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Oral Microbial Ecology of *Selenomonas noxia* and *Scardovia wiggsiae*

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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 SM and JM managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Objectives: *Selenomonas* species such as *S. noxia* are associated with poor oral health and oral prevalence of this organism may be useful as a biomarker to determine patient oral health. Current studies are now revealing novel insights into the epidemiology of the newly discovered oral cariogenic organism *Scardovia wiggsiae* (SW), although few studies have explored the oral microbial ecology with respect to this oral pathogen. Based upon the lack of information regarding the oral microbial ecology, the primary objective of this study was to screen an existing saliva repository to more accurately assess the microbial flora present (or absent) including SN and SW.
Experimental Methods: Previously collected saliva samples were evaluated for the DNA isolation and qPCR screening protocol. A total of 42 samples were identified and processed from both pediatric (n=28/42 or 67%) and adult (n=14/42 or 33%) patients.

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Results: The results of this screening demonstrate that of the SW-positive samples (n=27/42 or 64%) none harbored the oral microbe *Selenomonas noxia* (SN). Conversely, SN was identified only within the subset of SW-negative samples (n=15/42 or 35%). In addition, although *Aggregatibacter actinomycetemcomitans* (AA) was only present in a small subset of samples – this organism was only found among SW-positive samples. Other organisms, including *T. forsythia* (TF), and *F.nucleatum* (FN) were present in both SW-positive and SW-negative samples although their prevalence differed greatly.

Conclusions: This study may be the first to present oral microbial data which suggest SW may participate in direct or indirect bacterial interactions that influence the potential for other organisms to flourish within the oral microbiome. These data suggest that SN and SW may occupy distinct, non-overlapping niches, which may differ significantly from the interactions observed with AA, FN, and TF. Further research will be needed to more fully elucidate these interactions and to explore the potential ramifications for oral microbial ecology and the implications for predictive saliva screening.

Keywords: Scardovia wiggsiae; Selenomonas noxia; saliva screening.

ABBREVIATIONS

SW: Scardovia wiggsiae; SN: Selenomonas noxia; AA: Aggregatibacter actinomycetemcomitans; TF: T. forsythia; FN: F. nucleatum; 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

Selenomonas noxia is an organism within the *Veillonella* family, which is widely distributed among various animal species [1-3]. Although *S. noxia* is commonly found in the gastrointestinal tract, it is also found in the oral cavity and may be found in higher levels among patients with poor oral health [4-6]. The recent developments of a rapid PCR-based detection assay and anaerobic culturing conditions have made the screening for oral *S. noxia* more accessible and cost effective [7-9].

Many patients with poor oral health often harbor multiple disease-causing organisms that may contribute to one or more pathologies within the oral cavity [10-12]. Recently, a new oral pathogen *Scardovia wiggsiae* was discovered in patients with poor oral health [13,14]. Although some screenings for *Scardovia* are now beginning to emerge, much remains to be discovered about the oral ecology and microbial interactions that facilitate or inhibit the growth of this organism [15-17].

Based upon the lack of information regarding the oral microbial ecology of these specific organisms, the primary objective of this study was to screen an existing saliva repository to more accurately assess the microbial flora present (or absent) with *S. wiggsiae*, with specific emphasis on *S. noxia.*

2. METHODOLOGY

2.1 Project Approval

Approval for this study was granted by the Institutional Review Board (IRB) and the Office for the Protection of Human Subjects (OPRS) under Protocol #875879-1 "*Selenomonas noxia* prevalence in DNA previously isolated from pediatric patient saliva samples" in March 2016. The retrospective nature of this study qualified as "exempt" according to Federal regulatory statute 45CFR46.101(b).

The saliva repository was originally created under the OPRS-IRB Protocol #1305-4466M "The Prevalence of Oral Microbes in Saliva from the UNVL School of Dental Medicine Pediatric and Adult Clinical Population" approved in June 2013. In brief, a cross-sectional (one time) convenience sample of clinic patients were recruited at the University of Nevada Las Vegas (UNLV) School of Dental Medicine (SDM) pediatric dental clinic between June 2013 and June 2015. This involved the collection of unstimulated saliva from pediatric and adult patients from the University of Nevada Las Vegas (UNLV) School of Dental Medicine (SDM) dental clinic. Patients (and parents or guardians) were required to provide Informed Consent, while pediatric patients seven years or older were also required to provide Pediatric Assent (written consent to participate). Exclusion criteria

included any parent, guardian or patient (pediatric or adult) who declined to participate.

