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Prevalence of *Selenomonas noxia* among Pediatric and Adult Orthodontic Patients

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Authors' contributions

This work was carried out in collaboration among all authors. Author KK was responsible for sample collection and experimental protocol. Authors KK and ND were responsible for DNA isolation and PCR screening, as well as project design and funding. Authors ND, KK and KMH participated in data analysis and manuscript preparation. All authors read and approved the final manuscript.

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ABSTRACT

Pediatric patients face many challenges to oral and periodontal health, including the placement of fixed orthodontic appliances during adolescence. One of the more recently identified periodontal pathogens is the organism *Selenomonas noxia* or *S. noxia*.

Objectives: Due to the paucity of evidence regarding the oral prevalence of *S. noxia* and the lack of evidence regarding the prevalence among pediatric orthodontic patients, the main objective of this project was to evaluate the oral prevalence in a dental school setting.

Methods: Using an existing saliva repository, twenty five (n=25) orthodontic saliva samples were selected from patients between the ages of 13 – 24 with twenty five (n=25) age-matched non-orthodontic saliva samples. DNA isolation was performed and screened with primers specific for *S. noxia*. Chi square analysis of demographic groups was performed and descriptive statistics of all results was reported.

Results: Screening of each DNA derived from each saliva sample for *S. noxia* revealed the presence of this pathogen in a subset of the study population. More specifically, the majority of samples screened (60% or n=30/50) did not harbor DNA for this organism. Most of the *S. noxia*-

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positive samples were derived from adults (65% or n=13/20) with more females (60%) than males, which were nearly equally divided among Orthodontic and non-Orthodontic patients.
Conclusions: This study provides novel information regarding the oral prevalence of *S. noxia* among both pediatric and young adult populations, with and without orthodontic brackets. These findings demonstrate that higher percentages of adults than pediatric patients harbor this organism, which does not appear strongly correlated with orthodontic treatment. These data add to the growing body of evidence that may suggest the presence of this organism may be associated with many additional factors that influence oral health and disease.

Keywords: Selenomonas noxia; prevalence; orthodontic treatment; saliva screening.

ABBREVIATIONS

OPRS : Office for the Protection of Research Subjects
IRB : Institutional Review Board
DNA : Deoxyribonucleic Acid
PCR : Polymerase Chain Reaction
ATCC : American Type Culture Collection
GAPDH : Glyceraldehyde- 3- phosphate dehydrogenase
Tm : Primer Melting Temperature
LOD : Limit of Detection

1. INTRODUCTION

Pediatric patients face many challenges to oral and periodontal health, including the placement of fixed orthodontic appliances during adolescence [1,2]. Although many studies have evaluated the effectiveness of various interventions on the outcomes of caries and periodontal disease, fewer of these studies have focused specifically on particular pathogens [3,4]. The question then remains whether these previously identified periodontal pathogens are more prevalent during orthodontic treatment [5,6].

One of the more recently identified periodontal pathogens is the organism *Selenomonas noxia* or *S. noxia* [7,8]. *Selenomonas* species are gram-negative obligate anaerobic microbes, some of which have been identified as periodontal pathogens [9-11]. These organisms, including *S. noxia*, have been identified in patients with severe or aggressive periodontitis [12-14].

Interestingly, this organism has recently been associated with other health conditions, including obesity and arthritis-induced bone loss [15,16]. However, despite these many disease associations – few studies have evaluated the prevalence of this organism [17,18]. Due to the development of a rapid screening assay that can function using DNA isolated from saliva, recent efforts from this group have attempted to assess prevalence among a dental school population – although no evaluation of pediatric orthodontic patients has yet been attempted.

Due to the paucity of evidence regarding the oral prevalence of *S. noxia* and the lack of evidence regarding the prevalence among pediatric orthodontic patients, the main objective of this project was to evaluate the oral prevalence using saliva samples derived from these patient populations in a dental school setting.

2. METHODS

2.1 Study Approval

This retrospective study was reviewed by the Office for the Protection of Research Subjects (OPRS) and the Institutional Review Board (IRB) at the University of Nevada, Las Vegas (UNLV). The exemption for this study OPRS#880427-1 was titled “The prevalence of oral microbes in saliva from the UNLV School of Dental Medicine pediatric and adult clinical population.

