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Scardovia Wiggsiae Prevalence in Orthodontic Patients

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SCARDOVIA WIGGSIAE PREVALENCE IN ORTHODONTIC PATIENTS

By

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Bachelor of Arts – Medical biology
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Abstract

Scardovia Wiggsiae prevalence in orthodontic patients

By

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Dental caries has mainly been associated with *Streptococcus*, *Lactobacillus*, *Actinomyces* and *Veillonella* species. But in recent salivary studies a new cariogenic pathogen, has been identified. This new bacteria, *Scardovia wiggsiae (*SW*)*, is currently being tested within the UNLV School of Dental Medicine patient population. Although these current studies are being conducted to study its prevalence in both pediatric and adult populations, it has not been evaluated among patients with an altered oral environment as seen in patients with orthodontic appliances. Fixed orthodontic appliances increase the difficulty of removing daily plaque on and in between the teeth with standard oral hygiene practices. Approximately 73% of orthodontic patients get at least one new lesion during orthodontic care. Understanding the cause of cavities and the key bacteria involved in patients with orthodontic appliances will help us learn how to best evaluate the risk of caries during orthodontic treatment and design strategies for reducing or preventing this disease process. The initial focus of this study will be to assess health parameters

among orthodontic patient samples for comparison with non-orthodontic patients. We will also compare SW prevalence among the orthodontic patient samples with samples taken from non-orthodontic patients. Other microbial prevalence data will also be concurrently evaluated, including *S. mutans*, and *P. gingivalis* prevalence – which will also be analyzed in conjunction with the aforementioned health parameters.

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Chapter 1: Introduction

Background and Significance

Nearly all of the general population is affected by dental caries [1, 2] and it has been mainly associated with *Streptococcus*, *Lactobacillus*, *Actinomyces* and *Veillonella* species [3-5]. But in recent salivary studies a new cariogenic pathogen has been identified as acidophilic and acid producing bacteria, contributing to severe caries in individuals who test negative for the presence of *Streptococcus mutans* [6-7]. The prevalence of the recently discovered bacterium *Scardovia wiggsiae (*SW*)* within the UNLV School of Dental Medicine population has been tested. Although these current studies are being conducted to study its prevalence in both pediatric and adult populations, it has not been evaluated among patients with an altered oral environment as seen in patients with orthodontic appliances.

Fixed orthodontic appliances increase the difficulty of removing daily plaque on and in between the teeth with standard oral hygiene practices. They also impede the oral cavity's ability to remove plaque and food particulates through natural salivary flow and tongue and buccal tissue movements. When this plaque, filled with cariogenic bacteria and food particulates, is left on the teeth for longer than average periods of time, caries risk will be increased. Approximately 73% of orthodontic patients get at least one new lesion during orthodontic care [8, 9]. Understanding the cause of cavities and the key bacteria involved in patients with orthodontic appliances will help us learn how to best evaluate the risk of caries during orthodontic treatment and design strategies for reducing or preventing this disease process.

Previous studies at UNLV's School of Dental Medicine have collected oral and other health data, as well as saliva samples to further our understanding of oral health and disease. The initial focus of this study will be to assess these health parameters among orthodontic patient samples for comparison with non-orthodontic patients. These health parameters will provide a more

comprehensive analysis of health status and oral disease risk to provide a greater understanding of risk among UNLV-SDM orthodontic patients more specifically.

The primary focus of this study will then entail comparison of SW prevalence among the orthodontic patient samples for comparison with samples taken from non-orthodontic patients. Other microbial prevalence data will also be concurrently evaluated, including *S. mutans*, and *P. gingivalis* prevalence, which will also be analyzed in conjunction with the aforementioned health parameters.

Methods and Materials

A retrospective analysis of previously collected saliva samples from orthodontic patients will be used for comparison with age-matched samples from non-orthodontic patients. Samples from the previous study (Protocol OPRS#1305-4466M: The Prevalence of Oral Microbes in Saliva from the UNLV School of Dental Medicine pediatric and adult clinical population) approved May 22, 2013 will be used (n=190).

In brief, patients from the pediatric, orthodontic, and general UNLV-SDM clinics were asked to participate in the study. Subjects who agreed to participate were given a small, sterile saliva collection container, 50 mL sterile polypropylene tube (Fisher Scientific: Fair Lawn, New Jersey, USA) and asked to spit into it for a full minute. Samples were stored on ice until transport to a biomedical laboratory for analysis. Each saliva sample was assigned a unique, randomly-generated number to prevent research bias. On all subjects the following data was collected concurrently; gender, race/ethnicity, age, and number of decayed missing or filled teeth (DMFT). For this project, samples will be sorted by age into Preteen (<13), Adolescent (13-17) and Adult (>18) categories and then further separated into orthodontic and non-orthodontic patients. DNA will be isolated from these samples and will subsequently be screened for SW using polymerase

chain reaction (PCR) and primers specifically designed to distinguish this organism [10]. Results from the orthodontic patients will be compared to those from non-orthodontic patients and will be analyzed for any significance in presence based on gender, race/ethnicity, age, and number of decayed missing or filled teeth (DMFT), as well as other relevant health parameters.

Research Question

1. Does the health status or oral health parameters of UNLV-SDM orthodontic patients differ from those of age-matched non-orthodontic patients?

H_0: Orthodontic patients will have similar health and oral health parameters to non-orthodontic, age-matched controls

H_A: Orthodontic patients will have different health or oral health parameters to non-orthodontic, age-matched controls

2. Does the prevalence of S. wiggsiae vary between orthodontic and non-orthodontic patients?

H_0: Orthodontic and non-orthodontic patients will have similar prevalence of *S. wiggsiae*.

H_A: Orthodontic and non-orthodontic patients will have different prevalence of *S. wiggsiae*.

In addition to SW, other microbial agents can be evaluated and assessed to provide a more comprehensive and complete view of the oral health status of these patient samples. To date, no such preliminary analysis or pilot study has been attempted at UNLV-SDM.

Research Design

The primary research design of this study was retrospective and observational. An IRB exemption was filed to work with existing (already collected) saliva samples for analysis. The main outcome variable consisted of a binary PCR screening result: positive (+) or negative (-); Additional information can be evaluated regarding relative levels (CFU/mL of saliva). The main

predictor variable consisted of Orthodontic treatment. The confounding variables consisted of demographic variables including age, gender, race/ethnicity, and some basic clinical and health information (BMI, oral lesions, DMFT score).

Statistical Analysis

Because the difference in prevalence between groups (Orthodontic, non-Orthodontic) are to be measured from a cross-section of samples taken from a cohort or convenience sample, a preliminary analysis using a two-tailed t-test can be reasonably employed to discern any statistical difference. As long as the sample size is at least moderate from each group (~20), quite severe departures from normality make little practical difference in the conclusions reached from these analyses. In addition with a sample size of (~20) a chi-square can easily be used to discern any statistical correlation between prevalence and age of the patient.

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Chapter 2

Orthodontic care in a community of underserved patients: a public dental school analysis.

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Role of Authors:
Dr. Brandon Streiff designed the study, was the primary author, data collector and analyzer, and graphics generator. Dr Karl Kingsley was secondary author and assisted with data analysis.

Abstract

Orthodontic treatment in the United States has become commonplace, with the prevalence approaching one fifth of adolescents and teenagers. Ethnic minorities are significantly less likely to have orthodontic treatment, however these trends are starting to improve in recent years. Although much is known about adolescent oral and dental health during orthodontic treatment, much less is known about adult oral and dental health among the growing population of young minority adults seeking orthodontic treatment. Therefore, this study sought to analyze the demographic composition of the patient population within a recently opened public dental school-based Orthodontic program to determine if minority and low-income residents are being served and to evaluate some general parameters of oral health. Using Medicaid, Census and aggregate patient data, these analyses revealed that UNLV-SDM currently serves a large percentage of Medicaid and CHIP patients (>62%), much higher than in the local community (~37%). Moreover, minority patients in the Main (~59%), Orthodontic (~65%) and Pediatric (~82%) clinics are also much higher than the local population (~48%). These analyses strongly suggest that UNLV-SDM is currently meeting the mandate to provide services to low-income,

Medicaid and Minority patients. Finally, the analysis of oral health parameters revealed that Minority patients were more likely to have significantly elevated markers for oral disease than non-Minority patients. These data may be among the first to elucidate the oral health problems facing this patient population and may provide more in depth prevention and treatment options for patients that face barriers to heath information and social access.

Key words: Dental, Orthodontics, Underserved and Minority Patients

1. Background

Orthodontic treatment and care in the United States has become commonplace, with the prevalence approaching one fifth of adolescents and teenagers (1). In addition, nearly 1% of young adults (18 to 30 years old) surveyed were in orthodontic treatment in a recent cross-sectional analysis (2). Although males and females are nearly equally represented among those receiving orthodontic care and treatment, racial and ethnic minorities (Black and Hispanic children, in particular) were found to have significantly lower odds of having made any type of orthodontic visit (3).

