Investigation of the Transfer of Oral Bacteria
From the mouth of the Patient to the Nasal Vestibule of the Clinician
During Orthodontic Bracket Debonding

A Thesis
Submitted to
The Temple University Graduate Board

In Partial Fulfillment
of the Requirements for the Degree
MASTER OF SCIENCE
in Oral Biology

By
Meghan P. McInnes DDS
May, 2011

Elizabeth Spannhake DDS, MS, PA
Thesis Advisor
Orhan C. Tuncay, DMD
Thesis Co-Advisor

TABLE OF CONTENTS

ABSTRACT

Background: Despite the use of personal protective equipment (PPE), the possibility exists that dental aerosols generated during standard dental procedures may penetrate to the nasal regions of treating clinicians and staff. These aerosols may carry with them pathogenic oral bacteria. Orthodontic procedures, such as the removal of braces that employ the use of high-speed handpieces and water spray to remove excess bonding material, may release aerosols. When the procedure involves the removal of bracket adhesive material from subgingival areas of the tooth in patients with inflamed hyperplastic gingival, there is an increased risk that aerosols generated carry pathogenic aerobic and anaerobic oral bacterial. This study was designed to determine whether an orthodontist wearing an ear-loop mask is at risk of nasal inoculation of aerosols containing pathogenic oral bacteria released during routine removal of braces (debond). There were two phases to the present study. The first phase sought to (a) adapt DNA-based methods for the selective identification of particular bacterial strains in the mouth and nasal vestibule, (b) confirm the ability to detect these representative bacterial targets in patient saliva samples, and (c) develop an effective method for collecting samples from the nasal vestibular regions of the clinician. The second phase of the study was designed to test the ability of aerosols to transfer the patients' oral bacteria into the nasal vestibules of treating orthodontists during a routine debonding procedure.

Methods: In *Phase 1*, nasal and oral swab samples from the patients' mouths and clinicians' nasal vestibules were taken to establish sampling and assay protocols necessary to identify and assess the relative abundance of the selected target bacteria of oral origin. Bacterial DNA was extracted from the swabs using the Sigma-Aldrich GenElute Bacterial Genomic DNA kit and was analyzed through Real-time PCR. After developing the methods in *Phase 1*, *Phase 2* involved one oral sample taken from a patient's mouth and two nasal samples taken from the treating clinician's nasal vestibule. The first nasal sample was taken as the start of the day, prior to any clinical contact with patients. The second nasal sample was taken directly after the patient's braces were removed. At the same time, a sample was taken of the patient's oral cavity prior to any rinsing. These three samples constituted a "case" of which there were twenty-eight in the study. Clinicians followed standard health and safety procedures. Bacterial DNA was extracted from the swabs using the Sigma-Aldrich GenElute Bacterial Genomic DNA kit and was analyzed through Real-time PCR. Only the bacteria detected in the patients' oral cavities were analyzed in the nasal samples of the treating clinicians.

Results: In *Phase 1*, nasal swab samples were obtained from seven residents using various collection methods to determine which of them yielded an adequate amount of bacterial DNA. The results indicated that detectable levels of individual bacterial DNA could be extracted from nasal swabs using the full nostril technique, twirled

around ten times. The bacterial DNA signatures used as surrogates for the presence of orally derived bacteria were confirmed in the oral samples taken from the patients' mouths. In *Phase 2*, twenty sets were analyzed from ten "cases." The remaining eighteen "cases" were excluded because they didn't meet the inclusion criteria. Three out of the ten "cases" showed a transfer of bacteria from the patient's mouth to the clinician's nasal vestibule by a two-fold increase. In two out of the three cases, two bacteria *Actinomycetes israelii* and *Fusobacterium nucleatum* increased in relative abundance from the pre-nasal samples to the post-nasal samples, and in the third case, only *Actinomycetes israelii* showed an increase in relative abundance. Therefore, 30% of the "cases" showed a two-fold increase in relative abundance from the pre-nasal samples to the post-nasal samples. Two out of the ten "cases" showed a two-fold decrease in relative abundance.

Conclusions: Oral microorganisms that are part of the aerosol cloud generated during the debond procedure may have the ability to pass around the edges of the protective mask into the clinician's nasal vestibule and the results of this study show that a transfer of bacteria can occur. The use of personal protective equipment, if worn properly, may not always be effective in preventing aerosols that are generated during standard removal of braces, however it is important for clinicians to be aware of the potential health risk as a result of inhalation of aerosol contamination during dental procedures.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Elizabeth Spannhake, for your guidance and support throughout this project. Thank you for your patience and providing me with a wondering education.

I would like to thank Dr. Ernst William Spannhake, Professor at JHSPH, for suggestions regarding experimental design and data interpretation. Thank you for dedication to the project and providing me with a valuable experience.

I would like to thank Dr. Meixia Gao, Postdoctoral Fellow in the Department of Environmental Health Sciences at the Johns Hopkins Bloomberg School of Public Health (JHPH), for instruction and assistance in performing all laboratory DNA procedures and data analysis.

I would like to thank Dr. Michael Horton, Assistant Research Professor in the Department of Orthodontics, for the design of the primer-probe sets and gathering the initial supply list.

I would like to thank Global Life Technologies, Corporation for providing a seed grand that made this study possible.

I would like to thank Dr. Orhan Tuncay for believing in me and providing me with the chance to achieve my goals. I truly appreciate the time and effort you have selflessly dedicated to this program and its residents.

I would like to thank the faculty and staff at the Temple University Department of Orthodontics. Your enthusiasm for Orthodontics and dedication to this program is extraordinary. Thank you for all the wonderful memories and outstanding education you have given me.

I would like to thank my classmates, Class of 2010, for making my time here fun, enjoyable and entertaining. To the Class of 2011 and 2012, stick together, support one another and never stop laughing. Good luck to you all!

I would like to thank my brothers and sisters for your love and support, without it my dream would have never come true. You managed to get me through the tough times and celebrate the good. I love you all so much.

I would like to thank my parents, your unconditional love and support have made all my achievements possible. Thank you for never giving up on me, always encouraging me, and instilling in me strong values that guide my decisions today. You both have been the most incredible role models in my life and I am so lucky to have you as my parents. I love you.

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Introduction

According to the Centers for Disease Control (CDC) and the Occupational Safety and Health Administration (OSHA) in 2007, dental personnel are among the group of all health care workers considered to be at high risk of exposure to airborne infectious diseases. Awareness of the dangers associated with airborne material generated during routine procedures in a dental office is essential for healthcare professionals. Aerosols released during routine dental procedures may contain pathogenic bacteria that increase the risk of infection for the dental staff. It is important to understand dental aerosols to reduce the risk of infection by pathogenic microbes.

Respiratory diseases, including those transmitted by airborne sources, are responsible for a large proportion of morbidity and mortality in the United States. Chronic lower respiratory disease was ranked as the fourth leading cause of death in 2001, and pneumonia and influenza were the seventh leading cause of death (CDC 2002). There is a paucity of definitive epidemiologic research studying the direct association between dental aerosols and disease transmission. Since bacterial pathogens have been linked to airborne transmissibility such as in the epidemic of tuberculosis, many concerns are raised about the potential risk to health care providers as a result of inhalation of aerosol contamination during dental procedures

(Greco and Lai 2008). Bentley et al. (1994) reported that dental aerosols can contaminate the skin and mucous membranes of the mouth, respiratory passes, and eyes of dental personnel. A higher prevalence of aerosol related symptoms such as nasal, ocular and skin discharges was observed among dental hygienists (Basu et al. 1988). Examples of respiratory diseases caused by aerosols of infectious microorganisms range from episodes of inhalation anthrax in textile mill workers to the long and well-documented history of nosocomial infections (Spendlove and Fannin 1984). Carter and Seal (1953) showed a definite correlation between the incidence of epidemics of common colds and other respiratory diseases in new naval recruits and in the dental personnel who treated them.

Awareness of and knowledge about dental aerosol production contaminated with bacteria and blood is important. Miller (1979) reported that as many as 100,00 bacteria per cubic foot of aerosols can be generated from a patient's mouth that normally contains 300 to 500 different species of bacteria. All procedures performed with the use of dental unit handpieces cause the formation of aerosol and splatter which are commonly contaminated with bacteria, viruses, fungi, often also with blood (Bentley et al., 1994; Harrel & Molinari 2004; Sacchetti et al., 2006; Szymanska, 2007; 2005). Aerosols represent an important potential route for disease transmission and these concerns extend to the offices of dental specialists as well. In the case of orthodontists, such aerosols are generated during procedures that include removal of

bonded appliances and braces, Interproximal Reduction (IPR) and enamelplasty. The disease potential to health care providers from inhalation of aerosolized oral bacteria generated from dental procedures still remains unclear.

The purpose of this study is to explore if the orthodontic clinician is sufficiently protected from the nasal inhalation of oral microorganisms by the facial mask typically used in orthodontic offices and clinics. Despite the use of personal protective equipment (PPE), the possibility exists that aerosols generated during standard dental procedures may penetrate to the nasal regions of treating clinicians and staff and carry with them pathogenic oral bacteria. Orthodontic procedures, such as the removal of braces that employ the use of high-speed handpieces with carbide burs and water spray to remove bonding material, may release aerosols. When the procedure involves the removal of bracket adhesive material from subgingival areas of the tooth in patients with inflamed hyperplastic gingiva, there is an increased risk that aerosols generated carry pathogenic aerobic and anaerobic oral bacteria. This study is designed to determine whether an orthodontist wearing an ear-loop mask is at risk of nasal inoculation of aerosols containing pathogenic oral bacteria released during routine removal of braces.

The present study consisted of three phases.

Phase 1 sought to (a) adapt DNA-based methods for the selective identification of particular bacterial strains in the mouth and nasal vestibule, (b) confirm the ability

to detect these representative bacterial targets in patient saliva samples, and (c) develop an effective method for collecting samples from the nasal vestibular regions of the clinician. Bacterial strains that are normally found in the patient's mouth, but are not routinely found in the clinician's nasal flora were identified from the literature. DNA signatures unique to these target bacteria were used as surrogates for the presence of orally derived bacteria in the nasal vestibule. *Phase 1* involved the use of nasal and oral swab samples from the patients' mouths and clinicians' nasal vestibules to establish sampling and assay protocols necessary to identify and assess the relative abundance of the selected target bacteria of oral origin.

Phase 2 was designed to test the ability of aerosols to transfer the patients' oral bacteria into the nasal vestibules of treating orthodontists during a routine debonding (removal of braces) procedure. Clinicians followed standard health and safety procedures. The detection of oral microorganisms in the treating clinician's nose after, but not before the procedure, that were also found to be present in the patient's mouth was used as the indication that transfer of bacteria had taken place during the procedure.

In the event that the results of *Phase 2* were positive, *Phase 3* was designed to compare the effectiveness of adding one or two commercially available nasal interventions to standard protective procedures in reducing the oral to nasal transfer of bacteria during the debonding procedure.

CHAPTER 2

Review of the Literature

2.1 Definition and Sources of Aerosols in the Dental Work Environment

Aerosols are suspensions of fine solid particles or liquid droplets in a gas that are produced during a sneeze, cough or any activity that expels oral fluids into the air (Toroglu et al., 2001). If microorganisms or their byproducts are present in the aerosols then they are known as bioaerosols. The principal components of bioaerosols are bacteria, viruses, fungi, protozoa, and their metabolites (Dutil et al., 2009). Infectious bioaerosols are those generated as respiratory particles and can be inhaled by the practicing clinicians and dental personnel.

Throughout the course of a dental procedure, an aerosol cloud of particulate matter may be visible. This cloud is evident during the use of an air/water syringe, rotary instrumentation or air abrasion, during the use of an ultrasonic scaler and polishing. This aerosolized cloud consists of materials and matter that originate from the treatment site and from the dental unit water lines. With the advent of the Swine Flu and severe acute respiratory syndrome or SARS, many questions and concerns about the potential spread of infection through airborne transmission have arisen. Aerosols entering the upper respiratory tract of the clinician have the potential to cause infection.

A number of factors affect the infectious potential of an aerosol. These may include the nature of the aerosol generated by various dental procedures, the aerosol particle size and concentration, airflow, climate, infectivity and virulence of the pathogens, and host susceptibility (Greco and Lai 2008). Aerosols containing particles more than 50 micrometers in diameter are referred to as splatter, while particles measuring less than 50 micrometers are called droplet nuclei (Harrel et al., 1998 and Micik et al., 1969). Droplet nuclei 50 micrometers in diameter have the ability to stay airborne for an extended period of time before they settle on environment surfaces or enter the respiratory tract (Harrel and Molinari 2004). Particles that are 2.5 micrometers in diameter have the ability to circulate in the air for days, increasing their harmful effects on dental personnel (Greco and Lai 2008).