2.2 Sample Selection

The original protocol for saliva collection involved Informed Consent (adult) and Pediatric Assent (pediatric) prior to unstimulated saliva collection. The original collection period for these samples took place between July 2015 and July 2018. In brief, the inclusion criteria were pediatric patients aged seven (7) years or older and their parents or guardians who agreed to participate. Pediatric assent and Parental permission to consent for voluntary participation were obtained at the time of study enrollment. Adult patients were recruited from the general clinic and provided Informed Consent. Exclusion criteria included any person (pediatric or adult) that was not a patient of record at UNLV-SDM, any patients who declined to participate, and any parent or guardian that declined to let their child participate.

Saliva samples were obtained in sterile 50 ml collection tubes and transported to the biomedical laboratory for storage (-80C) and future analysis. Each sample was assigned a randomly generated, non-duplicated identifier that prevented any person from directly or indirectly linking a specific sample to any patient identifying information. Limited demographic information was concurrently collected, which provided Sex, Age, Race or Ethnicity (if voluntarily provided) and whether or not the patient had orthodontic brackets.

For this study, a total of fifty (n=50) saliva samples were selected for screening. This study population involved the first, randomly selected twenty five (n=25) orthodontic saliva samples with twenty five (n=25) age-matched non-orthodontic saliva samples, selected from patients between the ages of 12 – 24. Pediatric samples from patients aged 0-18 years and adults aged 19 to 91 were eligible for inclusion in this study.

2.3 DNA Isolation

The selected samples were thawed for subsequent DNA isolation using the GenomicPrep DNA isolation kit using the protocol outlined by the manufacturer, as previously described [19,20]. The DNA from each sample was then analyzed for purity and concentration using a NanoDrop spectrophotometer at absorbances of 230, 260 and 280 nm. Samples with a concentration > 1 ng/uL and A260:A280 ratio above 1.55 were then screened for *S. noxia*.

2.4 PCR Screening

In brief, qPCR used initial incubation of 50°C for 120 seconds, followed by denaturation at 95°C for ten minutes and 40 cycles, consisting of 95° C for 15 seconds and 60°C for 60 seconds. Positive control human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and standards were derived from American Type Culture Collection (ATCC) *S. noxia* reference strains ATCC-43541, -51893, and -700225), as previously described [18,19].

Positive control:

Glyceraldehyde- 3- phosphate dehydrogenase (GAPDH)
GAPDH 5'-ATCTTCCAGGAGCGAGATCC-3' (sense); 20 nt; 55% GC; Tm=66°C
GAPDH 5'-ACCACTGACACGTTGGCAGT-3' (antisense); 20 nt; 55% GC; Tm=70°C
Optimal PCR Primer melting temperature (Tm): 65°C.

Forward primer- SNF1,
TCTGGGCTACACACGTACTACAATG (25 bp)
Reverse primer- SNR1,
GCCTGCAATCCGAACTGAGA (20 bp)
SnP[6 ~
FAM]CAGAGGGCAGCGAGAGAGTGATCTTAA GC [TAMRA]

The selected probe (SnP) was 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

Basic demographic information regarding the study sample (age, sex, race or ethnicity) were compiled and presented using simple descriptive statistics (counts and percentages). Any statistical differences between the demographic groups were determined using Chi square analysis, which is appropriate for non-parametric data.

3. RESULTS

The demographic analysis of the study sample (n=50) was performed using the clinic population for reference (Table 1). These data demonstrated that the study sample was comprised of approximately half females (52%) and half males (48%), which was not significantly different than the overall percentages in the clinic population (50.9% and 49.1%, respectively), p=0.4865. However, the proportion of samples from minority patients in the study sample (72%) was significantly higher than the percentage from the clinic population (58.6%), p=0.0001. The majority of these patients were Hispanic in both the study sample (56%) and the clinic (35.9%).

The samples derived from pediatric patients ranged in age from 12 – 17 years with an average age of 13.25 years, which is slightly older than the pediatric clinic population average of 10.14 years. The average age of the adult samples was 21.57 years with a range of 18 – 24 years, which is much younger than the overall clinic population average of 52.3 years.