The data reflect another recent study that have found large shortages of minority graduate dental residents, which revealed nearly three quarters of all Orthodontic residents were non-Hispanic Whites (4). Furthermore, additional research regarding professional attitudes and behaviors of orthodontic residents found overall positive attitudes about treating poor patients, as well as ethnic and racial minorities compared with currently practicing orthodontists – although these attitudes did not indicate an increased willingness to treat pro bono patients or provide reduced fees or financial assistance if requested (5). The data may suggest that although many White

adolescents and teenagers seek orthodontic treatment at the behest of their parents, many minorities are significantly less likely to have access or knowledge of orthodontic care until adulthood, which may account for the large and growing population of young adults undergoing orthodontic treatment (2, 6).

Although much is known about adolescent oral and dental health during orthodontic treatment, much less is known about adult oral and dental health among the growing population of young minority adults seeking orthodontic treatment and care (6, 7). Some promising research has been undertaken in recent years to more thoroughly investigate the oral microbial burden among adult, minority orthodontic patients, which revealed elevated levels of pathogenic bacteria among this patient population (8). Although some research has suggested new caries testing and risk models for adolescent orthodontic patients, less may be known about adult patients, minorities in particular, and their oral health assessment needs (9, 10).

Many southwest in the U.S. have disproportionately large percentages of both low-income and minority families, which include Nevada (11, 12). To address the needs of low-income and minority residents of Southern Nevada, The University of Nevada – Las Vegas established a public dental school to serve and improve the oral and dental health of the underserved. Although an Advanced Education Program in Orthodontics was established in 2008, to date there have been no comprehensive evaluations of the patient population to determine if care is being provided to the needy and underserved within this community. In addition, although one study evaluated oral microbial burden in a small subset of patients– no thorough investigation of oral health status has yet been undertaken (8).

Based upon this information, the primary objectives of this study were: 1) to evaluate and analyze the demographic composition of the patient population within this orthodontic clinic to determine if the mission to serve minority and low-income residents is being met, and 2) to evaluate the general information collected about the oral health of these patients for comparison with young adult patients without orthodontic appliances.

2. Methods

2.1. Aggregate patient data

Selected demographic information, which included sex or gender, race or ethnicity, and insurance status (Medicaid/Children's Health Insurance Program or CHIP or Self-insured/private pay) was provided to the study authors by the Office of Information Technology. The data was provided as summary data, with no references or identifiers to any specific patient record or information. Overall number of UNLV-SDM patients: Main clinic N=71,051; Pediatric clinic N=3,042; Orthodontic clinic N=1,220.

2.2. Medicaid / CHIP and Census data

Aggregate data for both Medicaid and the Children's Health Insurance Program (CHIP) in Nevada were accessed from the Medicaid/CHIP State of Nevada website and the Center for Children and Families (CCF), Georgetown University Health Policy Institute State Resource Center (15, 16, 11, 12). Information from this website includes total number and percentage of insured, Medicaid, CHIP, and uninsured, which were originally compiled by the Nevada Division of Health Care Financing and Policy's Medicaid and Nevada Check Up Fact Book,

January 2013 (17). Aggregate data regarding sex and ethnicity were obtained from the U.S. Census Bureau State and County Quick Facts website (18).

2.3. Human subjects

The protocol for this study titled "The prevalence of oral microbes in saliva from the UNLV School of Dental medicine pediatric and adult clinical population" was filed, amended and approved by the University of Nevada, Las Vegas (UNLV) Office of Research Integrity and Protection of Research (Human) Subjects (OPRS#1502-506M) on February 6, 2015. This current study is a retrospective examination of previously collected saliva samples (n=183), originally obtained under a separate protocol approved on April 9, 2010 (OPRS#1002-3361). Orthodontic samples, n=54; Pediatric samples, n=76; Adult samples, n=53.

2.4. Convenience sample patient health data

In brief, in the previous study consented dental patients were given a sterile saliva 50 mL collection container for one sample (8; 13; 14). Each of these samples was given a unique, randomly generated number to prevent research bias and any identifying information from being disclosed. The patient demographic and corresponding oral and general health information was also collected and given the matching randomly generated number for analytical purposes, but no patient-specific identifying information was available to any research team member. This information included height, weight and body mass index (BMI), overt oral lesions, decayed missing and filled teeth (DMFT) score, depth of periodontal pockets, and number of sealants (pediatric patients only).

2.5. Statistical analysis

Demographic and insurance information from Nevada were compared with the overall demographic profile of the UNLV-SDM patient clinics using a chi-square ($\chi 2$) test, to determine if any characteristic (gender, race, age, Medicaid/CHIP status) was different than expected. Although data for gender, age and insurance status for all patients was available, only a subset had complete demographic information for all demographic variables, including race. A probability level of alpha (α) $\leq$ 0.05 was used to determine statistical significance. The differences between sample groups (patient health data) were measured using a t distribution, $\alpha=$ 0.05. All samples were analyzed using two-tailed t-tests as departure from normality can make more of a difference in a one-tailed. As long as the sample size is at least moderate (>20) for each group, quite severe departures from normality make little practical difference in the conclusions reached from these analyses.

For the clinic sample analysis, two-tailed t-tests were performed between Orthodontic and Pediatric samples, Orthodontic and Main clinic samples, as well as Pediatric and main clinic samples. Because these analyses involved multiple two sample t-tests, these results may have a higher probability of Type I error (incorrectly rejecting the null hypothesis, H_0). ANOVA was performed to more accurately assess these results and confirm significance. Significance level for these analyses was $\alpha=0.05$. To minimize reporting of multiple non-significant findings and results, only the lowest *p*-value results were reported.

3. Results

At the time of this analysis, a cross-sectional analysis of summarized patient demographic information was used to determine if the dental school clinic was providing care for the low-income and underserved population, as evidenced by current enrollment in Medicaid and other public assistance programs including CHIP (Figure 1). The most current Medicaid/CHIP information from Nevada demonstrated a participation rate of 73.7%, which is lower than the national participation rate of 88.3% - but represents an increase of nearly two-thirds since 2013 (Fig. 1A). The analysis of Medicaid or CHIP patients within the UNLV-SDM clinic (N=71,051) revealed almost two-thirds (62.1%) of all patients were enrolled in Medicaid/CHIP or other public assistance programs, which was significantly higher than their percentage statewide of only slightly more than one-third of residents or 36.8% (Fig 1B, $p<0.0001$).
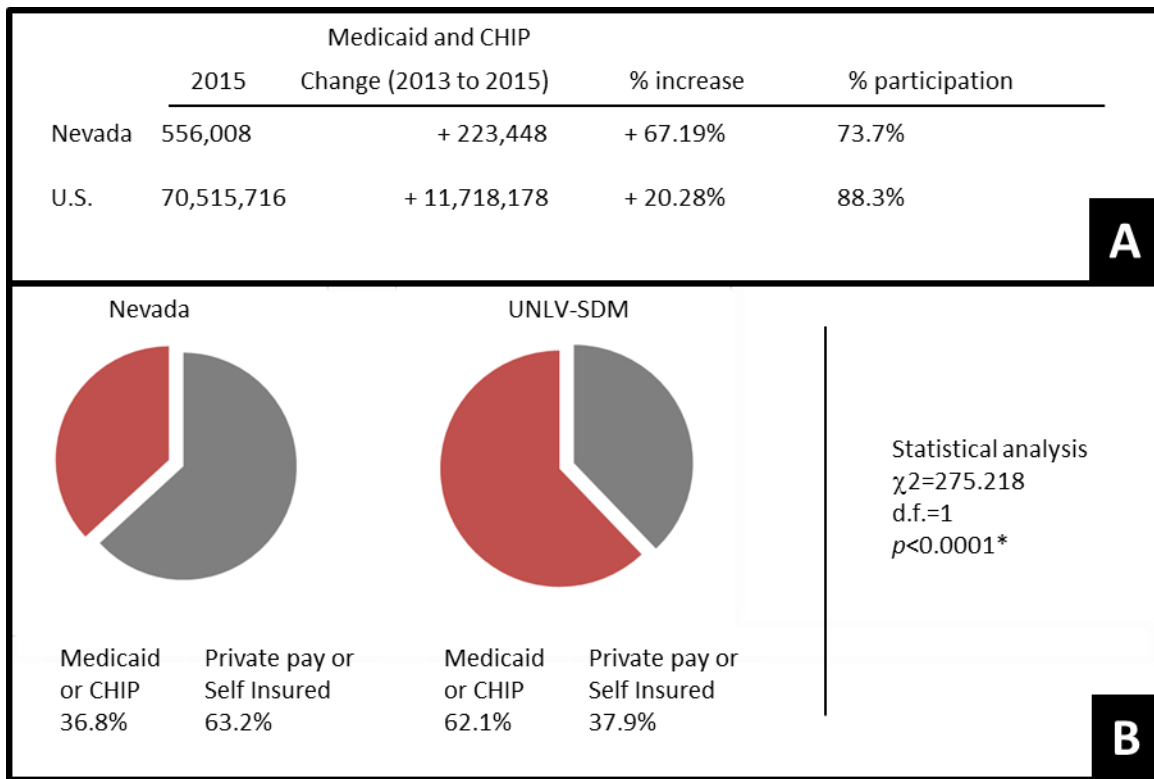
Figure 1. Comparison of Medicaid/CHIP/Public Assistance Programs in Nevada and UNLV SDM.