Inhaling particles larger than 10 to 15 micrometers is more closely associated with upper respiratory infections, whereas smaller droplet nuclei 0.5 to 3.0 micrometers in diameter have the ability to penetrate and deposit in smaller passages of the lungs and accumulate in the lower respiratory tract (Greco and Lai 2008, Harrel and Molinari 2004). Toroglu et al., 2003 concluded that most aerosol droplets generated during a dental procedure have a diameter of 5 micrometers or less and are concentrated within two feet of the patient's mouth. Because orthodontists work within close proximity to the patient's mouth, they are at high risk of inhaling these

small aerosol droplets generated during the process of removing the braces, or bonded orthodontic appliances.

Aerosols are not usually generated during routine orthodontic procedures such as wire insertion, ligature tying and bonding brackets to the tooth surface, because no high-speed instruments are used. Generation of aerosols can occur during air-rotor stripping, rotary trimming of removable appliances, the removal of residual bonding resin from the tooth surface after the removal of orthodontic brackets or bonded appliances (also known as a "debonding" procedure), or air-power polishing before tooth banding or bracket placement procedures (Toroglu et al., 2003). At the termination of orthodontic treatment, high-speed handpieces are used to remove attachments and gross amounts of remaining adhesive cement used to attach brackets and bands to the teeth. The removal of the adhesive resin is carried out with a carbide bur with a fast or slow speed dental handpiece. This procedure often generates aerosols, splatter and dust. It is not known if oral microorganisms are part of this cloud and whether they pass through the pores of the facial mask barrier or around the edges into the clinician's nasal cavity. There is a paucity of published reports on this subject.

Currently, four studies have been carried out assessing aerosol production during enamel cleanup after orthodontic fixed appliances were removed. Toroglu et al. (2003) showed that aerosols generated during the debonding process were

routinely contaminated with blood. Ireland et al. (2003) examined particle size and determined that a wide range of particles (2.5 to 10 micrometers) are produced during a debonding procedure. They stated that particles larger than 2.5 micrometers in diameter will reside in the upper respiratory tract while those smaller than 2.5 micrometers will reach the alveoli. Day et al., 2008 concluded that an operator, during a debonding procedure, can inhale the aerosol particulates regardless of the handpiece speed or the presence or absence of water coolant. Toroglu et al. (2001) determined that environmental aerosols significantly increased during the removal of adhesive cement remaining after bracket removal. They showed that a considerable amount of microorganisms were found on blood agar plates fixed on the orthodontist's face shield.

Orthodontists and assistants can be at high risk of infection in the dental office. Bioaerosols are generated everyday in the orthodontic office which can increase the risk of disease to these health care providers. The infectious nature of dental bioaerosols, their small diameter, and prolonged and chronic exposure increase sensitization or infectious risk. It remains unclear whether the oral bacteria from the patient's mouth can be detected in the nasal flora of orthodontists. Clark (1974) showed that 50% of the dental staff exhibited altered nasal flora throughout the course of a day. It still remains unknown if aerosols generated during standard dental

procedures can penetrate to the nasal regions of treating clinicians and staff and if these aerosols carry pathogenic oral microorganisms present in the oral cavity.

2.2 Bacteria in the Oral Cavity

2.2.1 Healthy Oral Cavity

The oral cavity provides an environment of different ecological situations which leads to changes in the composition of normal flora. At birth, the oral cavity presents as a sterile environment, composed of soft tissues, lips, cheeks, tongue and palate. Through contact with the mother during feeding, bacteria is quickly introduced to the infant's oral cavity (Bowden and Hamilton 1998). The earliest bacteria isolated from the cavities of the neonate and young infants are bacteria that can survive in the environment consisting of soft tissue surfaces. These bacteria include *Streptococcus* (*S. Mitis, S. Oralis* and *S. salivarius*), *Prevotella oris, Prevotella melaninogenica, Fusobacterium spp.,* and *Veillonella* (Könönen et al., 1992, 1994a, b; Pearce et al., 1995). After tooth eruption, the environment in the oral cavity of a child becomes very similar to an adult.

As infants mature, the formation of the gingival sulcus and the supporting non-shedding structures of the teeth create an environment where anaerobic bacteria can colonize (Caufield et al., 1993; Petit et al., 1993, 1994; Milnes et al., 1993a,b; Könönen et al., 1994a; McClellan et al., 1996). By the age of five, a complex oral flora exists and

the child's oral cavity provides a favorable environment for a wide variety of organisms. In addition to the bacteria present in the normal flora, central to the survival of bacteria in an open system like the mouth, is the formation of dental plaque on tooth surfaces (Bowden and Hamilton 1998). The plaque biofilm serves as a habitat for a complex bacterial community that has the potential of being aerosolized during previously mentioned dental procedures and inhaled by clinicians.

2.2.2 Diseased Oral Cavity

Throughout the course of orthodontic treatment, many patients experience significant buildup of dental plaque and calculus due to the inability to clean teeth effectively. Even though orthodontists routinely review oral hygiene procedures and provide small brushes to clean teeth, many patients continue to have trouble adequately brushing and maintaining good levels of oral hygiene. As a result, dental plaque may form around the braces, irritating the gingiva of many patients.

Plaque can be defined as "the community of microorganisms found on a tooth surface as a biofilm, embedded in a matrix of polymers of host and bacterial origin" (Marsh, 2006). This structurally- and functionally- organized biofilm forms in an ordered manner and has a diverse microbial composition that, in health, remains relatively stable over time. This is known as *microbial homeostasis* (Marsh 2006). According to Socransky and Haffajee (1994), 300 to 400 different bacterial species have the capability to colonize the oral cavity. Moore and Moore (1994) detected 509

distinct bacteria species from gingival crevices of 300 people. Plaque samples analyzed through molecular ecology approaches, have shown more than 600 bacteria present(Marsh, 2006).

Plaque biofilm forms in the oral cavity on the solid surfaces of teeth and contributes significantly to the survival of oral bacteria. The formation of the biofilm follows a series of stages (Bowden et al., 1979; Brecx et al., 1983; Gilbert et al., 1993; Bowden and Li, 1997). During the initial formation of plaque, a pellicle forms from components of the saliva and crevicular fluid as well as bacteria products (Gibbons, 1989). This pellicle forms on clean, smooth surfaces immediately after tooth brushing. As the biomass increases, the cells become surrounded by a matrix of biopolymers, normally comprised of carbohydrates, lipoteichoic acid, and protein (Bowdman and Hamilton, 1998). The second stage involves the adherence and co-adherence of bacteria to the teeth. This involves interactions between bacterial adhesions and macromolecules comprising the acquired pellicle (Gibbons, 1989). Gram-positive bacteria such as *Streptococcus sanquis, Streptococcus mutans* and *Actinomyces viscosus* are considered the primary colonizers of dental plaque. These bacteria adhere to the pellicle-coated tooth surface via bacteria surface molecules that interact with components of the pellicle. Many bacteria possess proteinaceous components, called "adhesions," on their surfaces, which bind in a steriochemically specific manner to complementary molecules, or "receptors," on the tissue surfaces (Jones and Isacsson,

1983; Gibbons 1989). Adhesins are often associated with surface fibrils called "fimbriae" or "pili." Once these specific and non-specific interactions between adhesions on the cell and receptors on the surface of the pellicle have been established, the microflora at the site remains relatively stable over time despite regular minor perturbations to the oral environment (March, 1989). The established microbial homeostasis results from a dynamic balance of microbial interactions, including both synergism and antagonism (Marsh, 1994).

Plaque increases on the tooth by two mechanisms after the initial colonization. These two mechanisms are: (1.) An increase in accumulation of the initial bacterial colonizers and (2.) Adherence and multiplication of new bacterial species to the bacteria already present on the tooth surface. As the plaque mass increases, saliva is less able to penetrate plaque and protect the enamel. (Marsh, 1994). The microbial homeostasis can become compromised and a major shift in the composition of the microflora can occur.

The accumulation of plaque around the gingival margin can lead to gingivitis. The host response to the microbial challenge is an inflammatory one, and an increased flow of gingival crevicular fluid (GCF) occurs. As a result, the composition of the subgingival plaque shifts away from a streptococci-dominated microflora (Slots, 1977) to one with higher levels of gram-negative, capnophilic and anaeobic species such as *Fusobacterium nucleatum*, *Prevotella intermedia* and *Capnocytophaga species* as well as higher

levels of *Actinomyces spp* (Savitt and Socransky, 1984; Moore et al., 1987). Gingivitis can lead to more advanced forms of periodontal disease, resulting in a more diverse oral microflora. Depending on the type of disease, bacteria present in the oral microflora may include *Porphyromonas gingivalis, Campylobacter rectus, Eikenella corrodens, Actinobacillus actinomycetemcomitans*, and the oral spirochetes (*Treponema* species) (Marsh, 1994).

As the plaque mass accumulates and matures, there is a gradual characteristic shift from gram positive facultative microorganisms to gram negative anaerobic microorganisms. This overall developmental progression of bacteria is reflected as a shift from health to disease.

Streptococcus mutans are gram positive, anaerobic bacteria commonly found in the oral cavity and are a main contributor to dental decay. *Streptococci* are one of the early colonizers of the tooth surface, including *S. mutans*. *S. mutans* metabolize sucrose to lactic acid, resulting in an acidic environment on the highly mineralized tooth surface that can lead to decay and are one of few bacterial organisms that have the capability of effectively adhering to the tooth surface via receptors. *S. mutans* utilize sucrose to create a sticky, extracellular, dextran-based polysaccharide that gives them the ability to cohere to each other forming plaque. *S. mutans* can form this sticky polysaccharide only through the utilization of sucrose. Other sugars such as fructose, lactose and glucose are digested by *S. mutans* and produce lactic acid as the end product. The lactic acid in combination with dental plaque leads to dental decay.

In addition to *S. mutans*, *Actinomyces israelii* is one of the early colonizers of dental plaque on the tooth surface. One of several species of *Actinomyces*, *A. israelii* is a gram positive facultative, anaerobic, filamentous bacterium present in human oral flora. High numbers of these bacteria are present in dental plaque, cemental caries and tonsillar crypts.

Eikenella corrodens is a fastidious gram negative facultative anaerobic bacillus and inhabits the mucous membrane surfaces in humans, especially the upper respiratory tract. If the immune system is weak, *E. corrodens* has the capability of causing an infection. These bacteria are also one of the many bacterial species thought to be involved with the etiology of periodontitis, as it is a putative periodontal pathogen. It is also present at considerable levels in dental plaque.

Actinobacillus actinomycetemcomitans is a non-motile, gram-negative, capnophilic, fermentative coccobacillus (Zambion, 1985). Early studies suggest that *A. actinomycetemcomitans* is present mainly as a human parasite with a preference for colonizing mucous membranes. The bacteria are considered a normally innocuous constituent of the oral microflora which could occasionally cause severe medical infections (Zambion, 1985). Slots et al. (1980) determined that the bacteria's main oral ecologic niche is dental plaque found in periodontal pockets and buccal mucosa. The primary environment that *A. actinomycetemcomitans* inhabits is the oral cavity and *A. actinomycetemcomitans* has been identified as an important microorganism in the etiology of localized juvenile periodontitis. They are found to colonize the palate, tongue,

buccal mucosa as well as supragingival and subgingival plaque of both periodontally healthy and diseased individuals (Gmur and Guggenheim, 1994). The prevalence of *A. actinomycetemcomitans*-infection has been reported to be 20 to 25% among young people in urban areas of the US (Slots et al. 1980).

Capnocytophaga species are gram negative bacteria and can cause serious oral and extraoral infections in a susceptible host. *Capnocytophaga* comprises part of the normal flora of dental plaque and appears to be a component in the pathogenesis of periodontal disease in immuno-compromised hosts (Wilson et al.).

Fusobacterium nucleatum are gram negative non-sporeforming anaerobic filamentous bacteria inhabiting the mucous membranes of humans. The bacteria are present in the normal flora of the oral cavity. *Fusobacterium nucleatum* are one of the etiological agents of dental plaque and has been shown to be associated with advanced lesions of localized periodontitis in adults. The presence of *F. nucleatum* in patients with gingivitis may indicate that the gingival lesion is at risk for periodontitis (Tanner and Stillman, 1993).

Peptostreptococcus anaerobius, an obligate anaerobic cocci, is genetically and phenotypically heterogeneous (Watt and Jack, 1977). This gram positive anaerobe has played a role in human health and disease. *Peptostreptococcus anaerobius* is recognized as part of the gastrointestinal flora and is commonly isolated from human clinical specimens, particularly from the abdominal cavity and genitouninary tract (Murdoch, 1998). Moore et al. (1991) demonstrated the presence of *Peptostreptococcus anaerobius* in

the microflora of periodontal sites showing active periodontitis. The presence of the anaerobe in gingival inflammation may indicate the gingival lesion is at risk for periodontitis (Tanner and Stillman, 1993).