Each of the samples was then processed to extract DNA for the subsequent screening

(Table 2). These data demonstrated that DNA was successfully extracted from all samples (n=50) resulting in a yield of 100 (n=50/50), which approximates the range estimated by the manufacturer protocol (90-95%). The concentration of the samples was approximately 500 ng/ul, which was similar from both the pediatric (502.1 ng/ul) and adult (493.2 ng/ul) patient samples. The purity of the DNA isolates measured by the absorbance ratio of A260 nm and A280 nm demonstrated that all samples were of sufficient quality to proceed with the PCR screening.

Screening of each DNA derived from each saliva sample for *S. noxia* revealed the presence of this pathogen in a subset of the study population (Fig. 1). More specifically, the majority of samples screened (60% or n=30/50) did not harbor DNA for this organism. In addition, most of the *S. noxia*-positive samples were derived from adult patients (65% or n=13/20). Finally, the majority of positive samples appeared in the 14-17 age range for pediatric patients and the younger age ranges 18 – 25 for the adult patients.

To more thoroughly evaluate these results, a demographic analysis of the *S. noxia*-positive and *S. noxia*-negative samples was performed (Table 3). This analysis revealed that the majority of positive samples were derived from adult patients (65%) rather than pediatric patient samples (35%). In addition, there was a slightly higher proportion of females with positive samples (60%) than males (40%). Slightly less than half of the positive samples came from Orthodontic patients (45%).

Table 1. Demographic analysis of sample study

	Study sample (n=50)	Clinic population	Statistical analysis
Sex			
Female	52.0% (n=26)	50.9%	χ2=0.484, d.f.=1
Male	48.0% (n=24)	49.1%	p=0.4865
Race / Ethnicity			
White	28.0% (n=14)	41.4%	χ2=74.014, d.f.=1
Minority	72.0% (n=36)	58.6%	p=0.0001
Hispanic	56.0% (n=28)	35.9%	
Black	8.0% (n=4)	13.1%	
Asian / Other	8.0% (n=4)	9.6%	
Age			
Pediatric	Range: 12 – 17 yrs.	Range: 0 – 17 yrs.	
(n-26)	Ave.=15.25 yrs.	Ave.=10.14 yrs.	
Adult	Range: 18 – 26 yrs.	Range: 18 – 91 yrs.	
(n=24)	Ave.=21.57 yrs.	Ave.=52.3 yrs.	

Table 2. DNA isolation and study sample analysis

	DNA concentration	DNA purity	Recovery/yield
Study sample	499.52 ng/ul +/- 70.3	A260:A280=1.72	100% (n=50)
Pediatric samples	502.1 ng/ul	A260:A280=1.71	
Adult samples	493.2 ng/ul	A260:A280=1.74	
Manufacturer range	100 – 1000 ng/ul	1.70-2.00	90-95%

Table 3. Demographic analysis of *S. noxia*-positive and *S. noxia*-negative samples

	S. noxia-positive (n=20)	*S. noxia*-negative (n=30)
Sex		
Female	60.0% (n=12)	46.7% (n=14)
Male	40% (n=8)	53.3% (n=16)
Age status		
Pediatric	35.0% (n=7)	63.3% (n=19)
Adult	65.0% (n=13)	36.7% (n=11)
Clinic status		
Orthodontic	45.0% (n=9)	53.3% (n=16)
Non-Orthodontic	55.0% (n=11)	46.7% (n=14)