A) Participation rates (and rate increases) for Medicaid/CHIP and other public assistance programs demonstrate a large increase in recent enrollments within Nevada, although participation rates remain lower than the national average. B) Summary data from the UNLV-SDM clinic (N=71,051) demonstrate that patient participation rates (62.1%) are significantly higher than statewide averages (36.8%), $p<0.0001$. [* denotes statistical significance].

To more accurately assess whether dental care was being provided to traditionally underserved minority populations, national, state, and clinic demographic information were also analyzed (Figure 2). The preliminary analysis of the data revealed that minorities (non-White) within Nevada (47.8%) comprise a significantly higher percentage of the population than nationwide (37.4%)(Fig 2A, $p<0.0001$). A more detailed analysis of clinic data revealed that minorities within the overall clinic (59.2%), as well as pediatric (81.6%) and orthodontic clinics (64.9%) comprise significantly higher percentages than expected – given their distribution within the overall population (Fig. 2B, $p<0.0001$). Furthermore, the data also strongly suggest that orthodontic treatment, while traditionally accessed at much lower rates among minorities populations (Okunseri et al., 2007; Okunseri et al., 2013), represent nearly two-thirds of all patients at this clinic. Overall number of UNLV-SDM patients from each clinic were as follows: Main clinic N=71,051; Pediatric clinic N=3,042; Orthodontic clinic N=1,220.
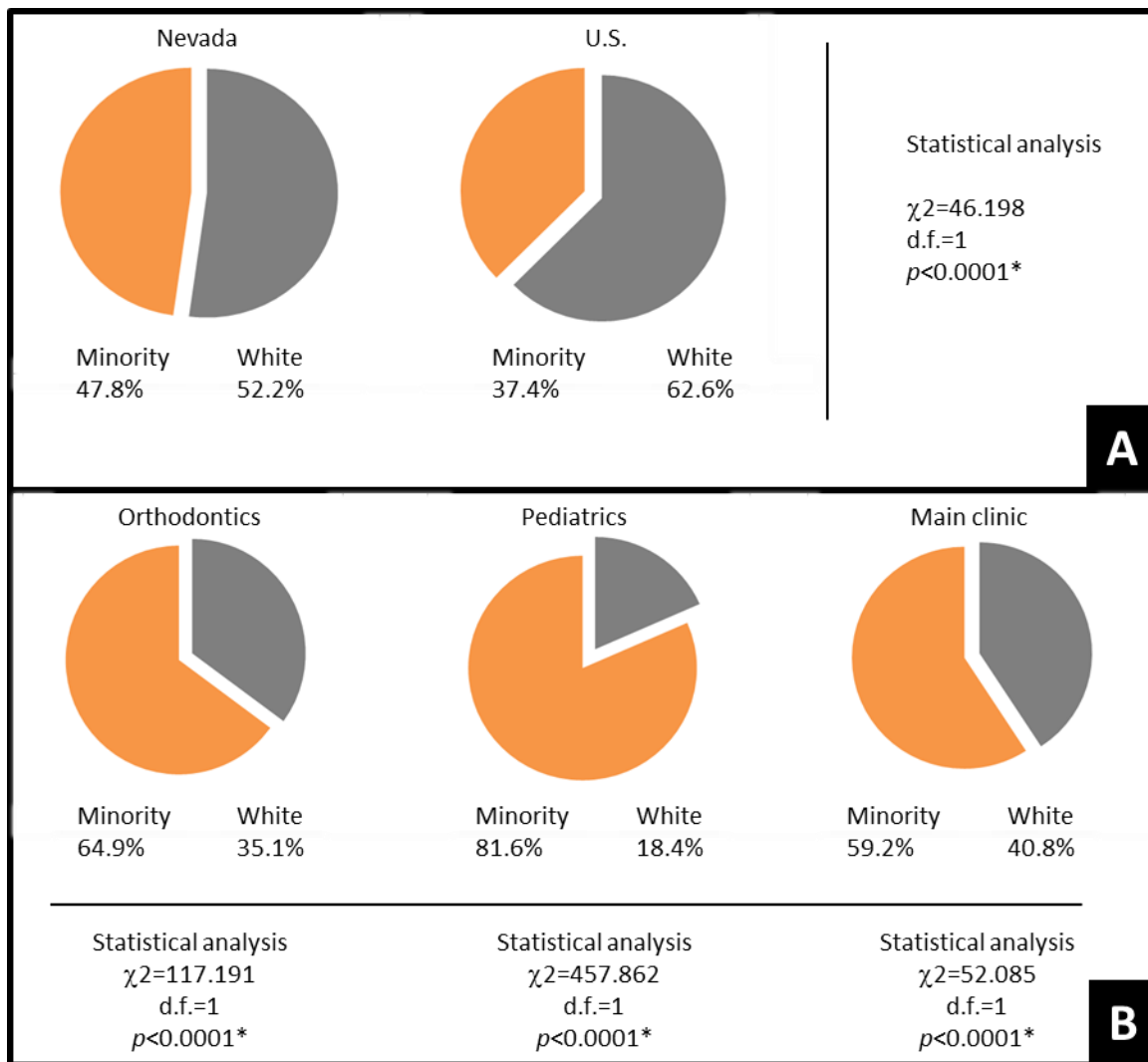
Figure 2. Racial and ethnic analysis of National, State, and Clinic populations.
A) State and national demographic data suggest the Nevada population may be comprised of significantly higher percentages of minority (non-White) residents ($p<0.0001$). B) Analysis of UNLV-SDM clinic summary data reveal that minority patients represent much higher percentages within the main (59.2%), orthodontic (64.9%) and pediatric (81.6%) populations than their overall percentage within the state population (47.8%), which was statistically significant $p<0.0001$. [* denotes statistical significance] Main clinic N=71,051; Pediatric clinic N=3,042; Orthodontic clinic N=1,220.

All of the remaining demographic information was also examined (Figure 3). The data demonstrated that the Nevada population is nearly equally distributed among males and females, similar to the overall U.S. population (Fig. 3A). Moreover, the data also reveal that the main and pediatric patient clinics are also nearly equally divided between male and females patients (Fig.

3B, $p>0.05$). However, the orthodontic clinic patient population has significantly higher percentages of females (61.3%) than males, which was significantly different from the other clinic, state and national demographic statistics ($p<0.0001$).
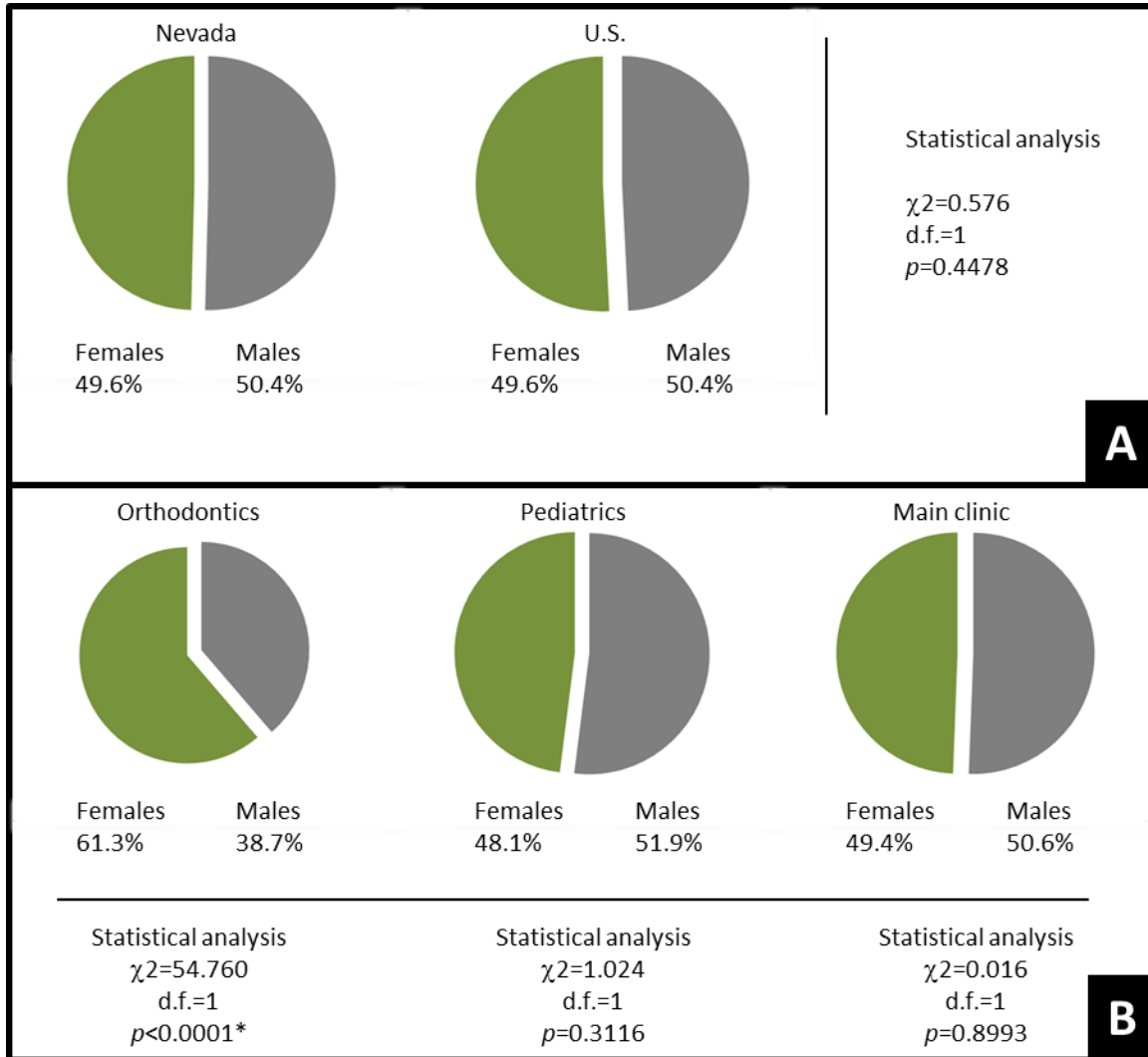
Figure 3. Sex or gender analysis of National, State, and Clinic populations.
A) Percentages of females and males within Nevada closely resemble nationwide statistics, $p=0.4478$. B) Analysis of UNLV-SDM clinic summary data reveal that female patients represent a significantly higher percentage within the orthodontic clinic (61.3%) than in the pediatric (48.1%) or main clinic (49.4%) populations, which was statistically significant $p<0.0001$. [* denotes statistical significance] Main clinic N=71,051; Pediatric clinic N=3,042; Orthodontic clinic N=1,220.