Staphylococcus aureus are gram-positive spherical bacteria that play an important role in human disease. The increasing number of methicillin-resistant *S. aureus* infections has raised awareness of the emerging problem of antibiotic resistance in pathogenic bacteria. Carriage of *S. aureus* in the nose appears to play a key role in the epidemiology and pathogenesis of infection. The ecological niche of *S. aureus* strains is the anterior nares. The nares prove to be the most consistant site from which this organism can be isolated (Williams, 1963). Three patterns of carriers have been distinguished over time. Approximately 20% of the population are persistent carriers, meaning they carry one type of strain. The majority of the population (about 60%) harbors *S. aureus* intermittently and the strains change with varying frequency. A small portion of the population, approximately 20%, are non-carriers and never carry *S. aureus* (Kluytmans et al., 1997).

The primary reservoir of *S. aureus* is the vestibulum nasi or anterior nares. The lining in this part of the nose contains fully keratinized epithelium with hairs, sebaceous glands and sweat glands. S. aureus is thought to flourish in this environment due to the absence of human defenses and are capable of withstanding the local antibacterial defenses. The bacteria attach firmly to human cell surfaces,

which prevents their elimination by most physicochemical mechanisms (Kluytmans et al., 1997).

It is a remarkable phenomenon that so many people harbor a microbe with such considerable powers of producing disease as staphylococcus. No other pathogenic bacteria are carried in such a large proportion of normal individuals. Healthy carriers can become a risk to others by transmitting the bacteria from themselves to another individual and thus causing potential health consequences.

2.3 Potential Health Consequences of Bacterial Inoculation of the Nose

2.3.1 Infection of the Exposed Individual

Airborne particulate matter, including fine and course water droplets, can contain pathogenic organisms and lead to inoculation of the nose, throat, upper airway and lung that can result in respiratory tract infections. Bacterial inoculation of the nose can result in a wide clinical spectrum of infections that range in severity from the common cold to life-threatening illnesses such as pneumonia (Busse, 1991).

In the pre-vaccination era, the dramatic spread of measles, and the high death rate caused by airborne anthrax define a clear threat of airborne infectious diseases. Other respiratory infections commonly transmitted through the airborne route include legionella, which is spread through aerosolization and aspiration of contaminated water (Yu, 1993).

Even though influenza has been demonstrated to spread through direct contact, widespread indoor transmission of influenza has been documented to spread beyond the physical limits of personal contact (Fiegel et al., 2006). Infections, such as small pox, tuberculosis, and rhinovirus, spread rapidly through large and fine airborne droplet transmission and severe acute respiratory syndrome (SARS) virus can spread through contaminated water and from person to person dispersion of droplet nuclei (Fiegel et al., 2006).

Transmission of airborne respiratory diseases poses a major public health threat especially to health care providers. Outcomes of exposure to infectious diseases not only involve clinical infection but also the risk of becoming a carrier. Although asymptomatic, carriers have the ability to transmit disease pathogens to the surrounding population at any point in time.

2.3.2 Exposed Individual Acting as a Carrier

"A carrier is someone who harbors and transmits pathogenic parasites without showing the usual evidences of infection." (Nichols, 1922) Thus, even though carriers tend to be symptom-free, they can be highly contagious and represent a health threat to others with whom they interact in public, work and private environments.

2.4 Nasal Cavity as a Repository for Bacteria

The upper respiratory tract is colonized by a large number of bacteria. The nares (nostrils) and the region just inside the nostrils (the nasal vestibule) are predominantly colonized by gram-positive, facultative anaerobic *Staphylococcus*

epidermidis and gram positive, aerobic or facultative anaerobic *corynebacteria.* About 20% of the human population serve as long-term carriers of *Staphylococcus aureus* with the bacteria being detected inside the nasal opening.

2.4.1 Role of the nose in filtering bacteria

The human immune system possesses many defense mechanisms against inhaled particles and aerosols that offer considerable protection in the respiratory tract (Kazantzis, 1961). The hairs of the nasal cavity filter larger particles while other particles that contact the mucous membrane of the nasal passages, pharynx and larynx will excite a sneeze or cough reflex, and be expelled.

The anterior portion of the nose, termed the nasal vestibule, is unciliated. When particles are deposited in this area, they remain at the deposition sites for an indeterminate period until they are mechanically removed by nose wiping, blowing or sneezing (Lippmann et al., 1947). Inspired particles and their contents not removed from this zone, can pass onto the ciliated portion of the nasal passage and also may be inspired deeper into the respiratory tract.

Inspired air passes through a web of nasal hairs and flows around the turbinates in the ciliated portion of the nasal passage directly beyond the nasal vestibule. The air is warmed, moistened and partially depleted of particles with aerodynamic diameters greater than 1μm by contact with the nasal hairs and at the bends in the air path. By the process of diffusion, particles in the inspired air that are less than 1μm can be deposited in this area. This portion of the nasal passage is lined

with mucus, most of which is driven toward the pharynx by the beating of the cilia, carrying with it deposited insoluble particles. The mucus has the ability to further dissolve the soluble particles still present. Some mucus, not propelled toward the pharynx, moves towards the anterior nares, carrying inhaled whole or dissolved particles into the zone of intermittent mechanical clearance, from which the clearance process is again attempted (Lippmann et al., 1947). Inhaled particles driven toward the nasopharynx from the nasal passage and particles inhaled through mouth and deposited in the oropharynx are generally swallowed. Regardless of their route of entry, particles are deposited on the larynx and carried rapidly to the esophagus by the mucus coming up from the trachea (Lippmann et al., 1947). Once particles have been deposited on the larynx, they have entered the lower respiratory tract.

2.5 Movement of bacteria from upper airway to lower airway

The basic anatomy of the respiratory tract provides an initial barrier against noxious and infectious agents. The tight cellular junctions and epiglottis help to protect the lower respiratory tract from penetration by harmful agents (Busse, 1991).

The anatomic branch design of the respiratory tract provides an effective initial mechanical barrier. The conducting airways branch into smaller and smaller units distally that allows for rapid and effective filtration of potentially harmful inhaled particles. The branch design greatly reduces the entry of noxious and infectious agent into the terminal alveoli.

As the individual airway diameters decrease, the total number of branching units increases, resulting in an increase in total cross sectional area and a decrease in air velocity. Particles too heavy to follow the bends of the air passage in the larger airways are deposited through impaction at the branch points. In the smaller airways, the low air velocity results in deposition by sedimentation and diffusion (Lippmann et al., 1994).

Inhaled air follows a tortuous path through the nose or mouth and branching airways in the lung. The three main mechanisms of deposition are impaction in the larger airways, sedimentation in the smaller bronchioles and diffusion in smallest airways and aveoli. Particles inhaled through the nose or from the mouth have the capability of entering the respiratory system. Bacteria can replicate in the mucosal tissues of both the upper and lower airways, resulting in the destruction of airway cells, denuded epithelium, and compromised normal ciliary activity. As a consequence, normal mechanical clearance of respiratory particles does not take place and bacterial colonization is enhanced (Busse, 1991).

The oral secretions of orthodontic patients with bonded appliances such as braces provide a rich source of potentially pathogenic bacteria. Generation of aerosols containing the patients' mouth fluids can occur during orthodontic procedures and can serve as a mechanism of dispersing these bacteria. Even though orthodontists wear personal protective barriers such as ear-looped facemasks, the possibility exists that these generated aerosols may penetrate to the orthodontists' nasal regions, either

through the mask material itself or through gaps in the contact between the mask edges and the face. Nasal passages exposed in this way could provide sites for the deposition and accumulation of potentially pathogenic bacteria. Although there is a lack of research that specifically links dental aerosols to disease transmission, evidence does indicate that pathogenic bacteria inoculating the nose have the potential to either move to through the respiratory tract or be transmitted to others who may be potentially more susceptible to infection. The use of effective means to reduce or eliminate exposure of the nose to dental aerosols is important to reduce disease risk in dental professionals.

CHAPTER 3

Phase 1: METHODS DEVELOPMENT

3.1 Specific Aims of *Phase 1*

(a) Adapt DNA-based methods for the selective identification of particular bacterial strains in the mouth and nasal vestibule,

(b) Confirm the ability to detect these representative bacterial targets in patient saliva samples, and

(c) Develop an effective method for collecting samples from the nasal vestibular regions of the clinicians.

3.2 Materials and Methods

In order to provide a source of DNA specific to each of the selected bacterial targets, cultures of the following bacteria were obtained from the American Type Culture Collection (ATCC) in Manassas, Virginia: *Actinobacillus actinomycetemcomitans, Actinomycetes israelii, Capnocytophaga sputigena, Eikenella corrodens, Fusobacterium nucleatum, Peptostreptococcus anaerobius,* and *Staphylococcus aureus*. Genomic DNA was extracted from the bacteria to provide standards with which to confirm that the designed primer-probe sets were specific for each target bacterial strain. The DNA for *Streptococcus mutans* was ordered from ATCC.

Genetic markers for the eight bacteria typically present in the oral subgingival flora were used as surrogates for the presence of orally-derived bacteria. Species-specific RT-PCR primers were designed on the basis of published data with the assistance of GeneBank; Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (NCBI), Bethesda, MD. Primer Express 3.0™ from Applied Biosystems was used to design the primer/probe sets. Forward and reverse signature sequence primers (approximately 20-base each) were designed (Table 1). TaqMan gene assays for the eight primer/probe sets were ordered from Applied Biosystems.

Table 1: Primer-Probe Sets for the 8 Bacteria

Bacterium	Aerobe	Anaerobe	Primer/Probe Set (F, R, Probe)	Amplicon (bp)
Actinobacillus (Aggregatibacter) actinomycetemcomitans	X		5'-CGGTTACCGAGGCGGTTATT -3" 5'-CCTGACGTTGTGCGTCGTTA -3" 5'-CTGTGCCGGCATACT -3"	57
Actinomycetes israelii		X	5'-CGGAGCATGCGGATTAATT -3" 5'-CCCATGTCAAGCCCTGGTAA -3" 5'-ATGCAACGCGAAGAA -3"	58
Capnocytophaga sputigena	X		5'-GGCGCACGGGTGCAT-3' 5'-TCGGGCTATCCCTATGTGAAA-3' 5'-ACGCGTATACAATCTG-3'	?
Eikenella corrodens		X	5'-CGTTGGTAATGCCTGGTGAA -3" 5'-CGCACAGCATCATCAGGAAT -3" 5'-CCGAAGAAAGCAAGCC -3"	72
Fusobacterium nucleatum		X	5'-CAGCAATGTCGCGGTGAAT -3" 5'-TGTGACGGGCGGTGTGT -3" 5'-CGTTCTCGGGTCTTG -3"	?
Peptostreptococcus anaerobius		X	5'-TTTTGGGAACGTTGGTGCTT -3" 5'-AGCAACAACTTTTCCGCCTAAC -3" 5'-CACAGTTAAGAACATCC -3"	?
Staphylococcus aureus	X		5'-ATGACCACCACGAGTCTTAGCA -3" 5'-ATCTAACGTACGCGCATTTGAG -3" 5'-CTTGTGCCAACTTC -3"	61
Streptococcus mutans	X		5'-AGAGGAGCTGCAAGCGAAGT -3" 5'-GAACCCCTGTGGAAATTTGTTTT -3" 5'-TCCGGAGCTTTTTG -3"	62

Real-time PCR relies on the release and detection of a fluorescent signal following the cleavage of a fluorescent labeled probe by the 5'exonuclease activity of

Taq polymerase. In the intact state, the fluorescent signal on the probe is quenched by the close proximity on the probe of the second dye. The release of the fluorescent dye during each round of amplification allows for the rapid detection and quantification of DNA without the need for post-PCR processing, such as gel electrophoresis and radioactive hybridization (Nadkarni et. al (2002)

PCR MicroAmp Fast 96-well reaction plates were prepared with the eight Genomic DNA samples in duplicate and analyzed using the eight primer-probe sets presented in Table 1. The target primer sequences must be unique in order to identify a specific organism or organism group. It is critical that the PCR primer be able to identify with high efficiency and specificity the target primer sequences in the specimen of interest (Espy et al., 2006).

The reaction plate was centrifuged and placed in a Biosystem RT-PCR 7000 System. The real time PCR conditions consisted of an initial step 50˚C for 2 minutes. The next step was 95˚C for 10 minutes followed by an amplification program for 40 cycles of 15 seconds at 95˚C, 30 seconds at 60˚C, and 30 seconds at 72˚C with fluorescence acquisition at the end of each extension. The amplification program was immediately followed by a melt down consisting of 60 seconds at 95˚C, 60 seconds at 65˚C, and a gradual increase to 90˚C at a rate of 0.2˚C/sec with fluorescence acquisition at each temperature transition. The run time for the PCR was 1hr and 50min and confirmed the primer-probe sets were specific for the bacteria DNA

templates for all eight bacteria. Using the above procedure, the specificity and absence of cross-reactivity of the primer-probe sets was confirmed using the strain-specific DNA.

The eight DNA samples were diluted to four different concentrations to standardize the bacterial primer and probe sequences. The DNA samples were diluted 10-fold, 100-fold and 1000-fold for Real-time PCR analysis. A one unit of increase in C_T value represents a doubling of the DNA present and about 3.34 cycles represents a 10-fold increase in the DNA present. The 10-fold dilution of the bacterial primer and probe sets at 10^{-1}, 10^{-2}, and 10^{-3} resulted in the expected C_T value difference of approximately three.