2.2 Saliva Repository

During the original saliva collection, demographic information (age, sex, and race/ethnicity) and saliva samples were obtained from 240 patients. In brief, each study participant was provided a sterile 50 mL saliva collection container with a target of collecting 5 mL. All samples were placed on ice until transfer and storage in the biomedical research laboratory. Each sample was given a unique, randomly generated unique identifier to prevent any personal or patient information from accompanying the sample outside of the clinic. No patient-specific identifying information was subsequently available to any member of this research project. No other oral health information was collected at the time of the original sample collection.

2.3 DNA Isolation

The current study involved a retrospective analysis of pediatric saliva samples currently available in the repository. All the available (remaining) samples (n=240) were located and then evaluated to ascertain if enough saliva remained for the DNA isolation required to perform the PCR screening. Out of the 240 samples identified, a smaller subset off n=162 contained sufficient volume (>0.5 mL) for inclusion in this study. Isolation of DNA was facilitated using the Amersham Bioscience (Buckinghamshire, UK) GenomicPrep DNA isolation kit. The DNA from each sample was suspended in 50 uL of DNA hydration solution from Amersham Bioscience (Buckinghamshire, UK) and stored at 4°C. Quality and quantity of the DNA isolated from each sample was calculated using measurements of absorbance at 260 and 280 nm to calculate the A260:A280 ratio.

2.4 PCR Screening

All DNA isolates with sufficient quantity (1 ng/uL or greater) and sufficient quality (A260:A280 ratio >1.65) were then screened using qPCR to assess the presence of several oral microbial species. The qPCR used an initial incubation of 50°C for 2 minutes, followed by 10 minute denaturation at 95°C and 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. The DNA positive controls were *S. noxia* reference strains obtained from American Type Culture Collection (ATCC)-43541,-51893,-700225), as previously described [7]. The positive DNA controls for *S. wiggsiae* were derived from previously identified SW-positive samples, as previously described [15,18,19]. TaqMan universal PCR master mix with the following primers from Eurofins MWG Operon (Huntsville, AL) was used, resulting in a final probe concentration of 0.2 uM with 5 uL of template (sample) DNA in each reaction. Sterile, nuclease-free distilled water from Promega (Madison, WI) was used to adjust the final reaction volume to 25 uL. Each screening was performed in duplicate.

S. noxia Forward primer- SNF1, TCTGGGCTACACACGTACTACAATG (25 bp)

S. noxia Reverse primer- SNR1, GCCTGCAATCCGAACTGAGA (20 bp)

SnP[6 ~ FAM]CAGAGGGCAGCGAGAGAGT GATCTTAAGC [TAMRA]

S. wiggsiae Forward primer-SW, GTGGACTTTATGAATAAGC (19 bp)

S. wiggsiae Reverse primer- SW, CTACCGTTAAGCAGTAAG (18 bp)

SwP[6 ~ FAM] 5'-AGCGTTGTCCGGATTTATT-3'G [TAMRA]

The selected probes (SnP, SwP) were labeled with the reporter dye 6-carboxyfluorescein (FAM) at the 5'-end and with the reporter dye tetramethyl-6-carboxyrhodamine (TAMRA) at the 3'-end.

2.5 Statistical Analysis

The information regarding this retrospective sample were summarized using simple descriptive statistics. Results were analyzed using Chi Square ($\chi 2$) analysis software from GraphPad (San Diego, CA) using a significance level of $\alpha = 0.05$.

To determine the appropriate sample size for this type of PCR screening for microbial composition using DNA extracted from saliva, the recovery rate from the sample-limited step of DNA extraction was used (90-95%) to establish the minimum expected difference of 0.10 or 10%. Using a significance level of $\alpha = 0.05$ and a power $p = 0.80$, a minimum sample size of forty (N = 40) was calculated [20].

3. RESULTS

All potential samples (n=162) were identified and their demographic information was compiled for analysis (Table 1). In brief, the study sample was comprised of nearly equal numbers of males and females, which is similar to the patient composition from the clinic population. The demographic analysis by race or ethnicity, however, revealed the overwhelming majority of samples were derived from minority patients (mostly Hispanic), which is higher than the proportion of minorities from the clinic population. The ages of patients included in the study sample ranged from 5 – 73 years of age, compared with 2 – 91 years of age from the general clinic population.