In addition, the general and oral health data from patient samples previously collected were also reviewed and analyzed (Table 1). This information was restricted to body mass index (BMI), decayed missing and filled teeth (DMFT) score, depth of periodontal pockets, and number of sealants (pediatric patients only). Analysis of the data revealed that there was no significant differences between BMI among Orthodontic and Pediatric patients samples, although adult patients from the main clinic had a slightly higher average BMI – this was not statistically significant (p=0.188). However, more detailed analysis of these data revealed that among all patients regardless of clinic, males had an overall average BMI higher than females. In addition, BMI averages among White patients from all three clinics were also found to be higher than those of Minority patients although these differences were not found to be statistically significant.

When the decayed missing and filled teeth (DMFT) scores were analyzed many differences were revealed, which may be expected. For example, DMFT scores among Pediatric patients (with the lowest average age) were the lowest (6.68), with slightly higher DMFT scores for Orthodontic patients (10.75) and significantly higher scores among adult patients (23.56). Furthermore, DMFT scores were similar between males and females in the Orthodontic and Adult clinics, but were much lower among females in the Pediatric clinic sample. Finally, DMFT scores from White patients were lower than those from Minority patients from all three clinics, on average, which was statistically significant (p<0.001).

When periodontal pocket depth (PPD) data were analyzed, several trends were observed. First, males in all three clinics (Orthodontic, Pediatric and Main or Adult) had greater PPD averages

than females. In addition, Minority patients had higher PPD scores in both the Orthodontic and Main clinic, but were similar among Pediatric patients. However, the average PPD scores in total were not significantly different from these three clinic samples (lowest *p*-value, *p*=0.169).

Finally, data was available regarding the number of sealants from patients under 18 years of age from the Orthodontic and Pediatric clinics. No clear patterns emerged from this analysis, however. For example, Males in the Orthodontic clinic had a slightly higher number of sealants (on average) than Females – although the opposite was found among Pediatric patients. Minority patients had a slightly higher average number of sealants in both the Pediatric and Orthodontic populations, but when the combined numbers for all patients within each clinic was analyzed, no statistically significant differences were found (lowest *p*-value, *p*=0.114).

Table 1. Analysis of general and oral health parameters from a study sample.

	Orthodontic (n=54)	Pediatric (n=76)	Main clinic (n=53)	Statistics two-tailed t-test
BMI (M)	28.17+/-2.83	26.44+/-4.82	27.89+/-7.94	
BMI (F)	24.01+/-4.78	25.1+/-4.36	27.09+/-8.19	
BMI (W)	26.34+/-6.72	25.41+/6.25	27.99+/-9.20	
BMI (Mi)	24.34+/-6.05	25.12+/-5.92	28.09+/-9.01	
BMI	25.67+/-6.36	25.64+/-4.53	27.54+/-8.12	$p=0.188$
DMFT (M)	11.4+/-1.23	8.17+/-2.21	24.65+/-6.25	
DMFT (F)	10.0+/-1.63	5.80+/-3.81	22.29+/-7.65	
DMFT (W)	9.40+/-1.08	5.32+/-4.94	20.78+/-5.71	
DMFT (Mi)	12.1+/-0.99	7.39+/-4.59	25.26+/-8.69	
DMFT	10.75+/-1.21	6.68+/-4.74	23.56+/-7.56	$p<0.0001$
PPD (M)	6.67+/-0.52	3.5+/-1.69	4.05+/-5.56	
PPD (F)	2.66+/-0.88	2.81+/-1.26	2.67+/-2.21	
PPD (W)	2.21+/-1.84	2.50+/-1.49	3.15/+/-1.84	
PPD (Mi)	4.67+/-1.15	2.47+/-1.87	3.84+/-5.38	
PPD	3.12+/-0.78	2.83+/-1.77	3.42+/-2.44	$p=0.169$
Sealants (M)	1.75	1.28	N/A	
Sealants (F)	1.15	2.64	N/A	
Sealants (W)	1.01	1.5	N/A	
Sealants (Mi)	1.80	2.82	N/A	
Sealants	1.60	2.03	N/A	$p=0.114$

4. Discussion and Conclusions

The primary objectives of this study were to evaluate and analyze the demographic composition of the patient population within this orthodontic clinic to determine if the mission to serve minority and low-income residents was being met, and to evaluate the general information collected about the oral health of these patients for comparison with young adult patients without

orthodontic appliances. These analyses revealed that UNLV-SDM currently serves a large percentage of Medicaid and CHIP patients (>62%), which represents a much greater share than expected in the local community (~37%). These results are also encouraging, despite the fact that Medicaid and CHIP participation rates are lower in Nevada than in the US, on average.

Moreover, the analysis of minority patients within the Main (~59%), Orthodontic (~65%) and Pediatric (~82%) at UNLV-SDM is also much higher than the local population (~48%). These analyses strongly suggest that UNLV-SDM is currently meeting the mandate to provide services to low-income, Medicaid and Minority patients. Finally, the ratio of females to males is nearly equal in both the Pediatric and Main patient clinics – although there are more females currently seeking Orthodontic care at UNLV-SDM. Finally, the overview of patient health revealed that BMI was not significantly different among the three clinic patient samples analyzed, although adults had slightly higher average BMI than either Orthodontic or Pediatric patients analyzed.

The analysis of oral health parameters revealed that Minority patients were more likely to have significantly elevated DMFT scores and PPDs than non-Minority patients. Due to their large percentages and representation in all UNLV-SDM clinics, including Orthodontics – the data is critical in order to provide more in depth prevention and treatment options for patients that may face greater barriers to health information and other types of social access.

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Chapter 3

Screening and prevalence of the novel cariogenic pathogen Scardovia wiggsiae among adult

orthodontic and non-orthodontic patient saliva samples

Abstract

Orthodontic therapy in the United States has become routine among teenagers and increasing among adults. Despite these positive developments, orthodontic treatment has often been associated with changes to the oral environment, which may increase disease risk. Although numerous studies have demonstrated the causal link between *Streptococcus mutans* and carious lesions, more recent evidence suggest that it only constitutes part of a much larger oral microbial community. Several recent studies have demonstrated the presence of newly characterized cariogenic pathogen, the anaerobic Gram-positive bacillus *Scardovia wiggsiae*. This retrospective study of previously collected saliva samples originated with a convenience sample of pediatric and adult patients, previously recruited from the University of Nevada Las Vegas-School of Dental Medicine (UNLV-SDM) clinics. More than one hundred saliva samples from

adult orthodontic (n=49) and non-orthodontic (n=52) patients were selected for inclusion in this study. All DNA extracted from these samples was subsequently screened using PCR, which revealed the presence of *S. mutans* (SM), *P. gingivalis* (PG), and *S. wiggsiae* (SW), which differed in prevalence among non-Orthodontic and Orthodontic patients. In non-orthodontic patients nearly all of the PG-positive and SW-positive samples were also SM-positive samples. However, among orthodontic patients, none of the SW-positive samples were either SM- or PG-positive, which suggest continued research in this area will be needed. In addition, further analysis of demographic variables revealed decayed-missing-filled teeth (DMFT) score, periodontal pocket depth (PPD), age, gender, and BMI did not vary between groups,

Key words: *Scardovia wiggsiae*, caries, Orthodontics

Introduction

Orthodontic therapy has become routine, with approximately 1% of all young adults (under 30 years of age) and nearly 20% of all teenagers undergoing some form of orthodontic treatment at any given time in the United States (1,2). Despite these impressive advances in the prevalence of orthodontic treatment in the U.S., many disparities remain to improve access for the underserved, including minorities, underinsured and uninsured (3-5). Many orthodontic and other dental specialty programs are now actively seeking low income, minority and underserved populations in an effort to improve not only access to oral healthcare, but to facilitate increased oral health awareness, education and other resources (6-8).