Study Subjects and Consent Procedures

IRB approval was obtained from Temple University Research Administration for the first phase of the study. Subjects for the first cohort consisted of two orthodontic patients scheduled to visit Temple University's Orthodontic clinic for removal of braces. Subjects that presented for removal of braces were identified in the daily schedule and when they arrived at the clinic for their appointment, they were approached and asked if they would like participate in the study. To participate in the study, subjects had to meet the following inclusion criteria: 18 years or older; no signs of respiratory infection or chronic sinusitis; no antibiotic use for two weeks before the appointment; non-smoker; and ready for removal of braces at that appointment. The

study and inclusion criteria were explained to them and if they wanted to participate and indicated that they met the inclusion criteria, an informed consent was signed. No medical information or records were obtained.

The second cohort consisted of seven orthodontic residents, age range 27-33 years, at Temple University's Orthodontic clinic. The study was described to the Temple University's Orthodontic residents and they were asked to participate in the study. To participate in the study, the residents had to meet the following inclusion criteria: no signs of respiratory infection or chronic respiratory sinusitis; no antibiotic use for two weeks before sample collection and a non-smoker. Residents were asked if they meet the requirements to participate in the study. No medical records were reviewed.

Phase 1 experiments posed no significant risk to the participating subjects. Slight discomfort may have occurred in residents when a sample of their nasal vestibules was taken. In order to maintain confidentiality, the resident and patient names were not recorded. No sensitive patient information was provided and no personal health information was obtained. In addition, bacterial samples remained anonymous.

Optimizing Nasal Sampling Methods

After a written informed consent was obtained, the tip of the collection swab was inserted approximately one centimeter into the nares of the resident and rolled around to collect the nasal bacteria present. Collection took place in the morning, prior to clinical contact with patients. The subject samples were numbered sequentially (Resident #1, Resident #2) and this number was the only identifier for the sample. These samples were used to verify that the target oral bacterial markers characterized in the patient cohort were normally absent or present at very low levels in the nasal vestibule. Different sampling methods were performed including full nostril swab with one roll, half-nostril swab with one roll, full nostril with swab rolled around 10 times, half-nostril with swab rolled around 10 times, full nostril with two swabs rolled around 10 times in the same nostril and half-nostril with two swabs rolled around 10 times in the same nostril (see Table 2).

Table 2: Variations in Nasal Sampling Methods

Patient #1 Mouth
Patient #2 Mouth
Resident #1 Half Nostril
Resident #2 Full Nostril
Resident #3 Full Nostril
Resident #3 Half Nostril
Resident #4 Full Nostril 10x + 2 swabs of same nostril
Resident #5 Half Nostril 10x + 2 swabs of same nostril
Resident #6 Half Nostril 10x
Resident #7 Full Nostril 10x

Sampling from the Patient Mouth

After consent was obtained from the patient, a single sample of tissue fluid along the gingival margin and buccal vestibule of the patient's mouth was collected by the principle investigator with a sterile cotton swab (Venturi Transystem; Copan Diagnostics, Corona, California) after the braces were removed. The subject samples were numbered sequentially (Patient #1, Patient #2) and this number was the only identifier for the sample. These samples were used to establish optimal procedures for sample preservation, DNA extraction, and target bacterial DNA amplification by RT-PCR methods. Only a few sample swabs were necessary to determine whether a single sample obtained would contain one or more of the eight bacteria and whether enough bacteria were present to analyze with the PCR technique.

The main purpose was to determine whether enough bacteria existed on the swab sample obtained and to test the sensitivity of the RT-PCR technique. Only the P.I. who was an orthodontic resident and study-affiliated faculty had access to the coded PCR assay data. Non-identified, coded samples of extracted DNA were stored during methods development and were destroyed when the *Phase 1* experiments were completed.

Bacterial DNA Extraction

The swab specimens were transported on ice and DNA was extracted for RT-PCR analysis within 24 hours of being collected. The following protocol was performed to extract the bacterial DNA from the collection swab using the Sigma-Aldrich GenElute Bacterial Genomic DNA kit.

The extraction protocol was modified as recommended by Sigma-Aldrich technical support to optimize recovery of low levels of DNA. Lysozyme solution was prepared with Gram-positive lysis solution and 200µL lysozyme solution was placed into each 1.5mL EP tube using a pipette. The collection swab was placed into the lysozyme solution and incubated at room temperature for two minutes then centrifuged at 12,000 X g for 1 minute. Each swab was twirled in the solution ten times and then excess solution was removed from the swab by twirling the swab firmly against the side of the tube. Each swab was then discarded, the tubes closed and vortexed briefly. The solution was then incubated for 30 minutes at 37°C. The

solution was treated with 20μL of RNase A solution and incubated for 2 minutes at room temperature. For cell lysis, 20μL of the Proteinase K solution was added to each sample followed by 200μL of Lysis Solution C. The solution was vortexed thoroughly (about 15sec) and incubated at 55°C for 10 minutes. A homogenous mixture was essential for efficient lysis. Each column was then prepared by adding 500μL of the Column Preparation Solution to each pre-assembled GenElute Miniprep Binding Column. It was centrifuged at 12,000 x g for 1 minute and the eluate was discarded. The Column Preparation Solution maximizes binding of the DNA to the membrane resulting in more consistent yields. To prepare for binding, 200μL of ethanol (95-100%) was added to the lysate and mix thoroughly by vortexing for 5-10 seconds. A homogenous mixture was essential. The entire contents of the tube were transferred into the binding column using a wide bore pipette tip to reduce shearing the DNA when transferring the contents into the column. The solution was centrifuged at ≥ 65000 X g for 1 minute. The eluate was discarded and the column was placed back into the same collection tube. The solution then underwent a First Wash where 500μL of Wash Solution 1 was added to each column and centrifuged for 1 minute at ≥ 65000 X g. The eluate was discared and each column was placed back into the collection tube. A Second Wash was performed by adding 500μL of wash solution to the column and centrifuging for 3 minutes at maximum speed (12,000-16,000 X g) to dry the column. Each column was centrifuged for an additional 1 minute at maximum

speed if residual ethanol was seen. The eluate was discarded and the column was placed into a new collection tube. To elute the DNA, 120μL of the Elution Solution was added directly onto the center of the column for the mouth sample and100μL of the Elution Solution was added directly onto the center of the column for the nasal sample. To increase the elution efficiency, each sample was incubated for 5 minutes at room temperature and then centrifuged for 1 minute at ≥ 65000 X g to elute the DNA. This step was repeated with the eluted solution at the bottom of the collection tube. The sample was stored at -20°C.

Real-time PCR

Eight 1.5mL EP tubes were prepared for all eight bacterial primer-probe sets. The Taqman MGB probes and primers provided were premixed to a concentration of 18uM for each primer and 5uM for the probe. This was a 20X mix so the final concentration in the reaction system was 900nM for the primer and 250nM for the probe. Taqman Master Mix (12.5μL/well) and water (6.25μL/well) were added to each probe (1.25μL/well) and the total volume of 20μL was obtained. Each bacterial primer-probe solution was added in rows to the MicroAmp Fast 96-well reaction plate in the preparation for RT-PCR. The bacterial DNA that was extracted from the first four samples (two nasal samples and two oral samples) were added to each well (5μL/well). Each sample was duplicated for replication. The total volume in each reaction well was 25μL. A second reaction plate was set up for remaining nasal

samples. Since these samples were all collected from the residents' nares, only *S. Aureus* was analyzed because out of all eight bacteria, *S. Aureus* is the only one that is predominantly present in the nasal vestibule.

The reaction plate was centrifuged and placed in a ABI Prism 7000 Sequence Detection System. This system is a Peltier-based thermal cycling system with a tungsten-halogen lamp excitation source. The real time PCR conditions consisted of an initial step 50˚C for 2 minutes. The next step was 95˚C for 10 minutes followed by an amplification program for 40 cycles of 15 seconds at 95˚C, 30 seconds at 60˚C, 30 seconds at 72˚C with fluorescence acquisition at the end of each extension. The amplification programs was immediately followed by a melt down consisting of 60 seconds at 95˚C, 60 seconds at 65˚C, and a gradual increase to 90˚C at a rate of 0.2˚C/sec with fluorescence acquisition at each temperature transition. The run time for the PCR was 1hr and 50min.

For the purpose of providing a representation of the relative abundance of bacteria present as a proportion, it was necessary to establish a value for the "total bacterial load present" in each nostril. Based on the C_T values, a calculation of the relative abundance of each target bacteria as a proportion of the total could be determined. This normalization provided the basis for controlling for the inadvertent removal of samples of differing size or content in the two samples being compared.

Nadkarni et al. (2002) constructed and evaluated a universal primer and probe set which specifically detected 16S rDNA of the Domain *Bacteria* to estimate total bacterial load by real-time PCR. Horz et al. (2005) confirmed that the universal assay designed by Nadkarni et al. (2002) was the best of ten primer/probe sets analyzed from the literature. Pre-optimized concentrations of the universal forward (5'-TCCTACGGGAGGCAGCAGT-3') and reverse (5'-GGACTACCAGGGTATCTAATCCTGTT-3') primers and the probe (5' [6-FAM]-CGTATTACCGCGGCTGCTGGCAC-3'-[TAMRA]) as designed by Nadkarni et al. (2002) were used to standardize the data.

3.3 *Phase 1* Results and Statistical Analysis

Nasal swabs samples were obtained from seven residents using various techniques to determine which of them yielded an adequate amount of bacterial DNA. The results indicated that detectable levels of individual bacterial DNA can be most consistently extracted from nasal swabs using the full nostril technique, twirled around ten times (See Table 3 and Table 4).

Actinobacillus actinomycetemcomitans, Actinomycetes israelii, Capnocytophaga sputigena, Eikenella corrodens, Fusobacterium nucleatum, Peptostreptococcus anaerobius, and *Streptococcus mutans* were identified in the oral samples (See Table 3).

Table 3: RT-PCR Results for *Phase 1*

Collection Method	Detector	CT	Average CT
Pt 1 Mouth	A.act	36.49	35.905
Pt 1 Mouth	A.act	35.32	
Pt 2 Mouth	A.act	34.91	35.875
Pt 2 Mouth	A.act	36.84	
Half Nostril	A.act	35.67	35.84
Half Nostril	A.act	36.01	
Full Nostril	A.act	Undetermined	
Full Nostril	A.act	Undetermined	
Pt 1 Mouth	C.spu	36.38	36.38
Pt 1 Mouth	C.spu	Undetermined	
Pt 2 Mouth	C.spu	26.68	26.68
Pt 2 Mouth	C.spu	Undetermined	
Half Nostril	C.spu	Undetermined	
Half Nostril	C.spu	Undetermined	
Full Nostril	C.spu	Undetermined	
Full Nostril	C.spu	Undetermined	
Pt 1 Mouth	E.cor	27.37	28.015
Pt 1 Mouth	E.cor	28.66	
Pt 2 Mouth	E.cor	19.98	21.615
Pt 2 Mouth	E.cor	23.25	
Half Nostril	E.cor	36.69	38.32
Half Nostril	E.cor	39.95	
Full Nostril	E.cor	Undetermined	
Full Nostril	E.cor	Undetermined	
Pt 1 Mouth	F.nuc	26.24	27.935
Pt 1 Mouth	F.nuc	29.63	
Pt 2 Mouth	F.nuc	17.68	19.4
Pt 2 Mouth	F.nuc	21.12	
Half Nostril	F.nuc	33	34.59
Half Nostril	F.nuc	36.18	
Full Nostril	F.nuc	37.2	37.2
Full Nostril	F.nuc	Undetermined	

Collection Method	Detector	CT	Average CT
Pt 1 Mouth	S.aur	36.72	37.41
Pt 1 Mouth	S.aur	38.1	
Pt 2 Mouth	S.aur	36.43	36.34
Pt 2 Mouth	S.aur	36.25	
Half Nostril	S.aur	34.01	34.385
Half Nostril	S.aur	34.76	
Full Nostril	S.aur	36.6	36.77
Full Nostril	S.aur	36.94	
Pt 1 Mouth	A.isr	22.98	23.415
Pt 1 Mouth	A.isr	23.85	
Pt 2 Mouth	A.isr	24.59	30.655
Pt 2 Mouth	A.isr	36.72	
Half Nostril	A.isr	38.38	38.38
Half Nostril	A.isr	Undetermined	
Full Nostril	A.isr	Undetermined	
Full Nostril	A.isr	Undetermined	
Pt 1 Mouth	P.ana	Undetermined	
Pt 1 Mouth	P.ana	Undetermined	
Pt 2 Mouth	P.ana	28.92	31.31
Pt 2 Mouth	P.ana	33.7	
Half Nostril	P.ana	Undetermined	
Half Nostril	P.ana	Undetermined	
Full Nostril	P.ana	Undetermined	
Full Nostril	P.ana	Undetermined	
Pt 1 Mouth	S.mut	23.3	23.42
Pt 1 Mouth	S.mut	23.54	
Pt 2 Mouth	S.mut	28.92	29.08
Pt 2 Mouth	S.mut	29.24	
Half Nostril	S.mut	Undetermined	
Half Nostril	S.mut	Undetermined	
Full Nostril	S.mut	39.3	39.3
Full Nostril	S.mut	Undetermined	

Table 4: RT-PCR Results for *Phase 1*

Collection Method	Detector	CT	Average CT
Full Nosril	S.aur	30.98	30.925
		30.87	
half Nostril	S.aur	31.53	31.335
		31.14	
Full Nosril with 2 swabs	S.aur	37.4	36.68
		35.96	
half Nostril with 2 swabs	S. aur	36.46	36.725
		36.99	
Full Nosril 10x	S.aur	34.34	34.55
		34.76	
half Nostril 10x	S.aur	37.15	37.15
		Undetermined	

Preliminary tests were run using the Universal Primer/Probe sets. The Universal Primer/probe set was diluted 10 fold and 100 fold to determine what the appropriate dilution would be for the nasal samples with the universal probe. After verifying that the Universal Probe was active, using the mouth derived DNA as a template, the proper dilution of nasal sample DNA was determined. A total of seven samples from both mouth and nasal sources were tested to determine the proper dilution. The results showed that a 10 fold DNA dilution at $10^{-1}$and 10^{-2} was approximately 3.40 with a standard error 0.15580. The binding of the probe was successful and because of the relatively low threshold C_T values obtained from the nasal samples using the Universal Probe, the decision was made to assay the DNA samples undiluted.