DNA from each of the study samples was then extracted, which revealed many samples had either insufficient quantity or insufficient quality for further processing (Table 2). More specifically, out of the total number of potential samples identified, only n=42/162 or 25.9% had sufficient DNA quality with an absorbance ration (A260:A280) ratio within the manufacturer range 1.70 – 2.00 and sufficient DNA quantity (100-1000 ng/uL) for further processing.

Each of the samples containing DNA of sufficient quantity and quality was then processed using qPCR (Fig. 1). The analysis of this screening revealed that only a small proportion of the overall study sample harbored DNA specific for *S. noxia* (n=6/42 or 14.3%). These data were significantly different for DNA specific to *S. wiggsiae*, which was found in a much higher number of patient samples (n=27/42 or 64.3%). These results were then verified by subsequent screening in duplicate. Chi-square ($\chi 2$) analysis revealed these differences are statistically significant ($p<0.0001$).

More detailed analysis revealed that none of the samples that tested positive for *S. wiggsiae* harbored DNA for *S. noxia* (Fig. 2). Conversely, the samples that tested positive for *S. noxia* also tested negative for *S. wiggsiae*. In addition, although *A. actinomycetemcomitans* (AA) was only present in a small subset of samples – this organism was only found among SW-positive samples. Other organisms, including *T. forsythia* (TF), and *F. nucleatum* (FN) were present in both SW-positive and SW-negative samples although their prevalence differed greatly.

4. DISCUSSION

The primary objective of this study was to screen an existing saliva repository to more accurately assess the microbial flora present (or absent) with *S. wiggsiae*, with specific emphasis on *S. noxia.* This screening revealed that more than half of the samples evaluated harbored *S. wiggsiae*, with a much smaller subset of samples testing positive for *S. noxia*. Interestingly, none of the *Scardovia*-positive samples had detectable levels of DNA for *S. noxia*, while none of the *Selenomonas*-positive samples tested positive for *S. wiggisae*.

Table 1. Study sample demographic information

	Sample (n=162)	UNLV-SDM clinic
Sex		
Female	n=87 (53.7%)	50.9%
Male	n=75 (46.3%)	49.1%
Race/Ethnicity		
White	n=26 (16.0%)	41.4%
Minority	n=136 (83.9%)	58.6%
Hispanic	n=91 (56.2%)	35.9%
Black	n=24 (14.8%)	13.1%
Asian/Other	n=21 (13.0%)	4.2%
Age range	5 – 73 yrs.	2 – 91 yrs.

Table 2. Analysis of DNA from study sample

	DNA recovery	DNA quantity	DNA quality
Study samples	n=42/162 (25.9%)	631.2 ng/uL+/-51.3	A260:A280 ave=1.72
Manufacturer	90-95%	100-1000 ng/uL	A260:A280 1.70-2.00