Despite these positive developments and the incremental steps towards improved access, orthodontic treatment has often been associated with changes to the oral mucosa, gingiva and microbial communities, which may increase disease risk (9,10). More specifically, many studies have generated and evaluated the evidence regarding increased risk for caries lesions during orthodontic therapy, which may disproportionately affect these underserved, low income and minority populations (11-13). In fact, this research group has facilitated several studies of oral health among minorities and under-served within the pediatric, adult and orthodontic populations over the past few years that clearly demonstrate these patients may be at increased risk for oral complications due to barriers to access, lower levels of health literacy, lowered access to preventive dental care, and increased burden of cariogenic, periodontal and other oral pathogens (6,7,14-16).

Although numerous studies have demonstrated the causal link between *Streptococcus mutans* and caries lesions, more recent evidence suggest cariogenic pathogens, such as *S. mutans,* constitute part of a much larger oral microbial community that may disproportionately influence caries risk when minor constituents become imbalanced – a process known as dysbiosis (17,18). Many of these microbial species are well-known and have been extensively studied in the context of the cariogenic process, including *Streptococcus, Lactobacillus, Veillonella* and *Actinomyces* species (19). However, several recent studies have demonstrated the presence of newly characterized cariogenic pathogen, the anaerobic Gram-positive bacillus *Scardovia wiggsiae* (20-22).

Despite the breadth of caries research, these more recent studies have demonstrated that these previously undetected (novel) cariogenic pathogens, such as *S. wiggsiae*, may be present in the oral cavity and may confer additional risks and might alter the current understanding of caries screening (23-25). However, only a few preliminary studies have focused on this newly detected organism, and the cariogenic potential this organism may pose during orthodontic treatment when cariogenic risk may be comparatively higher (23,25). Based upon the paucity of evidence, the goal of this study was to screen saliva from adult orthodontic patients to evaluate the prevalence of *S. wiggsiae* for comparison with a group of adult patients without orthodontic appliances. The data collected may be among the first to evaluate the presence of this organism within an orthodontic population at a public dental school that serves predominantly minority and underserved patients.

Material and Methods

Human Subjects

The protocol for this retrospective study of previously collected saliva samples titled "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" (Protocol#1502-5068M) was reviewed and approved by the UNLV Office for the Protection of Research Subjects (OPRS) Institutional Review Board (IRB) on February 6, 2015.

Study Design

This retrospective study of previously collected saliva samples originated with a convenience sample of pediatric and adult patients, previously recruited from the UNLV-SDM clinics. In

brief, all participants had previously provided informed consent prior to collection of demographic information and saliva samples. Exclusion criteria included patients (or their appointed guardian) who declined to participate.

Saliva Collection

In the original study protocol, consented dental patients were given a sterile saliva 50 mL collection container for one sample. Samples were stored on ice until transfer to a biomedical laboratory for screening and analysis. Each of these samples were given a unique, randomly generated number to prevent research bias and any identifying information from being disclosed. The patient demographic and health information was also collected and given the matching randomly generated number for analytical purposes, but no patient-specific identifying information was subsequently available to any research team member.

Cell counting and DNA isolation

For the purposes of this study, all previously collected saliva samples, which contained both shed epithelial cells and bacterial cells, were centrifuged for 10 minutes at 2,100 g (RCF) and the pellet washed with 1X phosphate-buffered saline (PBS) (HyClone: Logan, Utah, USA) and resuspended in 5 mL of 1X PBS. Epithelial cell number was determined using Trypan Blue (Fisher Scientific: Fair Lawn, New Jersey, USA) using a Zeiss Axiovert 40 inverted microscope (Carl Ziess, Inc: Thornwood, New York, USA) and a hemacytometer (Fisher Scientific: Fair Lawn, New Jersey, USA). To determine if any samples harbored the cariogenic pathogen of interest – *S. wiggsiae* or SW, DNA was isolated from the saliva sample using the GenomicPrep DNA isolation kit (Amersham Biosciences: Buckinghamshire, United Kingdom) and the procedure recommended by the manufacturer for blood and tissue, which recommends a

minimum of 3.5 x 10^5 cells (14-16). DNA was resuspended and stored in 50 μL DNA Hydration

Solution (Amersham Biosciences: Buckinghamshire, United Kingdom) at 4C. DNA purity was

calculated using ratio measurements of absorbance at 260 and 280 nm (A260/A280 ratio

between 1.59 and 2.0).

Polymerase chain reaction (PCR) primers

DNA from each sample was then used to perform PCR with the Fisher exACTGene complete

PCR kit (Fisher Scientific: Fair Lawn, New Jersey, USA) and a Mastercycler gradient

thermocycler (Eppendorf: Hamburg, Germany) using the following primers for *S. wiggsiae*, *S.*

mutans, *P. gingivalis*, 16S rRNA and glyceraldehyde- 3- phosphate dehydrogenase (GAPDH)

(SeqWright: Houston, Texas, USA) (14,16,20,21):

GAPDH forward primer, ATCTTCCAGGAGCGAGATCC;

GAPDH reverse primer, ACCACTGACACGTTGGCAGT;

16S rRNA universal primer, ACGCGTCGACAGAGTTTGATCCTGGCT;

16S rRNA universal primer, GGGACTACCAGGGTATCTAAT;

S. mutans forward primer, GCCTACAGC TCAGAGATGCTATTCT;

S. mutans reverse primer, GCCATACACCACTCATGAATTGA;

P. gingivalis forward primer, TACCCATCGTCGCCTTGGT;

P. gingivalis reverse primer, CGGACTAAAACCGCATACACTTG;

S. wiggsiae forward primer, GTGGACTTTATGAATAAGC;

S. wiggsiae reverse primer, CTACCGTTAAGCAGTAAG;

DNA standard: GAPDH

DNA standards obtained from standardized control cells, human gingival fibroblasts (0.3-0.5 x 10^6 cells/mL), approximating the range of cell concentrations observed in the saliva samples were used to establish the minimum threshold (CT) and saturation (CS) cycles required for calibration and concentration comparisons using relative endpoint PCR (RE-PCR). GAPDH signal detection above background or CT required a minimum of ten cycles (C10), with saturation or CS observed at C50.

DNA standards: SM and PG

In addition, the oral bacterial cell lines *Streptococcus mutans* (*S. mutans* or SM) (NCTC-10449) and *Porphyromonas gingivalis* (*P. gingivalis* or PG) (FDC-381) were also obtained from ATCC (Manassas, VA). In brief, cells were thawed, streaked and cultured on their respective agar plates from Difco (Sparks, MD) according to the manufacturer protocol (14). Colonies of each were then plated and grown overnight at 37C on Trypticase soy agar; SM plates were supplemented with 5% defibrinated sheep's blood and PG plates were supplemented with 1% yeast extract. Single plate colonies were then selected and inoculated into liquid broth cultures; Trypticase soy broth for SM and supplemented tryptic soy broth for PG and then incubated overnight at 37°C. Aliquots of bacterial suspensions were subsequently used to inoculate growth standards. Standard curves were created using spectrophotometric absorbance measurements of optical density (OD) at 650 nm and enumeration of colony forming units (CFU) (14, 24).

Turbidity resulting in an OD of 0.8 corresponded to 5.0 x 10^7 CFU/mL for both bacterial cell lines used. Serial dilutions were prepared for final concentrations of 5.0 x 10^6, 10^5, 10^4 and 10^3

CFU/mL to establish RE-PCR standards for SM and PG corresponding with the most current understanding of microbial saliva concentrations as biomarkers for disease (including caries) risk, which are: 10^6 CFU/mL = very high risk; 10^5 CFU/mL = high risk; 10^4 CFU/mL = moderate risk, and $< 10^3$ CFU/mL = normal or average risk (14,26,27). SM and PG signal detection or CT above normal or average risk required a minimum of twenty five cycles (C25), with saturation or CS observed at C45. Based upon the CT data for GAPDH at C10 and for SM and PG at C25, RE-PCR was performed at C30, above the lower detection limit (CT), but below the observed saturation limits (C45-C50).