Having successfully achieved the three goals of *Phase* 1, the feasibility of pursuing the goals of *Phase* 2 was established. *Phase 2* was designed to determine if a transfer of bacteria from the patient's mouth to the clinician's nose during the removal of braces can be detected using bacterial DNA markers.

CHAPTER 4

Phase 2: Assessment of Bacterial Transfer

4.1 Specific Aims of *Phase 2*

(a) Determine if a transfer of bacteria from the patient's mouth to the clinician's nose during the removal of braces can be detected using bacterial DNA markers,

(b) Determine the relative abundance of each target bacteria as a proportion of the total present in the before nostril and the after nostril samples, based on the C_T values. This normalization provided the basis for controlling for the inadvertent removal of samples of differing size or content in the two samples being compared and,

(c) Determine what bacteria are present in higher numbers in the oral cavities of patient receiving orthodontic treatment.

4.2 Materials and Methods

IRB approval was obtained from Temple University Research Administration for the second phase of the study. The same inclusion requirements established for Residents and patients in *Phase 1* were applied to *Phase 2*, with the exception that

patients of all ages were accepted in *Phase 2* and either an informed consent form or child assent form was signed by every patient or guardian.

Nasal Sampling Method

In *Phase 1*, Residents' noses were sampled one time in the morning for the purpose of determining proper methods of sample collection for bacterial detection. Having established these methods, *Phase 2* was designed to determine if a transfer of bacteria from the oral cavity of the patient to the nasal vestibule of the treating clinician could be detected under the conditions of the study. A total 12 residents were recruited to participate in the study. Each resident presented for one to five debonding cases, from which samples were taken from the resident and the patient. The residents' noses were sampled twice. The first sample was taken from the right nostril at the start of the day before any clinical contact with patients. A second sample was taken from the left nostril immediately after the resident removed the braces. Both samples were full swabs of each nostril, twirled around ten times. The first sample taken was the control sample for each Resident.

Sampling from the Patient Mouth

A third sample was collected from the patients' un-rinsed gingival margins immediately following bracket removal. These three samples constituted a "Case," of

which there were 28 in the study. Each Case centered on a different patient, which individual residents were involved in one or more Cases.

Sampling Protocol

A total of 28 patients presenting to the Department of Orthodontics for the removal of their braces were included in the study. After obtaining required consents, baseline nasal samples were collected from the right nasal vestibule of the treating resident at the start of the day before any clinical contact with patients. The residents were provided a new mask from the box before starting the debonding appointment. Henry Schein's earloop masks are a low barrier masks, having greater than 95% bacterial filtration efficiency. Residents were asked to chew a stick of sugar-free chewing gum during the procedure to encourage nasal breathing. They were instructed to use a carbide trimming and finishing bur #7801, to focus breathing through their noses, and not to touch the outside of their masks. After the removal of composite with the high-speed handpiece and carbide bur was completed, the patients' gingival margins and sulcus were swabbed prior to rinsing or brushing their teeth. The swabs were labeled and stored at -4 ˚C. The masks were removed with clean hands from the residents' faces and the residents' left nostrils were swabbed. The swabs were labeled and stored.

The swabs were transferred to the laboratory on ice and the DNA was extracted from the swabs within 24 hours using the methods established in *Phase 1*.

RT-PCR analysis was first performed on each mouth sample to determine which of the seven orally-derived bacterial species were present in that patient and could potentially be transferred to the nasal vestibules of the treating clinician. These bacterial targets were then analyzed for in the corresponding nasal sample in that Case.

TaqMan technology determines the PCR cycle at which the increase in fluorescence of the reporter dye reaches a threshold cycle (C_T). C_T is proportional to the log of the amount of target DNA and hence the log of the number of bacteria in the sample, provided there is only one copy of the reported sequence within the genome. Threshold cycle C_T values were determined for each individual sample run in duplicate for each of the seven bacteria that were present in the mouth of each case. *S. aureus* was not selected because *S. aureus* is predominantly present in the nose and the goal of the present study was to determine a mouth to nose transfer. This resulted in the analysis of 1, 092 samples. In addition to these samples, some samples were re-assayed to confirm the results and the universal probe was analyzed as well. This resulted in an excess of 1,200 assays performed on the mouth and nasal samples in *Phase 2*.

In order to provide a representation of the relative abundance of bacteria present, it was necessary to establish a value for the "total bacterial load present" in each nostril before and after the debond takes place in a given case. Based on the C_T

values, a calculation of the relative abundance of each target bacteria as a proportion of the total present in the pre-nasal and the post-nasal samples could be determined. This standardization provided the basis for controlling for the inadvertent removal of samples of differing size or content in the two samples being compared.

Nadkarni et al. (2002) constructed and evaluated a universal primer and probe set which specifically detected 16S rDNA of the Domain *Bacteria* to estimate total bacterial load by real-time PCR. In the present study, total bacterial loads of each nostril before and after the debond in a given case were determined using pre-optimized concentrations of the universal forward (5'-TCCTACGGGAGGCAGCAGT-3') and reverse (5'-GGACTACCAGGGTATCTAATCCTGTT-3') primers and the probe (5' [6-FAM]-CGTATTACCGCGGCTGCTGGCAC-3'-[TAMRA]) as designed by Nadkarni et al. (2002).

Each of the 28 "cases" consisted of "sets" of measurements for each bacterium. Each "set" consisted of paired samples from the mouth, pre-nose and post-nose samples. Inclusion criteria for the universal primer and probe analysis included: Both samples of a pair must have a detectable C_T level for the samples to be used and "detectable" was defined as any number provided by the instrument, regardless of how high; If not detectable in the mouth, none of the samples for that set (mouth/pre-nose/post-nose) can be used for that bacterium; If a bacterium is present in the mouth and the post nose samples, the data set can be used if the pre-

nose samples are both undetermined. Twenty-three out of the twenty-eight cases met the criteria and were analyzed using Real-time PCR.

Real-time PCR for the determination of total bacterial load

Amplification and detection of DNA by real-time PCR were performed using the optical grade 96-well plates. Duplicate samples were routinely used for the determination of DNA by real-time PCR. The PCR reaction was performed for twenty-three out of the twenty-eight cases in a total volume of 25μL using the Taqman Universal PCR Master Mix (Applied Biosystems), containing 18uM of each of the universal forward and reverse primers and 5uM for the probe. This was a 20X mix so the final concentration in the reaction system was 900nM for the primer and 250nM for the probe. No dilution was necessary as established in *Phase 1*.

4.3 *Phase 2* Results and Statistical Analysis

All of the seven selected oral bacterial targets were present in oral cavities of the 28 cases, some more prevalent than others (see Table 5). *Fusobacterium nucleatum* was the most prevalent bacteria of the seven selected, present in all 28 cases. *Captnocytophaga sputigena* was the least prevalent, only present in 14 cases.

Table 5: Prevalence of each bacterium in the oral cavity among all 28 cases

	Bacteria in Oral Cavity						
	A. comm	*C. sputa*	*E. corr*	*F. nucl*	*A. israel*	*P. aner*	*S. mutans*
Total cases	22	14	26	28	26	23	21

All cases in which any of the Universal Primer/Probe set values came back "Undetermined" were arbitrarily dropped from the study. Therefore, cases 1, 21 and 23 set values came back "Undetermined" and were eliminated with their associated bacterial sets (numbering 9 total). The number of bacterial sets went from 57 sets from 23 study cases to 48 sets from 20 study cases. These 48 bacterial sets were normalized. Each Cycle Threshold (C_T) value was converted to a value that represents "DNA relative abundance." The formula takes into account that each one unit increase in C_T value represents a doubling of the DNA present and about 3.34 cycles equals a 10-fold increase in the DNA present ($2^{-3.34}= 10$). A negative C_T value was used since it is inversely related to the amount of DNA present.

The 48 bacterial sets and their associated Universal Primer/Probe set data were converted from C_T values to DNA relative abundance using the formula:

$$\text{Relative Abundance} = 2^{Ct}\,(k),$$

where k represents a constant to bring the number into a decimal range convenient to work with. Each pre-sample bacterial abundance and each post-sample bacterial abundance were divided by its corresponding pre- or post- Universal abundance for

that case to normalize the values to the total DNA present (See Tables 6-12 in Appendix A).

The relative abundance of representative bacteria in the nasal samples, selected based on their presence in the patient's mouth, was determined in pre- and post-procedure samples. Figure 1A shows the non-normalized, relative bacterial DNA abundance in pre- and post-procedure samples taken from the nasal vestibule, calculated from individual C_T values, as described in the Methods section. In addition, the distribution of the individual bacterial strains are presented in Figures 1B-1H.

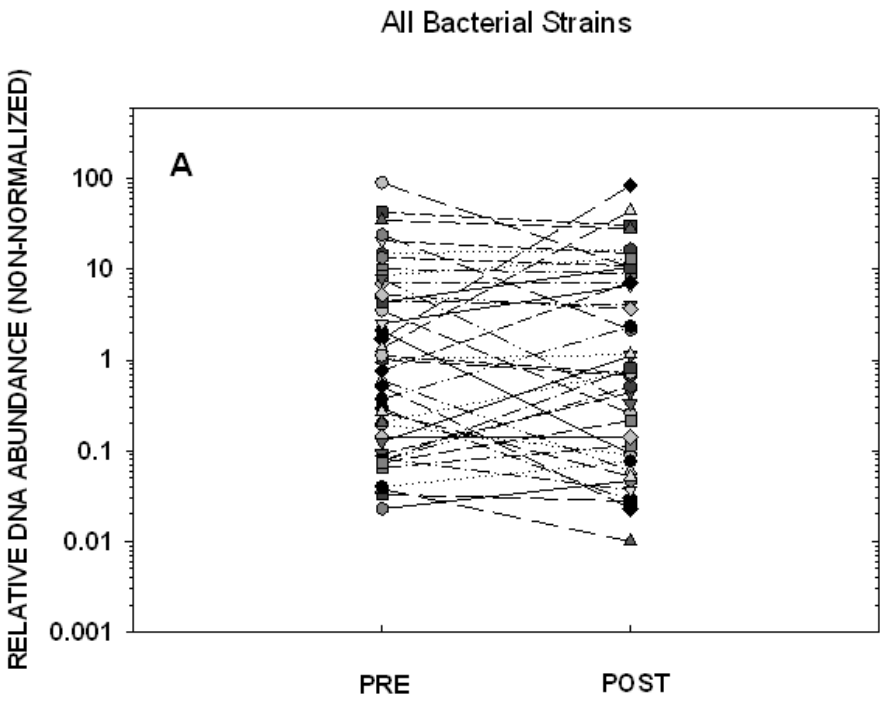

Figure 1A: Composite of pre and post nasal samples from 23 study cases whose non-normalized data met the criteria for inclusion