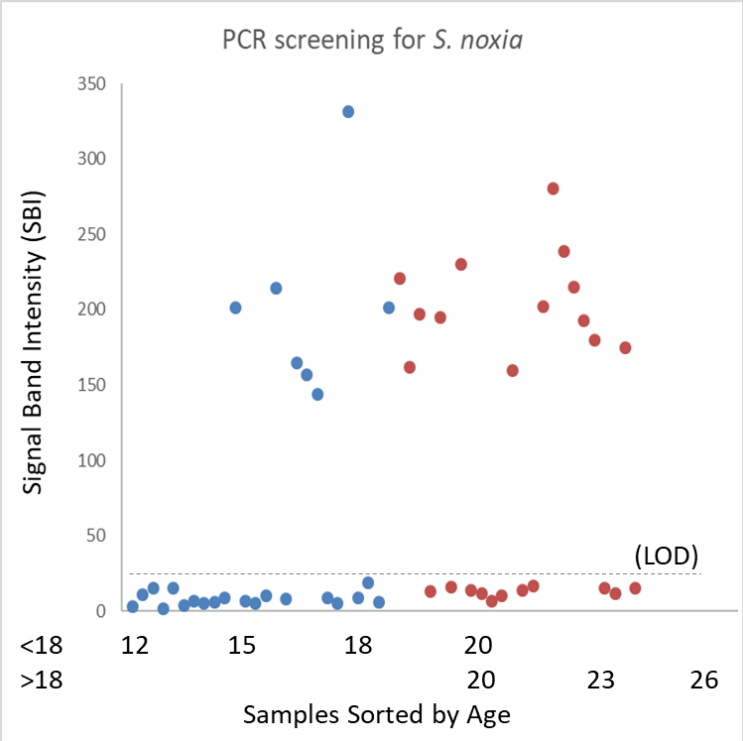

Fig. 1. PCR screening of samples for *S. noxia*. The majority of samples were S. noxia-negative (60%, n=30/50). The *S. noxia*-negative samples were nearly equally divided among females and males, as well as orthodontic and non-orthodontic patients. However, the majority of *S. noxia*-positive samples above the reliable limit of detection (LOD) were derived from adult (65% or n=13/20) versus pediatric (35% or n=7/20) patients

However, the analysis of negative samples revealed that most of these samples were derived from pediatric patients (63.3%). In addition, slightly more than half were also Orthodontic patients (53.3%). Finally, slightly more than half of the negative samples were derived from male patients (53.3%).

4. DISCUSSION

Due to the paucity of evidence regarding the oral prevalence of *S. noxia* and the lack of evidence regarding the prevalence among pediatric orthodontic patients, the main objective of this project was to evaluate the oral prevalence using saliva samples. This retrospective study was successful in identifying existing saliva samples from dental school patient populations, isolating DNA from each sample and subsequently screening for *S. noxia* using PCR.

These results have some similarities and differences with recent studies from this institution. For example, one recent study found no *S. noxia* among 54 pediatric patient samples – although the average ages of those patients were significantly younger (9.25 yrs.) than patients in the current study (15.25 yrs.) [19]. This may suggest that this organism (like many other periodontal organisms) appears in greater numbers during the onset of puberty and adolescence [11-13]. In addition, the lack of association with orthodontic treatment may also suggest the presence of this organism may not be strongly correlated with these procedures and that other factors, such as hormone levels or oral hygiene practices may, in fact, be stronger predictors [4-6].

Although this study provides novel information regarding the oral prevalence of this organism in these patient populations, there are some limitations inherent to this type of study that should also be considered in context. First, this was a retrospective study of previously collected salivary samples. Although every effort was made to reduce research bias of any kind, many types of bias exist in cross sectional (one-time sampling) studies – including the lack of temporal (before and after) information regarding patient health and microbial levels. In addition, the willingness of patients to participate in any study

(pediatric or adult) may also lead to selection bias that could also significantly influence the results from this type of study. Finally, the lack of other health information (such as weight, body mass index, or neck circumference) was not available, with some studies suggesting that *S. noxia* may be more strongly associated with obesity and periodontal disease than periodontal disease alone [21,22].

5. CONCLUSIONS

This study provides novel information regarding the oral prevalence of *S. noxia* among both pediatric and young adult populations, with and without orthodontic brackets. These findings demonstrate that higher percentages of adults than pediatric patients harbor this organism, which does not appear strongly correlated with orthodontic treatment. These data add to the growing body of evidence that may suggest the presence of this organism may be associated with many additional factors that influence oral health and disease.

CONSENT

Pediatric assent and Parental permission to consent for voluntary participation were obtained at the time of study enrollment. Adult patients were recruited from the general clinic and provided Informed Consent.

ETHICAL APPROVAL

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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