Polymerase chain reaction (PCR)

One μg of template DNA was then used for each reaction. The initial denaturation step ran for three minutes at 94°C. A total of 30 amplification cycles (C30) were run, consisting of 30 second of denaturation at 94°C, 60 seconds of annealing at 58°C, and 30 seconds of extension at 72°C. Final extension was run for five minutes at 72°C. The PCR reaction products were separated by gel electrophoresis using Reliant 4% NuSieve® 3:1 Plus Agarose gels (Lonza: Rockland, Maine, USA). Bands were visualized by UV illumination of ethidium-bromide-stained gels and captured using a Kodak Gel Logic 100 Imaging System and 1D Image Analysis Software (Eastman Kodak: Rochester, New York, USA).

Statistics

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% (28) . Using a significance level of $\alpha = 0.05$ and a power $p = 0.80$, a minimum sample size of fifty (N = 50) was calculated (29). Descriptive statistics regarding the sample population were reported, and chi-square (χ^2) analysis was performed to determine any significant differences in demographics between the sample group and the clinic population.

Results

More than one hundred saliva samples from adult orthodontic (n=49) and non-orthodontic (n=52) patients were selected for inclusion in this study (Table 1). The demographic breakdown for all samples combined was nearly equally divided between males and females (51.5%, 49.5%), and between non-minority (white) and minorities (Hispanic, Black, Asian/Other) (55.4%, 44.6%). More detailed analysis of the distribution from the orthodontic clinic revealed the sample to contain more females (61.2%) than males (38.8%), which closely resembled the overall distribution within the orthodontic clinic population (p=0.9482). An evaluation of the racial and ethnic demographics revealed nearly half of the samples were from non-minority patients (48.9%), which was significantly higher than in the overall orthodontic clinic population (35.1%) and was statistically significant (p<0.0001).

The comparison or control group of adult non-orthodontic samples was comprised of fewer females (42.3%) than males (47.5%), which was different than their nearly equal distribution among the main clinic population, in general (p<0.0001). In addition, this analysis revealed the majority of samples were from non-minorities (61.5%), which was dissimilar from the main clinic population and also statistically significant (p<0.0001).

Table 1. Demographic analysis of study samples

	Orthodontic sample (n=49)	Orthodontic clinic	Statistical analysis
Sex			
Female	61.2% (n=30)	61.3%	$\chi2=0.004$, d.f.=1
Male	38.8% (n=19)	38.7%	$p=0.9482$
Race/Ethnicity			
White	48.9% (n=24)	35.1%	$\chi2=83.600$, d.f.=1
Minority	51.1% (n=25)	64.9%	$p<0.0001$
Hispanic	32.7% (n=16)	53.9%	
Black	10.2% (n=5)	9.8%	
Asian/Other	8.2% (n=4)	1.3%	
	Non-Orthodontic sample (n=52)	**Main clinic**	**Statistical analysis**
Sex			
Female	42.3% (n=22)	49.4%	$\chi2=20.206$, d.f.=1
Male	57.5% (n=30)	50.6%	$p<0.0001$
Race/Ethnicity			
White	61.5% (n=32)	40.8%	$\chi2=177.402$, d.f.=1
Minority	38.5% (n=20)	59.2%	$p<0.0001$
Hispanic	13.5% (n=7)	25.5 %	
Black	23.1% (n=12)	12.2%	
Asian/Other	1.9% (n=4)	1.9%	
	Combined samples		
Sex			
Female	51.5% (n=52)		
Male	49.5% (n=49)		
Race/Ethnicity			
White	55.4% (n=56)		
Minority	44.6% (n=45)		
Hispanic	31.7% (n=32)		
Black	16.8% (n=17)		
Asian/Other	7.9% (n=8)		

Each of the selected saliva samples were processed to isolate DNA for subsequent screening using PCR (Table 2). Using previously established protocols and methods, DNA was successfully isolated from all orthodontic and non-orthodontic saliva samples, yielding a recovery rate of 100% (n=101), which was within the acceptable range for DNA recovery

according to the manufacturer (95-100%) and similar to results from previous studies (14-16,24).

DNA concentrations ranged from an average of 163.1 ng/uL from the orthodontic samples to

172.4 ng/uL from the non-orthodontic patient samples. DNA purity was calculated using ratio

measurement of absorbance at 260 and 280 nm (A260/A280 ratio), which ranged between 1.59

and 2.0. The processing of all samples using PCR revealed the presence of both human

(GAPDH) and bacterial (16S rRNA) DNA.

Table 2. DNA isolation and recovery

	DNA recovery	Unsuccessful	Analysis/Recovery
Orthodontic samples	n=49	n=0	100% (n=49/49) ave.= 163.1 ng/uL A260/A280: 1.71-2.0
	n=49; GAPDH n=49; 16S rRNA		
Non-orthodontic samples	n=52	n=0	100% (n=52/52) ave.= 172.4 ng/uL A260/A280: 1.59-2.0
	n=52; GAPDH n=52; 16S rRNA		

All DNA extracted from these samples was subsequently screened using PCR, which revealed

the presence of *S. mutans* (SM), *P. gingivalis* (PG), and *S. wiggsiae* (SW), which differed in

prevalence among non-Orthodontic and Orthodontic patients (Figure 1). For example, the

proportion of patients with SM was significantly higher among Orthodontic patients (69%) than

the control group (58%). Similar results were observed with PG, revealing a significantly larger

percentage of Orthodontic patients with PG (55%) compared with non-Orthodontic patient

samples (25%). However, the results of the SW screening revealed the prevalence was lower

among the Orthodontic patient samples (14%) than the non-Orthodontic control group (19%).

These differences between sample groups (Orthodontic, Non-Orthodontic) were found to be statistically significant (*p*<0.05).

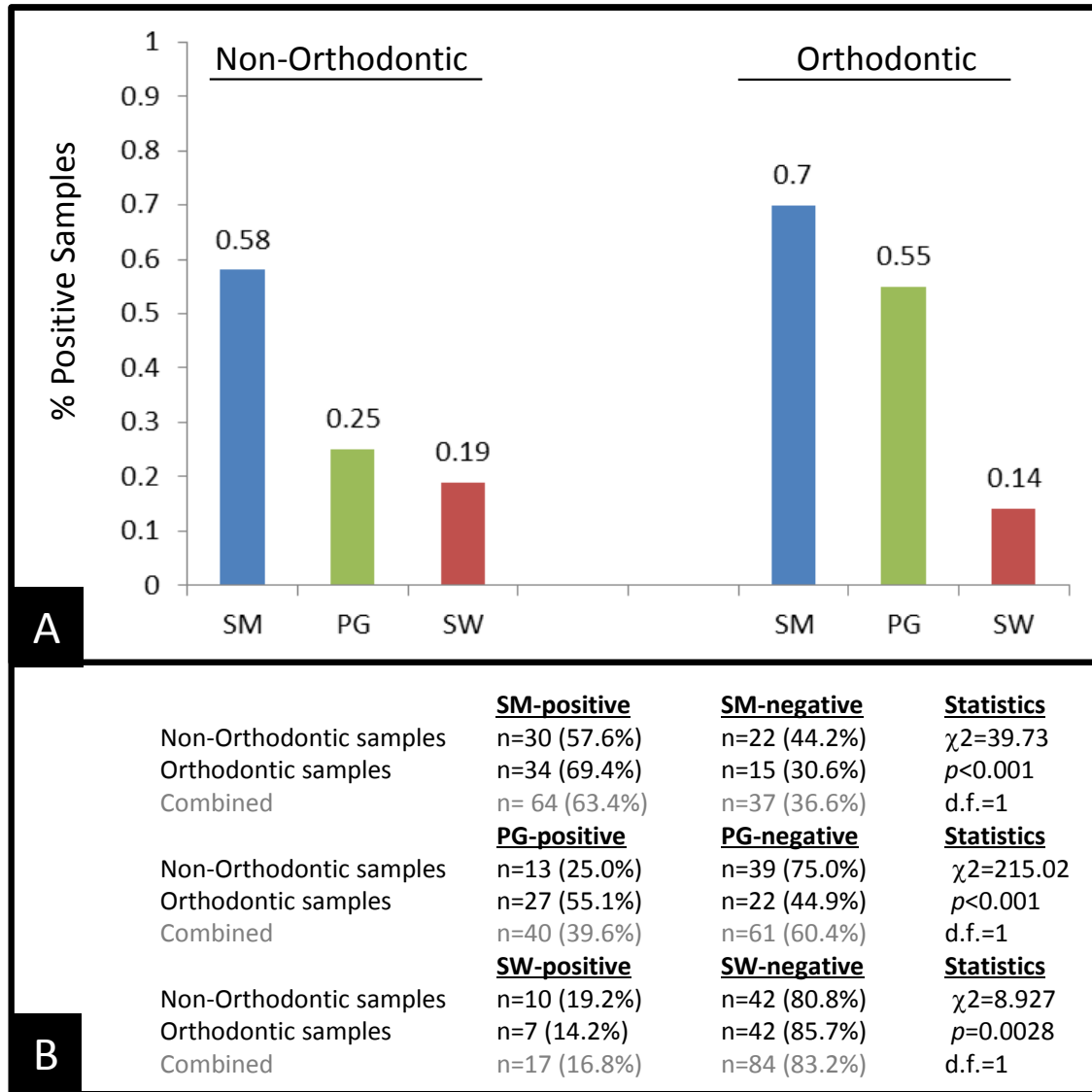Figure 1. Presence of S. mutans (SM), P. gingivalis (PG), and S. wiggsiae (SW) among Non-Orthodontic and Orthodontic patients