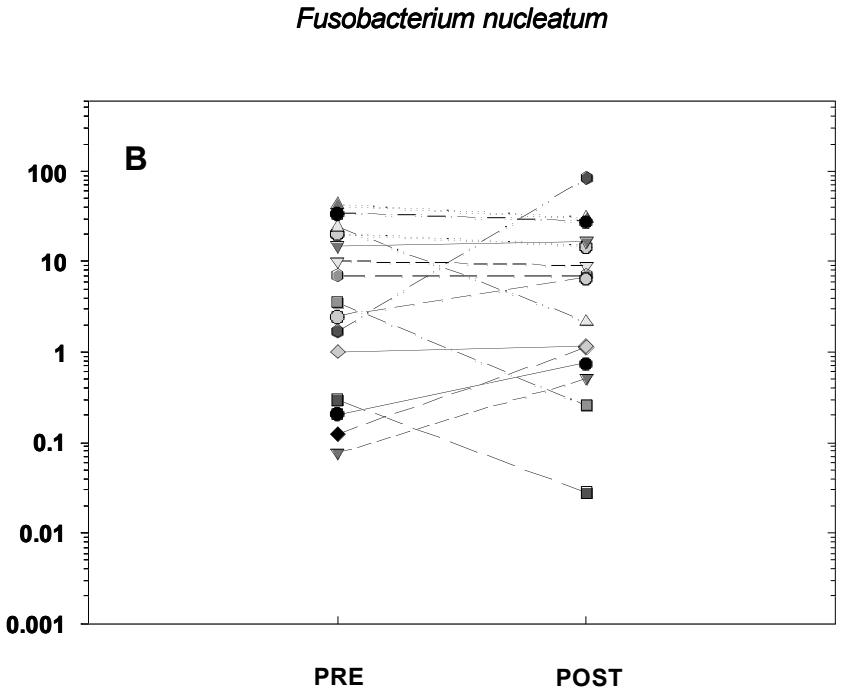

Figure 1B: Individual pre and post nasal sample data for *F. nucleatum* bacterial strains surveyed

Actinomycetes israelii

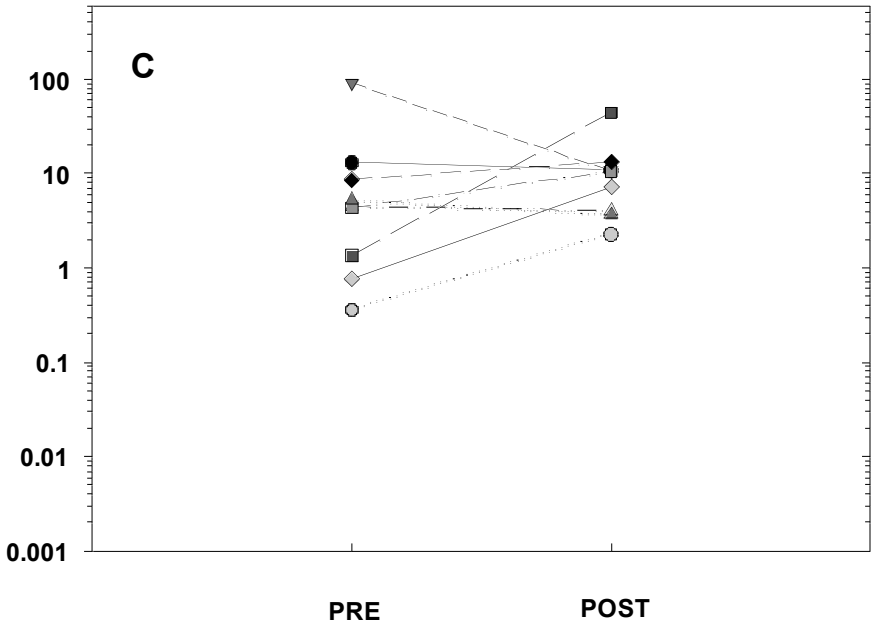

Figure 1C: Individual pre and post nasal sample data for *A. israelii* bacterial strains surveyed

Peptostreptococcus anaerobius

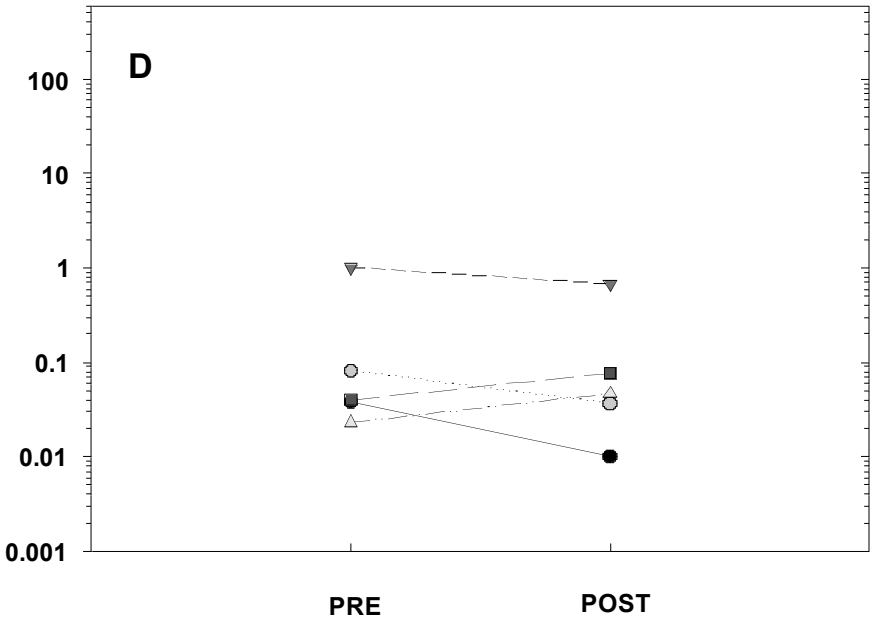

Figure 1D: Individual pre and post nasal sample data for *P. anaerobius* bacterial strains surveyed

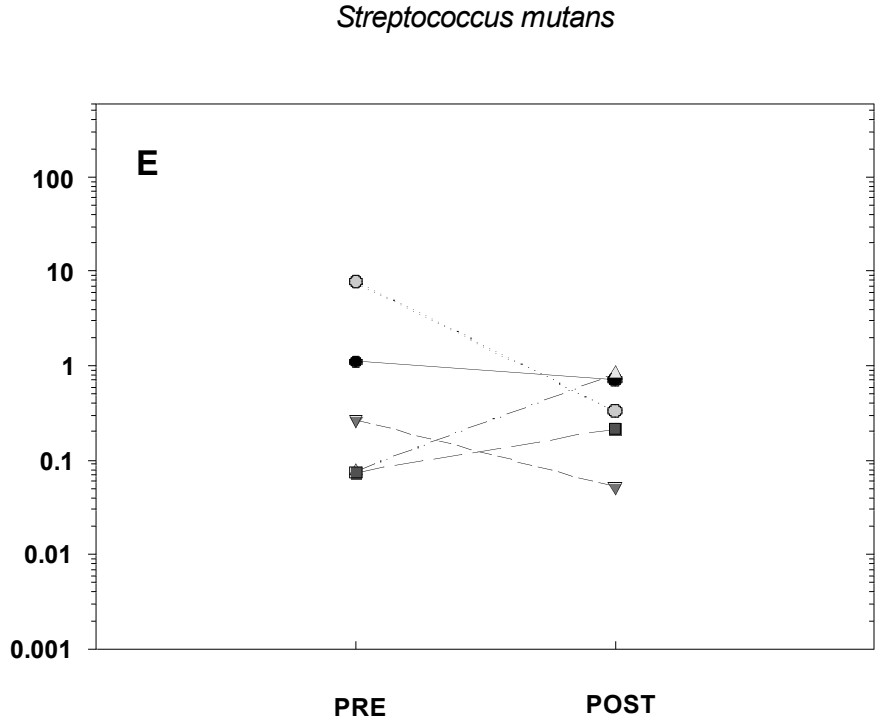

Figure 1E: Individual pre and post nasal sample data for *S. mutans* bacterial strains surveyed

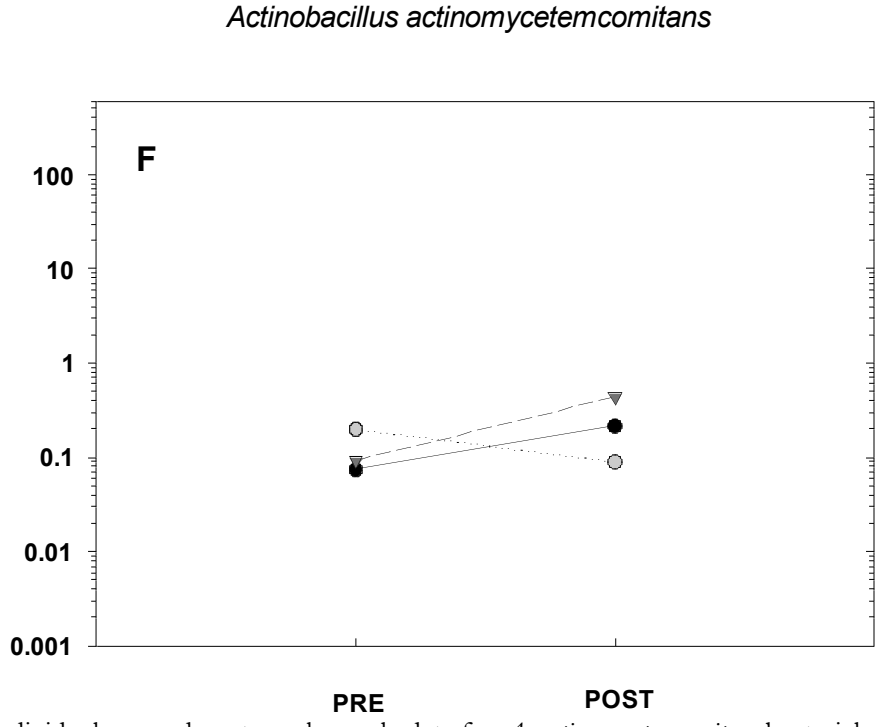

Figure 1F: Individual pre and post nasal sample data for *A. actinomycetemcomitans* bacterial strains surveyed

Capnophytophaga sputigena

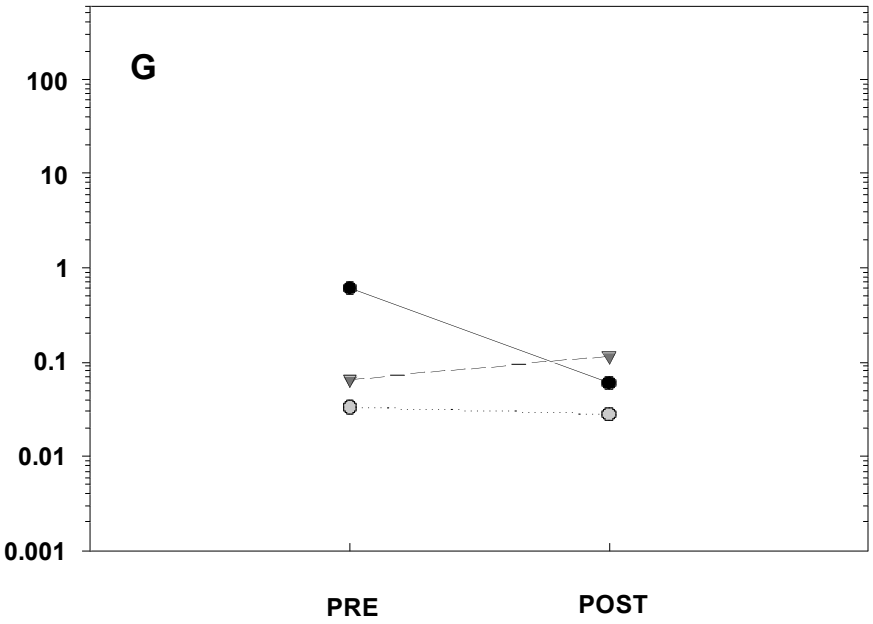

Figure 1G: Individual pre and post nasal sample data for *C. sputigena* bacterial strains surveyed

Eikenella corrodens

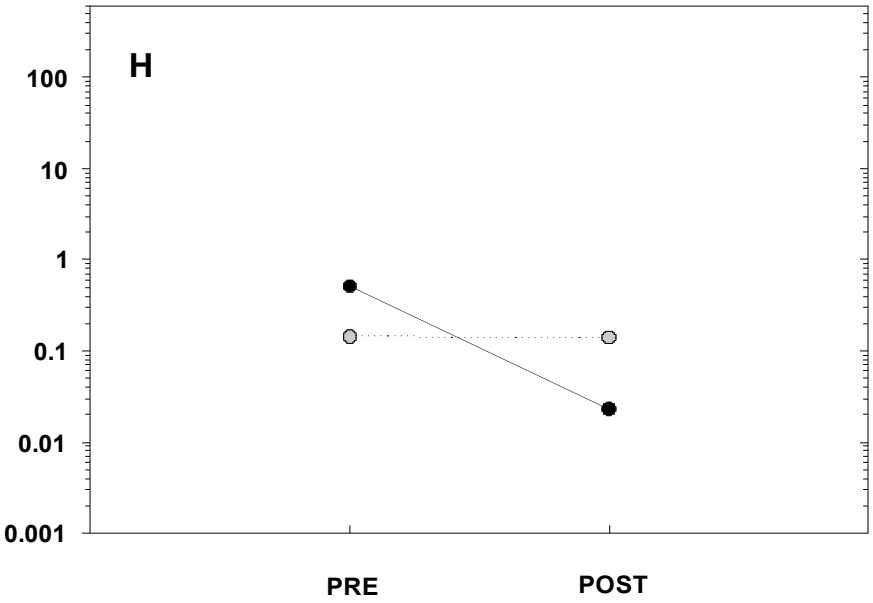

Figure 1H: Individual pre and post nasal sample data for *E. corrodens* bacterial strains surveyed

The relative abundance (non-normalized) values were then normalized to the total amount of bacteria present in each sample, utilizing a "universal" bacterial primer/probe set and presented in Figure 2. Normalization was performed in an attempt to remove any remaining random variation in the values due to sampling technique.