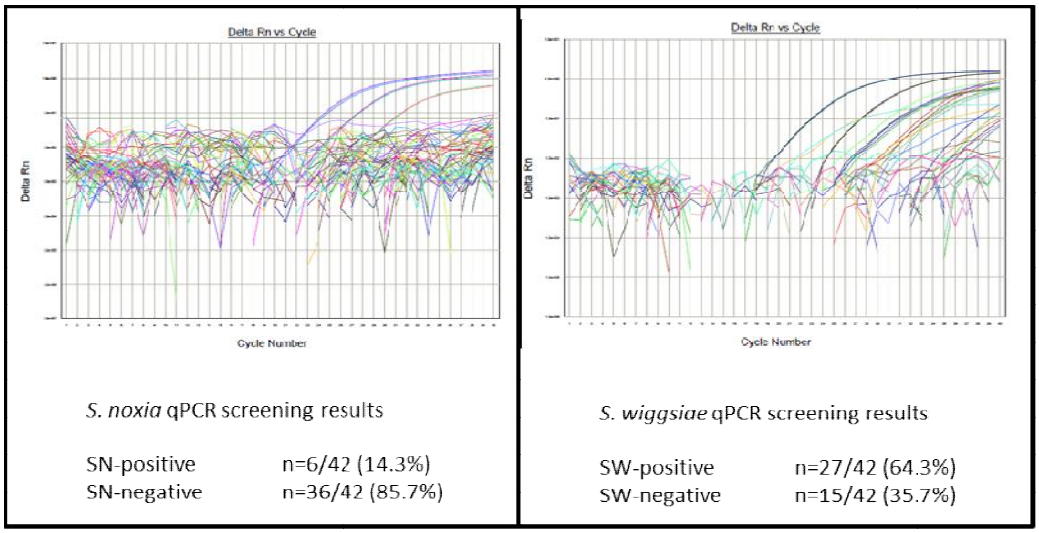
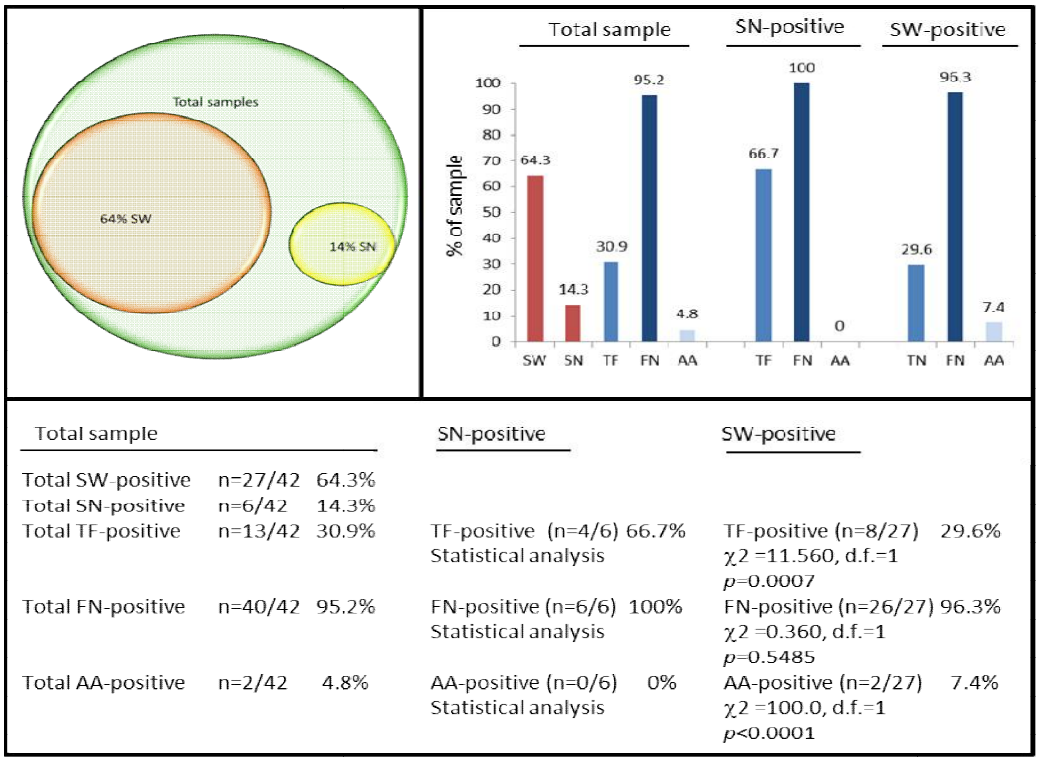


Fig. 1. qPCR screening for Selenomonas and Scardovia. qPCR screening of patient samples revealed differing prevalence of these organisms, with 14.3% of patient samples testing positive for DNA from *S. noxia*, while 64.3% tested positive for *S. wiggsiae*

Chi-square ($\chi 2$) analysis revealed these differences are statistically significant, $\chi 2=108.507$, d.f.=1, $p<0.0001$



Total sample			SN-positive	SW-positive
Total SW-positive	n=27/42	64.3%		
Total SN-positive	n=6/42	14.3%		
Total TF-positive	n=13/42	30.9%	TF-positive (n=4/6) 66.7% Statistical analysis	TF-positive (n=8/27) 29.6% $\chi 2$ =11.560, d.f.=1 p=0.0007
Total FN-positive	n=40/42	95.2%	FN-positive (n=6/6) 100% Statistical analysis	FN-positive (n=26/27) 96.3% $\chi 2$ =0.360, d.f.=1 p=0.5485
Total AA-positive	n=2/42	4.8%	AA-positive (n=0/6) 0% Statistical analysis	AA-positive (n=2/27) 7.4% $\chi 2$ =100.0, d.f.=1 p<0.0001