To further analyze these results, each of the samples was cross referenced to determine if any samples were positive for one or more (multi-positive) of the pathogens evaluated (Figure 2). These results demonstrated that among the 58% of Non-Orthodontic (control) samples testing

positive for SM, 21% were multi-positive for both SM and PG, while another 15% were SM and SW positive and a small fraction were SM-PG-SW positive (Fig 2A). Nearly all of the PG-positive and SW-positive samples were found among the SM-positive samples (Fig 2B), which suggests nearly two thirds (36% of 58%) demonstrated multi-positive results.

In contrast, a much higher proportion of the Orthodontic samples tested positive for SM (70%), although only a fraction (22%) were also found to be multi-positive (Fig 2C). In addition, the much higher percentage of PG-positive samples (55%) among the Orthodontic samples only partially overlapped with the SM-positive samples. Unexpectedly, none of the SW-positive samples were found to be SM- or PG-positive (Fig 2D).
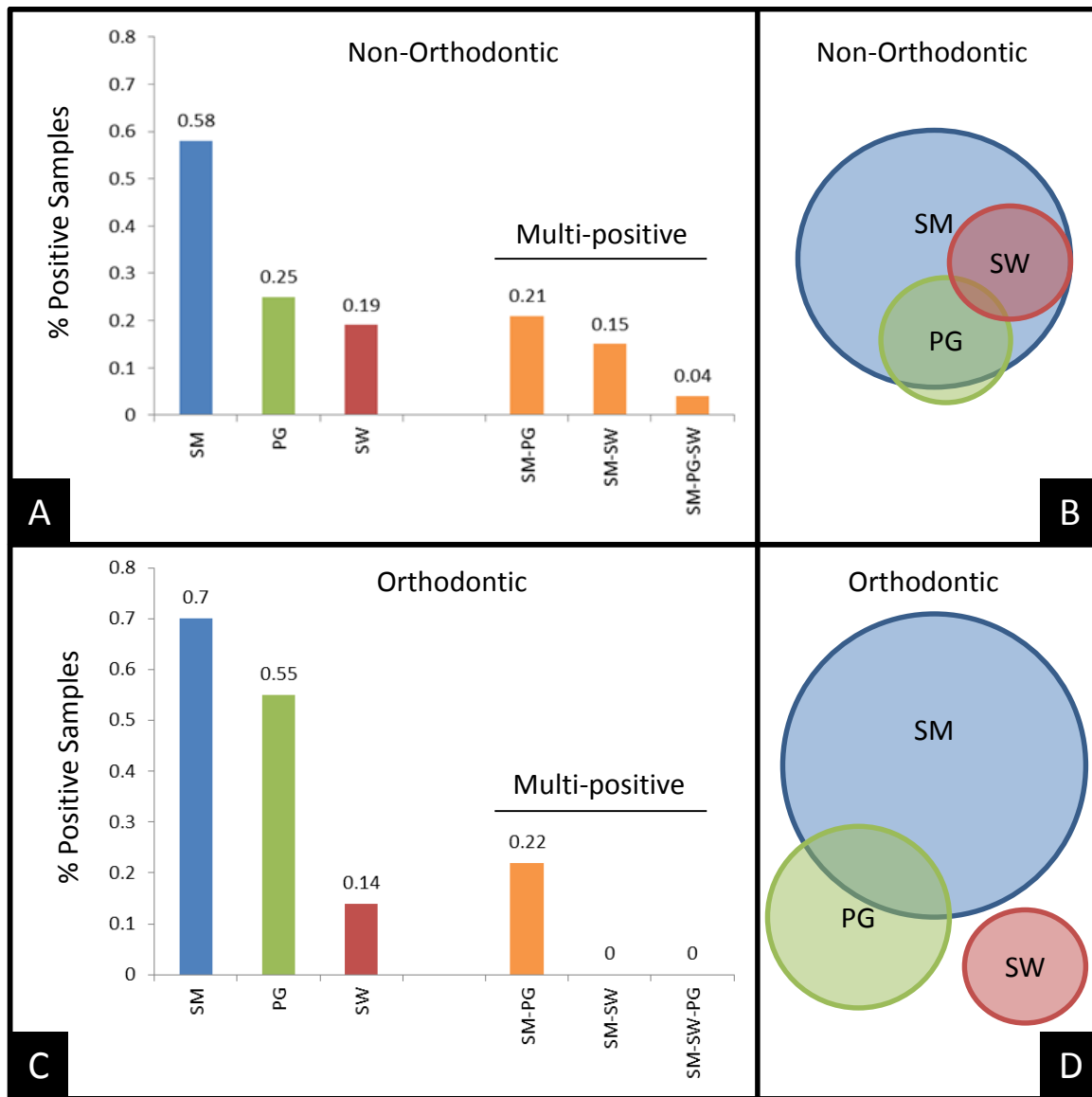
Figure 2. Multi-Positive Cross Reference

To determine the association and relationship with other demographic and health variables, data were sorted according to PCR screening results (Table 3). This analysis revealed that decayed-missing-filled teeth (DMFT) score did not vary significantly between the SM-, PG-, or SW-positive samples from the Non-Orthodontic and Orthodontic samples. In addition, no significant differences were found between periodontal pocket depth (PPD) when comparing the SM-, PG-, and SW-positive samples from these two groups. Further analysis, however, revealed that both

DMFT score and PPD among the PG-positive samples were higher when compared with SM-, or multi-positive samples – regardless of whether the sample came from Orthodontic or Non-Orthodontic samples. Finally, the DMFT scores among the SW-positive samples were higher than those from SM-, PG- and multi-positive samples and PPDs greater than SM- or multi-positive samples. No other variables, including age, gender or BMI were differed significantly from the overall sample demographics.

Table 3. DMFT Score and PPD in Non-Orthodontic and Orthodontic populations

	Non-Orthodontic	Orthodontic	Statistical analysis
DMFT score			
SM-positive	21.688	20.112	p>0.05
PG-positive	25.031	28.132	p>0.05
SW-positive	30.667	32.166	p>0.05
SM-PG	21.778	21.8	p>0.05
SM-SW	31	N/A	
SM-SW-PG	20	N/A	
PPD			
SM-positive	3.25	3.1	p>0.05
PG-positive	4.69	4.27	p>0.05
SW-positive	4.0	4.3	p>0.05
SM-PG	3.1	3.3	p>0.05
SM-SW	3.2	N/A	
SM-SW-PG	2.66	N/A	

Discussion

The goal of this study was to screen saliva from adult orthodontic patients to evaluate the prevalence of SW for comparison with a group of adult patients without orthodontic appliances. The results of this study clearly demonstrate that differences in the prevalence of oral pathogens differed significantly among these two patient samples – revealing a comparatively higher prevalence of SM and PG in the Orthodontic sample. Interesting, SW prevalence was

significantly lower in the Orthodontic sample – a finding which may be counterintuitive based upon the available information about this cariogenic organism (22,25).

Further analysis revealed that many of these samples in the Non-Orthodontic group were multi-positive, with several differing combinations of pathogens detected. Moreover, the vast majority of samples that were PG- or SW-positive were also found to be SM-positive – with the only major, single-positive group harboring SM. In contrast to the findings among the Orthodontic sample, however, none of the SW-positive samples were found to harbor either SM- or PG. In addition, a much greater proportion of PG-positive samples did not harbor SM. When combined with the overall higher proportion of SM and PG within this group, these data may suggest a disruption in the overall oral ecosystem that may facilitate the overgrowth of specific types of oral microbes, with corresponding declines in the overall prevalence of competing organisms.

Finally, the data revealed that DMFT score and PPD were higher among the PG- and SW-positive samples compared with SM- and multi-positive samples. As previous data have demonstrated that DMFT score and PPD may often be indicators of poor oral health and may be higher among underserved and minority patients, the data collected from the study may serve to reinforce and strengthen the resolve for oral health providers to increase knowledge and awareness of these important issues and to lower barriers to dental access and care (5-8). Although the data does not suggest an increase in SW prevalence within the orthodontic population, these may be among the first to reveal that orthodontic therapy may be sufficient to disrupt the oral ecology and ecosystem enough to create conditions that favor one major type of cariogenic organism, such as SM or SW – but not both. The data is especially valuable as it

serve to evaluate the presence of SW within an orthodontic population at a public dental school that serves predominantly minority and underserved patients (6,7,14,15).