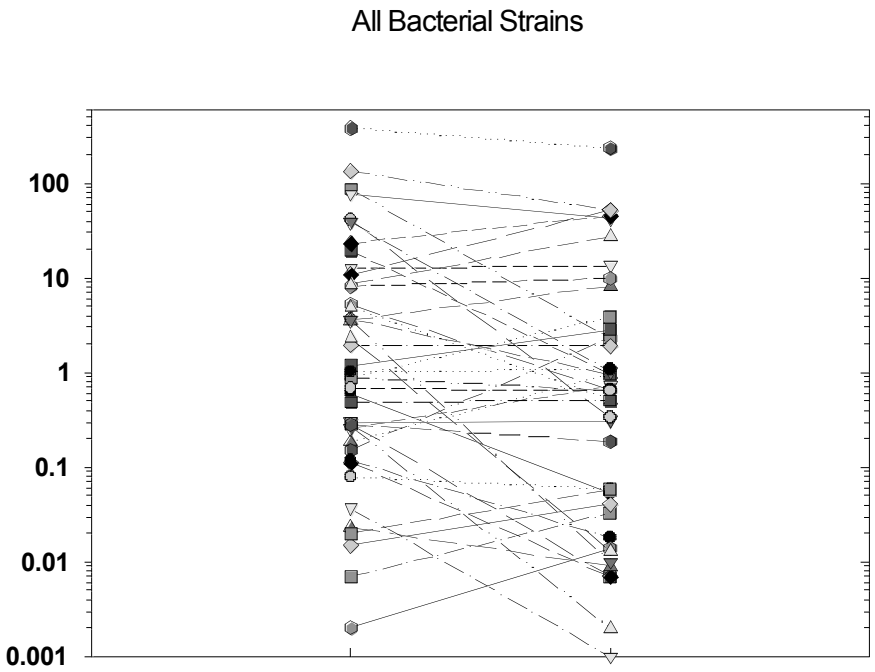

Figure 2. Relative bacterial DNA abundance in the same pre- and post-procedure samples represented in Figure 1A after normalization to the relative abundance of all bacteria present in each sample , as described in the Methods section.

Comparison of the abundance data in non-normalized and normalized forms (Figure 1A and Figure 2) indicates that the variance of the data is greater in the normalized form ((S.D. pre: 63.2; post: 37.9) than in the non-normalized form S.D.

pre: 16.2; post: 15.4). The standard deviation around the mean for the pre and post relative abundance values in Figure 1A and Figure 2 are presented in Table 13. The data after "normalizing" to the universal probe data indicate more variance. This observation suggests that this approach to normalization was not effective in reducing random errors in the data, such as those associated with sampling technique. Furthermore, it suggests that normalizing to the abundance of total bacteria present in each nostril interjected a new source of error into the data. Thus, the decision was made to focus further analysis on the non-normalized values of bacterial abundance.

Table 13: The Standard Deviation around the mean for the pre and post relative abundance values in Figure 1A and Figure 2

	Standard deviation around the mean	
	Pre-nasal Samples	Post-nasal Samples
Non-normalized data	16.21	15.43
Normalized data	63.18	37.87

There is less confidence associated with relative abundance estimates derived from high CT values so an arbitrary threshold above the limits of detection of the instrument was selected at C_T = 34 cycles or less. This inclusion criterion was applied to RT-PCR data. All sample sets with one or both samples having a C_T value greater than 34 cycles were excluded from further analysis. This resulted in the exclusion of

28 sets of low relative DNA abundance. The remaining 20 sample sets are presented in Figure 3.

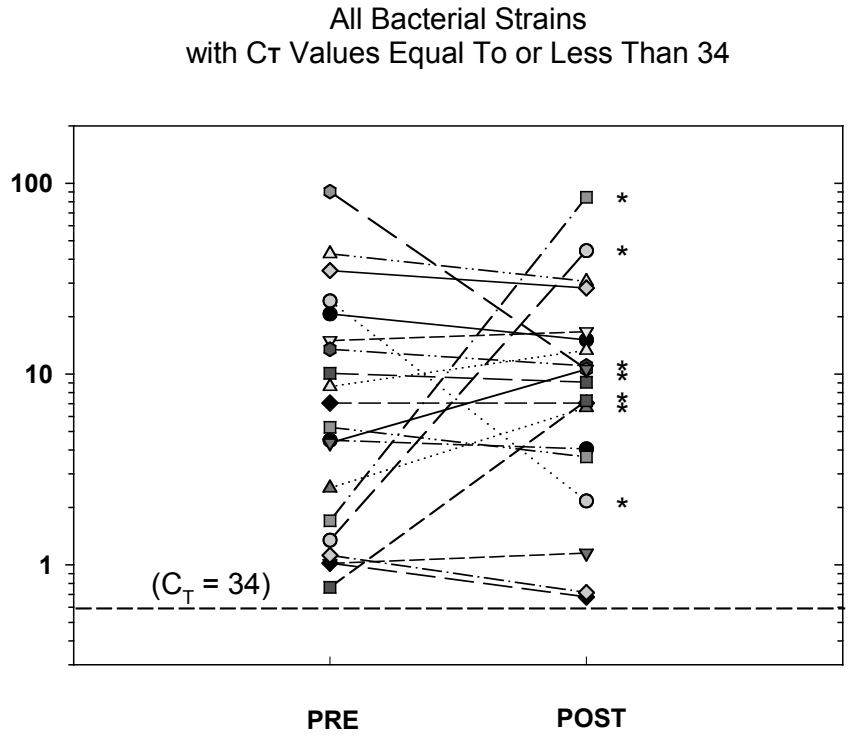

Figure 3. Non-normalized, relative bacterial abundance values selected from Figure 1A based on both pre- and post-procedure values having a CT equal to or less than 34. Asterisks indicate those sets demonstrating a 2-fold or greater change in relative abundance pre-to-post.

In order to increase confidence in selecting changes in pre-to-post measures that are true changes, only the cases with a two-fold or greater change were selected. The asterisks in Figure 3 indicate the seven sets out of twenty that met this criterion. Out of the seven sets, five (25%) demonstrated an increase in pre-to-post relative

abundance of targeted bacteria from the mouth. Two of the seven sets showed a decrease in pre-to-post relative abundance.

Given the low levels of bacterial DNA recovered through the sampling method used and in the absence of modifying the method to enhance bacterial recovery, *Phase 3* of this study could not be pursued. *Phase 3* would have sought to determine if methods beyond proper use of the mask exist to reduce nasal infection risk and to determine the effectiveness of adding a commercially available nasal intervention to standard protective procedures in reducing the oral to nasal transfer of bacteria during a debonding procedure. However, the ability to detect a reduction with the given the amount of DNA recovered would be unlikely.

CHAPTER 5

Discussion

Oral bacteria and pathogens have been implicated as potential sources of systemic infections. Awareness of the dangers associated with airborne material generated during routine procedures in a dental office is essential for healthcare professionals. Orthodontist wearing ear-looped masks may be at risk of nasal inoculation of aerosols containing pathogenic oral bacteria released during routine removal of braces. At the termination of orthodontic treatment, high-speed handpieces are used to remove attachments and gross amounts of remaining adhesive cement used to attach brackets and bands to the teeth. This procedure often generates an aerosol cloud that contains bacteria from the patient's oral cavity. It is crucial to determine whether bacteria from the patient's mouth can penetrate the nasal vestibule of treating clinicians during routine procedures in the office.

5.1 Personal Protective Wear

The results presented in this study indicate that the use of personal protective equipment (PPE) is effective in preventing aerosols that are generated during standard removal of braces from penetrating to the nasal regions of treating clinicians and staff. Oral microorganisms that are part of the aerosol cloud generated during the debond procedure may have the ability to pass around the edges of the mask and into the clinician's nasal cavity.

The type of masks used in the present study were Henry Schein's earloop procedure masks which meets all current ASTM F2100-04 performance criteria for low barrier masks. Henry Schein's ear-looped masks have a greater than 95% bacterial filtration efficiency. The bacterial filtration efficiency results demonstrate the effectiveness of facemask materials to trap particles with a fixed nonviable particle size of 1 to 5μm (Greco & Lai, 2008). Differential pressure measures the masks breathability. It measures how easily air passes through the mask. The masks are rated on a scale from 0-5, zero being most breathable and five being the least. Henry Schein's earloop masks, worn by the treating clinicians in the present study, have a breathability rating of less than $4.0mmH_20/cm^2$, indicating a low barrier mask. The results in this study suggest that the earlooped masks provide adequate protection against aerosols generated during standard removal of braces in the majority of the cases.

Facial hair and the adaptation of the mask to the Resident's face affect protection levels. In the present study, the Residents were not instructed on proper mask wear. Although the number of positive transfers was not sufficient to attempt to link transfer to specific mask-wearing behaviors, improper mask adaptation to the clinician's face can increase the risk of nasal inoculation, allowing bacteria to penetrate around the edges of the mask. The facial masks were sampled in this study using the swab and rolling it systematically across the mask after the debond took place. Samples of the facial mask were taken in an attempt to manipulate Toroglu et al.

(2001) results where they showed a considerable increase of microorganisms on blood agar plates fixed on the orthodontist's facial shield. Due to the absorbance of the facial masks in this study, a transfer could not be detected. A future study might consider sampling the facial area around the mask, such as the cheeks, forehead and hair, to see if orally-derived bacteria can be detected.

5.2 Oral Bacteria

All of the seven selected oral bacterial targets were present in the oral cavities of the participating patients. The most abundant species present in the oral cavities of all 28 cases was *Fusobacterium nucleatum*. *Actinomycetes israelii* and *Eikenella corrodens* were present in 26 out of the 28 cases. Figure 1 B and Figure 1C show a high overall relative abundance for *Fusobacterium nucleatum* and *Actinomycetes israelii*. These two bacteria are the two bacteria whose transfer to the nasal vestibule of the treating clinician could be detected. The other bacteria were consistently low in relative abundance and perhaps in part to this limitation, no transfer could be detected.

5.3 Limitations to the Study

5.3.1 Sampling Methods

The intent of the sampling protocol was to obtain pre- and post- debonding nasal samples that would allow an increase in bacterial presence to be detected that could be linked closely to the procedure. In practice, this was not optimally achieved. Residents' nasal vestibules were swabbed at the beginning of the day prior to any clinical contact with patients, regardless of when the debonding procedure took place.

Bacteria from other patient's oral cavities who were scheduled before the debond appointment may have penetrated the nasal vestibule of the treating clinician. Two of the seven sets in Figure 3 showed a decrease in pre-to-post relative abundance of targeted bacteria from the mouth. Loss of bacterial content in the nose from the baseline values could have occurred through several mechanisms, including inadvertent wiping or blowing of the nose prior to post-procedure sampling. Although not a sampling method error, safety of the samples could have been compromised during transport or preparation even though DNA was extracted from the samples within 24 hours. Degradation of the DNA over the period of time it was stored could have occurred as well but no specific evidence suggests that the DNA was damaged or that degradation occurred.

A better sampling method would be to have the Residents blow their nose right before sampling the first nasal vestibule and sample the Residents' nasal vestibules right before the debonding procedure begins. An accurate representation of the bacteria present in the Residents' nares prior to the removal of braces would then be obtained. By blowing their noses right before sampling, any inadvertent intervention could have been eliminated.

Another limitation with the sampling technique present in this study is the amount of bacteria detected on the one nasal swab was very minimal. Sampling methods need to be modified so that an optimal amount of bacteria from the nasal vestibule can be detected on one nasal swab collection.

5.3.2 Normalized vs. Non-normalized Data

In the present study, the comparison of the relative abundance data in non-normalized and normalized forms (Figure 1A and Figure 2) indicates that the variance of the data is greater in the normalized form than in the non-normalized form. This observation suggests that this approach to normalization was not effective in reducing random errors in the data, such as those associated with sampling methods. Furthermore, it suggests that normalizing to the total bacteria present interjected a new source of error into the data. Thus, further analysis of the data focused on the non-normalized values of bacterial abundance. Since the relative abundance results were used, it was assumed that the amount of bacteria present in the pre-nasal sample was the same as the amount present in the post-nasal sample. However, this may not be the case.