Fig. 2. Analysis of SW- and SN-positive samples. Detailed analysis revealed that SW-positive samples (64.3%) did not harbor SN, while SN-positive samples (14.3%) did not harbor SW. Differential results were obtained from screening for other organisms, including *T. forsythia* (TF; 30.9% overall, 66.7% SN$^+$, 29.6% SW$^+$), *F. nucleatum* (FN; 95.2% overall, 100% SN$^+$, 96.3% SW$^+$) and *A. actinomycetemcomitans* (AA; 4.8% overall, 0% SN$^+$, 7.4% SW$^+$)

Chi square analysis revealed these differences were statistically significant for both TF and AA but not FN ($p<0.05$)

These results may suggest that some oral microbial communities may facilitate the growth of specific species, while inhibiting the growth of others [21,22]. This study found that *A. actinomycetemcomitans* was found only among the *Scardovia*-positive samples. However, differential results were observed for *F. nucleatum*, and *T. forsythia*. These findings only strengthen the evidence for the hypothesis that specific microbial constituents such as *Scardovia* may compete with or inhibit the growth of some species, while facilitating or enhancing the growth of others [23,24]. While more evidence will be needed to validate these findings, this study may be the first to present oral microbial data which suggest that either *S. wiggsiae* or *S. noxia* (or possibly both) may participate in direct or indirect bacterial interactions that influence the potential for other organisms to flourish within the oral microbiome.

Due to the retrospective nature of this study, some limitations were inherent and should also be considered. For example, the length of storage time for these saliva samples may have varied greatly, which has been demonstrated to significantly affect both the quality and quantity of DNA isolates [25,26]. Future studies to confirm these observations may be prospective in nature, which would reduce the complications associated with long-term storage and sample degradation - and would also allow for the collection of oral (and other) health information, which may be useful to other oral health researchers. In addition, the higher proportion of minority patient samples from this public dental school patient population may also have influenced these results – mainly due to the limited resources and low socioeconomic status of the majority of these clinic patients [27-29].

5. CONCLUSIONS

These data suggest that *S. noxia* and *S. wiggsiae* may occupy distinct, non-overlapping niches, which may differ significantly from the interactions observed with *A. actinomycetemcomitans*, *F. nucleatum*, and *T. forsythia*. Further research will be needed to more fully elucidate these interactions and to explore the potential ramifications for oral microbial ecology and the implications for predictive saliva screening.

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.
   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.
   PMID: 2777378
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.
   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.
   PMID: 8814590
5. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25(2):134-44.
   PMID: 9495612
6. Boström L, Bergström J, Dahlén G, Linder LE. Smoking and subgingival microflora in periodontal disease. J Clin Periodontol. 2001;28(3):212-9.
   PMID: 11284533
7. Cruz P, Mehretu AM, Buttner MP, Trice T, Howard KM. Development of a polymerase chain reaction assay for the rapid detection of the oral pathogenic bacterium, *Selenomonas noxia*. BMC Oral Health. 2015;15:95.
   DOI: 10.1186/s12903-015-0071-1
   PMID: 26272608
8. Tanner AC. Anaerobic culture to detect periodontal and caries pathogens. J Oral Biosci. 2015;57(1):18-26.
   PMID: 25678835
9. 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 Car. 2017;1:1009.