Most other studies of SW to date have involved either the identification or detection of this organism from children with Severe Early Childhood Caries (SECC) (20-22). In addition, the most recent studies of SW involve detection of SW directly from caries lesions (30-32), while only one other study to date has included any patients with orthodontic appliances (25). However, this study evaluated the presence of SM and SW and association with white spot lesions (WSL) - a known risk factor for caries development, but did not provide any data to analyze the presence of SM or SW from individual patients. The current study is the only study to date that provides any evidence to describe the oral microbial composition from individual SW-positive orthodontic patients, which has provided some evidence to suggest a novel, previously unrecognized phenomenon that this type of disruption in the oral ecology may be sufficient to create conditions favoring SM or SW growth, but not both.

Although these findings may be among the first to screen for SW among Orthodontic and Non-Orthodontic patients, there are some limitations to consider for future studies of this nature. First, the use of an existing saliva repository restricts some of the conclusions that can be drawn from these observations based upon the retrospective study design. In addition, although the original collection protocol included both Orthodontic and Non-Orthodontic patients, these studies relied upon a convenience sample of willing participants that were not randomly selected and may therefore have implicit self-selection bias (12,14). Furthermore, these patient samples were

collected at a single time point, which suggests no temporal conclusions can be made regarding the observed oral microbial prevalence.

Despite these limitations based upon the study design, these findings are novel and may be clinically significant, which suggests the planning and implementation of prospective studies to evaluate any temporal changes within these populations. Data that can elucidate this phenomenon and describe any temporal changes over time are of the upmost importance. As the first known study of this type, a pilot study design using existing saliva samples was the best available option to provide an initial baseline screening within this patient population and provides crucial evidence to suggest the need for continued research in this area.

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Chapter 4: Summary and Conclusions.

The initial focus of this study was to assess the health parameters among orthodontic patient samples for comparison with non-orthodontic patients. However, the data sets obtained allowed for a much more in depth analysis of the demographic composition of the patient population within a public dental school-based orthodontic program.

The first manuscript "Orthodontic care in a community of underserved patients: a public dental school analysis," sought to analyze the demographic composition of the patient population within the Orthodontic program to determine if minority and low-income residents are being served and to evaluate some general parameters of oral health. These analyses revealed:

- UNLV-SDM currently serves a much higher percentage of Medicaid and CHIP patients than the local community.

- Minority patients in all three clinics (Main, Orthodontic, and Pediatric) are also much higher than the local population.

- UNLV-SDM is currently meeting the mandate to provide services to low-income, Medicaid and minority patients.

- Minority patients were more likely to have significantly elevated markers for oral disease than non-minority patients.

- Minority patients were more likely to have significantly elevated DMFT scores and PPDs than non-minority patients.

- The ratio of females to males is nearly equal in both the Pediatric and Main patient clinics while there are more females seeking Orthodontic care at UNLV-SDM.

- BMI was not significantly different among the three clinics analyzed, although adults had slightly higher average BMI than either Orthodontic or Pediatric patients analyzed.

These data may be among the first to elucidate the oral health problems facing this patient population. Due to minority's large percentages and representation in all UNLV-SDM clinics, including Orthodontics – the data is critical in order to provide more in depth prevention and treatment options for patients that may face greater barriers to health information and other types of social access.

The primary focus of the remainder of this study entailed comparison of SW prevalence among the orthodontic patient samples for comparison with samples taken from non-orthodontic patients. Other microbial data was also evaluated, including S. mutans, and P. gingivalis prevalence in conjunction with multi-positive oral environments.

The second manuscript "Screening and prevalence of the novel cariogenic pathogen Scardovia wiggsiae among adult orthodontic and non-orthodontic patient saliva samples" screened saliva from orthodontic patients to evaluate the prevalence of SW for comparison with a group of patients without orthodontic appliances. The results of this study demonstrated:

- The prevalence of oral pathogens differed significantly among the patient samples.

- SM and PG prevalence were much higher in the Orthodontic sample.

- SW prevalence was significantly lower in the Orthodontic sample.

- Non-Orthodontic samples were multi-positive, with several differing combinations of pathogens detected.

- Orthodontic samples with positive SW did not harbor either SM or PG.

- Orthodontic samples that were PG positive alone were much greater than the group that was multi-positive for both PG and SM.

- Data may suggest a disruption in the overall oral ecosystem that may facilitate the overgrowth of specific types of oral microbes with corresponding declines in the overall prevalence of competing organisms.

- DMFT score and PPD were higher among the PG- and SW-positive samples compared with SM- and multi-positive samples.

The data collected from the study may serve to reinforce and strengthen the resolve for oral health providers to increase knowledge and awareness of these important issues. This study will also help to lower barriers that minorities have to dental access and care. Although the data does not suggest an increase in SW prevalence within the orthodontic population, this study may be among the first to reveal that orthodontic therapy may disrupt the oral ecology and ecosystem driving microbial overgrowth of some species and creating separate and distinct types of cariogenic risk that have not previously been identified. The data is especially valuable as it serves to evaluate the presence of SW within an orthodontic population at a public dental school that serves predominantly minority and underserved patients.

Limitations and Recommendations

As the first known study of this type, a pilot study design using existing saliva samples was the best available option to provide an initial baseline screening within the patient population described and provides crucial evidence that suggests the need for continued research in this area. Although these findings may be among the first to screen for SW among Orthodontic and Non-Orthodontic patients, there are some limitations to consider for future studies of this nature. First, the use of an existing saliva repository and intake forms restrict some of the conclusions that can be drawn. From the viable samples with completed intake forms, we were left with adult

orthodontic patients. The original samples relied upon a convenience sample of willing participants that were not randomly selected and may therefore have implicit self-selection and cultural bias. Furthermore, these patient samples were collected at a single time point, which suggests no temporal conclusions can be made regarding the observed oral microbial prevalence. Although these findings are novel and clinically significant, limitations based upon the study design suggests the planning and implementation of prospective studies to be of the upmost importance to evaluate any temporal changes within these populations.

To gain an in depth picture of how SW is affected by orthodontic appliances future studies should include a longitudinal study that includes set intervals throughout treatment. Furthermore, with majority of orthodontic patients in the general population being adolescences and teens, a sample group of age matched samples would reveal an accurate depiction of how orthodontic appliances change the oral ecology and ecosystem in younger patients. Additionally, no information on which types of appliances were used or how long they have been present at the time of saliva collection were provided. It would be enlightening to know how different appliances affect the oral ecosystem. Finally, recognizing that the appliances seemed to give a competitive advantage to certain bacterial species either by creating a more favorable environment for one or a inhibitory environment for another, it would be beneficial to examine all bacteria species present before treatment and observe the changes in prevalence throughout orthodontic treatment.

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.

Appendix B

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Orthodontic Care in a Community of Underserved Patients: A Public Dental School Analysis

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Abstract
Orthodontic treatment in the United States has become commonplace, with the prevalence approaching one fifth of adolescents and teenagers. Ethnic minorities are significantly less likely to have orthodontic treatment, however these trends are starting to improve in recent years. Although much is known about adolescent oral and dental health during orthodontic treatment, much less is known about adult oral and dental health among the growing population of young minority adults seeking orthodontic treatment. Therefore, this study sought to analyze the demographic composition of the patient population within a recently opened public dental school-based Orthodontic program to determine if minority and low-income residents are being served and to evaluate some general parameters of oral health. Using Medicaid, Census and aggregate patient data, these analyses revealed that UNLV-SDM currently serves a large percentage of Medicaid and CHIP patients (>62%), much higher than in the local community (~37%). Moreover, minority patients in the Main (~59%), Orthodontic (~65%) and Pediatric (~82%) clinics are also much higher than the local population (~48%). These analyses strongly suggest that UNLV-SDM is currently meeting the mandate to provide services to low-income, Medicaid and Minority patients. Finally, the analysis of oral health parameters revealed that Minority patients were more likely to have significantly elevated markers for oral disease than non-Minority patients. These data may be among the first to elucidate the oral health problems facing this patient population and may provide more in depth prevention and treatment options for patients that face barriers to heath information and social access.

1. Background

Orthodontic treatment and care in the United States has become commonplace, with the prevalence approaching one fifth of adolescents and teenagers (1). In addition, nearly 1% of young adults (18 to 30 years old) surveyed were in orthodontic treatment in a recent cross-sectional analysis (2). Although males and females are nearly equally represented among those receiving orthodontic care and treatment, racial and ethnic minorities (Black and Hispanic children, in particular) were found to have significantly lower odds of having made any type of orthodontic visit (3).

These data reflect another recent study that have found large shortages of minority graduate dental residents, which revealed nearly three quarters of all Orthodontic residents were White, non-Hispanic (4). Furthermore, additional research regarding

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