analysis of the qPCR screening results demonstrates a statistically significant difference was observed among the *Scardovia*-negative samples, with a much lower percentage also harboring *S. mutans* (p<0.0001)

patients and are therefore unlikely to be exclusively competitive [25,26]. However, the discrepancy in the prevalence of *S. mutans* among *S. wiggsiae*-positive and –negative samples may suggest alternative factors may be influencing the microbial composition of patient oral flora [27,28].

Recent studies have now demonstrated that the salivary microbiota differ significantly among patients with different caries risk and experience [29,30]. This evidence has provided increasing support for the hypothesis that co-association of cariogenic and pathogenic oral microbes, may be understood more clearly as moving in tandem and providing commensal opportunities rather than merely as competitors for limited resources and available space [31-34]. Although much remains to be discovered regarding the epidemiology of these organisms in oral disease and prevention, these data suggest a more thorough understanding of prevalence will help clinicians and healthcare providers in both disease prevention and treatment.

Although these findings represent novel information regarding co-association and prevalence of *Scardovia* in relationship to *S. mutans*, there are some limitations of this study that must be considered. First, the retrospective nature of this study did not allow for the collection of samples based upon caries risk or caries experience, which may represent a significant confounding variable. In addition, the original establishment of the saliva repository was done as a convenience sample at a low-income, public University-based dental school – which may represent patients with more limited access to healthcare and more likelihood to have low levels of health literacy and insurance [35-37].

5. CONCLUSIONS

The limited numbers of studies available regarding *S. wiggsiae* prevalence have suggested that *S. wiggsiae* and *S. mutans* may inhabit similar and overlapping niches within the oral microbiome, which has been suggested by other studies of initial and mature plaques and oral biofilms [38-41]. In fact, some work has suggested the potential for competition and interactive inhibition between these organisms within the oral cavity, such as the production of

enzymes and proteases that may facilitate the growth of commensal species while inhibiting the growth or metabolism of others [42,43]. The preliminary data from this pilot study suggest *S. mutans* and *S. wiggsiae* may, in fact, be present in the same patients and may not therefore be exclusively competitive – at least in this cross sectional study. However, due to the large differences observed among these samples, further research will be needed to further elucidate and validate these findings.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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