10. Tanner AC, Mathney JM, Kent RL, Chalmers NI, Hughes CV, Loo CY, Pradhan N, Kanasi E, Hwang J, Dahlan MA, Papadopolou E, Dewhirst FE. Cultivable anaerobic microbiota of severe early childhood caries. J Clin Microbiol. 2011;49(4):1464-74.
DOI: 10.1128/JCM.02427-10
PMID: 2128915
11. Tanner AC, Kent RL Jr, Holgerson PL, Hughes CV, Loo CY, Kanasi E, Chalmers NI, Johansson I. Microbiota of severe early childhood caries before and after therapy. J Dent Res. 2011;90(11):1298-305.
DOI: 10.1177/0022034511421201
PMID: 21868693
12. Tanner AC, Sonis AL, Lif Holgerson P, Starr JR, Nunez Y, Kressirer CA, Paster BJ, Johansson I. White-spot lesions and gingivitis microbiotas in orthodontic patients. J Dent Res. 2012;91(9):853-8.
DOI: 10.1177/0022034512455031
PMID: 22837552
13. Downes J, Mantzourani M, Beighton D, Hooper S, Wilson MJ, Nicholson A, Wade WG. *Scardovia wiggsiae* sp. nov., isolated from the human oral cavity and clinical material, and emended descriptions of the genus Scardovia and *Scardovia inopinata*. Int J Syst Evol Microbiol. 2011;61(Pt 1):25-9.
DOI: 10.1099/ijs.0.019752-0
PMID: 20139283
14. Henne K, Rheinberg A, Melzer-Krick B, Conrads G. Aciduric microbial taxa including *Scardovia wiggsiae* and *Bifidobacterium* spp. in caries and caries free subjects. Anaerobe. 2015;35(Pt A):60-5.
DOI: 10.1016/j.anaerobe.2015.04.011
PMID: 25933689
15. 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.
PMID: 27805882
16. Richards VP, Alvarez AJ, Luce AR, Bedenbaugh M, Mitchell ML, Burne RA, Nascimento MM. Microbiomes of site-specific dental plaques from children with different caries status. Infect Immun. 2017;85(8). pii: e00106-17.
DOI: 10.1128/IAI.00106-17
PMID: 28507066
17. Eriksson L, Lif Holgerson P, Johansson I. Saliva and tooth biofilm bacterial microbiota in adolescents in a low caries community. Sci Rep. 2017;7(1):5861.
DOI: 10.1038/s41598-017-06221-z
PMID: 28724921
18. Catmull J, Row L, Repp MR, Heslington C, Miller T, Diamond J, Howard KM, Kingsley K. Newly identified cariogenic pathogen *Scardovia wiggsiae* detected by polymerase chain reaction in saliva of teenagers and adults in Southern Nevada. Forum for Dental Student Research and Innovation (FDSRI). 2014;22-29.
19. Streiff BJ, Seneviratne M, Kingsley K. Screening and prevalence of the novel Cariogenic pathogen *Scardovia wiggsiae* among Adult Orthodontic and Non-Orthodontic Patient Saliva Samples. International Journal of Dentistry and Oral Health (IJDOH). 2015;1(6).
20. Hays WL. Statistics. 5. International Thomson Publishing. Inferences about population means. 1994;311–42.
21. Zaura E, Keijser BJ, Huse SM, Crielaard W. Defining the healthy "core microbiome" of oral microbial communities. BMC Microbiol. 2009;9:259.
DOI: 10.1186/1471-2180-9-259
22. Lazarevic V, Whiteson K, Hernandez D, François P, Schrenzel J. Study of inter- and intra-individual variations in the salivary microbiota. BMC Genomics. 2010;11:523.
DOI: 10.1186/1471-2164-11-523
23. Wade WG. The oral microbiome in health and disease. Pharmacol Res. 2013; 69(1):137-43.
DOI: 10.1016/j.phrs.2012.11.006
24. Zarco MF, Vess TJ, Ginsburg GS. The oral microbiome in health and disease and the potential impact on personalized dental medicine. Oral Dis. 2012;18(2):109-20. DOI: 10.1111/j.1601-0825.2011.01851.x
25. Zarco MF, Vess TJ, Ginsburg GS. The oral microbiome in health and disease and the potential impact on personalized dental medicine. Oral Dis. 2012;18(2):109-20. DOI: 10.1111/j.1601-0825.2011.01851.x
26. Ng DP, Koh D, Choo SG, Ng V, Fu Q. Effect of storage conditions on the extraction of PCR-quality genomic DNA

from saliva. Clin Chim Acta. 2004; 343(1-2):191-4.
27. Streiff BJ, Kingsley K. Orthodontic care in a community of underserved patients: A public dental school analysis. Health Sciences Research 2015;2(4):19-24.
28. Derisse D, Archer W, Kingsley K. From Theory to practice: Analysis of a model to provide access to Preventive Dental Care (PDC) services for medicaid, low-income, and minority children at a Nevada Dental Scholl-Based Clinic. Journal of Theory and Practice of Dental Public Health. 2013; 1(4):11-15.
29. Jang S, Spader ET, Thacker M, Cochran CR, Bungum TJ, Chino M, Kingsley K. Access to care for pediatric, medicaid-insured patients in Clark County, Nevada. Journal of Theory and Practice of Dental Public Health. 2013;1(2):37-43.

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