5.4 Strengths of the Study

The present study was structured in a way that oral samples were screened to form a link between the patient donor and what to look for in the nasal vestibules of the targeted clinician. Seven bacteria typically present in the oral subgingival flora were selected and the genetic markers were used as surrogates for the presence of orally-derived bacteria. The eighth bacterium, *S. aureus,* was selected to determine sampling methods in the nose, since it is mainly present in the nasal vestibules. This selected group of bacteria was determined not to be present in the nose but present in the

mouth in *Phase 1* of the study. In *Phase* 2, each "case" included the resident's pre-nasal sample, post-nasal sample, and the sample from the oral cavity of the patient having their braces removed. Only the bacteria detected by PCR analysis to be present in the patient's oral cavity were used in the PCR analysis of the pre- and post- nasal samples of that patient's treating clinician. The other bacteria were not assayed. This "matching" between source and recipient is a valuable strength to this study in that it provided multiple and case-specific bacterial targets with which to assess potential transfer.

5.5 Further Investigation

In view of the evidence in support of the presence of transfer from patient to clinician in several cases, further investigation is warranted because of the possibility that strongly pathogenic bacteria, even if transferred to the nasal vestibule of treating clinicians at low levels, may pose a serious health risk. It may be important to consider the use of commercially available nasal products to supplement the protection achievable by face mask use and to protect against these pathogenic bacteria. By adding an additional nasal intervention to standard protective procedures, oral to nasal transfer of bacteria during the debonding procedure may be further reduced or eliminated. The use of non-antibiotic preparations to eliminate the possibility of fostering the development of antibiotic-resistant strains in the user would be an important consideration in selecting such a product. Two examples of available nasal products are:

Nozin Nasal Sanitizer is a product that benefits users by providing extra protection against bacteria and viruses when potential exposure to these pathogens is likely. Nozin has a unique non-antibiotic antimicrobial formulation that sanitizes the nasal vestibule and helps control pathogen populations that come to reside there. Laboratory tests have dmeonstrated that Nozin Nasal Sanitizer antiseptic is effective in killing at least 99.99% of bacterial and viral pathogens such as *staphylococcus aureus, streptococcus pneumonia, streptococcus pyogenes, rhinovirus type-14* (often responsible for the common cold), *human coronavirus* (responsible for the deadly SARS outbreak) and *influenza type A* (influenza contracted by humans and birds).

Xlear Nasal Spray is a combination of xylitol (a naturally-occurring sugar) and saline spray that has shown to be effective in soothing and moisturizing the sinuses and nasal passages. Xylitol decreases the adherence of harmful bacteria to the cells in the nasal vestibule by occupying the bacteria's binding sites and decreasing the ability of the bacteria to attach to the body's cells.

Unfortunately, the results of the present study do not warrant a *Phase 3* to examine whether commercially available products applied to the nasal vestibule can be beneficial to healthcare providers. The likelihood of being able to detect a treatment associated reduction in transfer with the relatively low incidence of transfer measured using the current sampling methods would likewise be low.

Clinicians should be reminded that bacteria and viruses are common constituents of aerosolized saliva and be made aware that nasal inoculation by these pathogens present in the patient's oral cavity can occur. In addition to wearing protective masks with the best facial adaptation, clinicians should also consider the use of high-speed suction during all debonding procedures to reduce the risk of nasal penetration of aerosolized pathogens. Supplemental protection using non-antibiotic nasal preparation might also be considered when working with patients who have a higher risk of being a source of pathogenic bacteria and viruses.

CHAPTER 6

Conclusions

The conclusions of this study are as follows:

- Personal Protective Equipment when worn properly can be effective in preventing aerosols that are generated during standard removal of braces from penetrating to the nasal regions of the treating clinicians and staff.

- A transfer of bacteria from the patient mouth to the nasal vestibule of the treating clinician is possible and clinicians should be aware that viruses are small in particle size and are commonly aerosolized.

- Clinicians should not only wear protective masks with the best facial adaptation but also consider the use of a high speed suction during all debonding procedures to reduce the risk of nasal penetration of aerosolized pathogens.

- Limitations to this study are mainly due to sampling methods. Instead of sampling the nasal vestibules of the treating clinicians at the start of the day, the pre-nasal sample should have taken place immediately prior to the debonding procedure, eliminating any inadvertent interventions that could have taken place.

REFERENCES

Basu MK, Browne RM, Potts AJ, Harrington JM. A survey of aerosol-related symptoms in dental hygienists. *Journal of Soc Occup Med* 38 (1998): 23-25.

Bentley CD, Burkhart NW, Crawford JJ. " Evaluating spatter and aerosol contamination during dental procedures." *Journal of American Dental Association*. 125 (1994): 579-584.

Bowden G, Hamilton I. "Survival of Oral Bacteria." *Critical Reviews in Oral Biology and Medicine* 9 (1) (1994): 54-85

Bowden GH, Ellwood DC, Hamilton IR. "Microbial ecology of the oral cavity. In: Advances in microbial ecology. Alexander M, editor. New York: Plenum, pp. 135-217.

Bowden GHW, Li YH. " Nutritional influences on biofilm development." *Adv Dent Res* 11 (1997): 81-99.

Brecx M, Theilade J, Attstrom R. "An ultrastructual quantitative study of the significance of microbial multiplication during early plaque growth." *Journal of Periodontal Research* 18 (1983): 177-186.

Busse, W. "Pathogenesis and Sequelae of Respiratory Infections." *Reviews of Infectious Diseases* 13, Suppl 6 (1991): S477-485.

Carter, WJ, Seal JR. "Upper respiratory infections in dental personnel and their patients. Research Project Report NM005 051 14 03. Nacal Medical Research Unit No. 4, Administrative Command, US Naval Training Center, Great Lakes, I11, Feb. 25, 1953.

Carranza F. Glickman's Clinical Periodontology. Seventh Edition. Philadelphia: W.B. Saunders Company, 1990.

Caufield PW, Cutter GR, Dasanayake AP. " Initial acquisition of mutans streptococci by infancts: evidence for a discrete window of infectivity." *Journal of Dental Research* 72 (1993): 37-45.

Clark A. "Bacterial colonization of dental units and the nasal flora of dental personnel." *Proc. Roy. Soc. Med* 67 (1974): 1269-1270.

Day CJ, Price R, Sandy JR, Ireland AJ. "Inhalation of aerosols produced during the removal of fixed orthodontic appliances: A comparison of 4 enamel cleanup methods." *American Journal of Orthodontics and Dentofacial Orthopedics* 133 (2008):11-17.

Dutil S, Meriaux A, Latremoille M, Lazure L, Barbeau J, Duchaine C. "Measurement of airborne bacteria and endotoxin generated during dental cleaning." *Journal of Occupational and Environmental Hygiene* 6 (2009): 121-130.

Espy MJ, Uhl JR, Sloan LM, Buckwalter SP, Jones MF, Vetter EA, Yao JDC, Wengenack NL, Rosenblatt JE, Cockerill III FR, Smith TF. "Real-Time PCR in Clinical Microbiology: Applications for Routine Laboratory Testing." *Clinical Microbiology Reviews* 19 (1) (2006): 165-256.

Fiegel J, Clarke R., Edwards D. "Airborne infectious disease and the suppression of pulmonary bioaerosols." *Drug Discovery Today* 11 (2006):51-57.

Gibbons RJ. "Bacterial adhesion to oral tissues: a model for infectious diseases." *Journal of Dental Research* 68 (1989): 750-760.

Gilbert P, Evans DJ, Brown MRW. "Formation and dispersal of bacterial biofilms *in vivo* and *in situ*." *Journal of Applied Bacteriology* 74 (Suppl) (1993): 67S-78S.

Gmur R., & Guggenheim, B. "Interdental Supragingival Plaque- A natural habitat of *Actinobacillus actinomycetemcomitans, Bacteroides forsythus, Campylobacter rectus,* and *Prevotella nigrescens*." *Journal of Dental Research* 73(8) (1994):1421-1428.

Greco PM, Lai C. "A new method of assessing aerosolized bacteria generated during orthodontic debonding procedures." *American Journal of Orthodontics and Dentofacial Orthopedics* 133 (2008): 79-87.

Harrel SK, Barnes JB, Hidalgo FR. "Aerosol and splatter contamination from the operative site during ultrasonic scaling." *Journal of American Dental Association* 129 (1998): 1241-1249.

Harrel SK, Molinari J. "Aerosols and splatter in dentistry." *Journal of American Dental Association* 135 (2004): 429-437.

Hortz HP, Vianna ME, Gomes BPFA, Conrads G. "Evaluation of universal probes and primer sets for assessing total bacterial load in clinical samples: general

implication and pratical use in endodontic antimicrobial therapy. *Journal of Clinical Microbiology* 43 (10) (2005): 5332-5337.

Ireland AJ, Moreno T, Price R. "Airborne particles produced during enamel cleanup after removal of orthodontic appliances" *American Journal of Orthodontics and Dentofacial Orthodpedics* 124 (2003): 683-686.

Kluytmans J., van Belkum A., Verbrugh H. "Nasal Carriage of *Staphylococcus aureus*: Epidemiology, Underlying Mechanisms, and Associated Risks. *Clinical Microbiology Reviews* 10 (1997): 505-520.

Könönen E, Asikainen S, Jousimies-SOmer H. "The early colonization of Gram-negative anaerobic bacteria in edentulous infants." *Oral Microbiology and Immunology* 7 (1992): 28-31.

Könönen E, Asikainen S, Saarela M, Karjalainen J, Jousimies-Somer H. "The oral Gram-negative anaerobic microflora in young children: longitudinal changes from edentulous to dentate mouth." *Oral Microbiology and Immunology* 9 (1994a): 136-141.

Könönen E, Saarela M, Karjalainen J, Jousimies-Somer H, Alaluusua S, Asikainen S. "Transimission of ral *Prevotella* melaninogenica between a mother and her child." *Oral Microbiology and Immunology* 9 (1994b): 310-314.

Lindhe J, Lang N, Karring T. Clinical Periodontology and Implant Dentistry. Fifth Edition. Oxford: Blackwell Publishing Ltd, 2008.

March PD. "Host defenses and microbial homeostasis: role of microbial interactions." *Journal of Dental Research* 68 (1989): 1567-1575.

Marsh PD. "Microbial ecology of dental plaque and its significance in health and disease." *Advanced Dental Reseach* 8 (1994): 263-271.

Marsh PD. "Dental plaque as a biofilm and a microbial community-implications for health and disease." *BMC Oral Health*; 6 Suppl I (2006):S14

Micik RE, Miller RL, Mazarella MA, Gunnar R. "Studies on dental aerobiology: I. Bacterial aerosols generated during dental procedures." *Journal of Dental Research* 48 (1969): 49-56.

McClellan DI, Griffen AL, Leys EJ. "Age and precalence of *Porphyromonas gingivalis* in children." *Journal of Clinical Mircobiology* 34 (1996): 2017-2019.

Miller RL. "Generation of airborne infection by high speed dental equiptment." *J Am Soc Prev Dent* 125 (1979): 14-17.

Milnes AR, Bowden GH, Gates D, Tate R. "Normal microbiota on the teeth of preschool children." *Mirco Ecol Hlth Dis* 6 (1993a): 213-227

Milnes AR, Bowden GH, Gates D, Tate R. "Predominant cultivable oragnaisms on the tongue of preschool children." *Mirco Ecol Hlth Dis* 6 (1993b): 229-235

Moore LVH, Moore WEC, Cato EP, Smibert RM, Burmeister JA, Best AM, et al. "Bacteriology of human gingivitis." *Journal of Dental Research* 66 (1987): 989-995.

Moore WEC, Moore, LVH. "The bacteria of periodontal diseases." *Periodontology 2000* 5 (1994): 66-77.

Moore WEC, Moore LH, Ranney RR, Smibert RM, Burmeister JA, Schenkein HA. "The microflora of periodontal sites showing active destructive progression." *Journal of Clinical Periodontology* 18 *(*1991): 729-739.

Murdoch DA. "Gram-Positive Anaerobic Cocci." *Clinical Microbiology Reviews* 11 (1998): 81-120.

Nadkarni MA, Martin FE, Jacques NA, Hunter N. "Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set." *Microbiology* 148 (2002): 257-266.

National Center for Health Statistics, National Center for Human Services, Centers for Disease Control and Prevention. Deaths- leading causes for US in 2002. Available at: http://www.cdc.gov/nchs/fastats/deaths.htm. Assessed December 1, 2004.

Nelson, KE., Williams CM. Infectious Disease Epidemiology: Theory and Practice. Second Edition. Boston: Jones and Bartlett Publishers, 2007.

Newman MG, Sutter VL, Pickett MJ, Blachman U, Greenwood JR, Grinenko V, Citron D. "Detection, Identification, and Comparison of *Capnocytophaga*, *Bacteroides ochraceus*, and DF-1." *Journal of Clinical Microbiology* 10 (1979): 557-562.

Pearce C, Bowden GH, Evans M., Fitzsimmons S, Johnson J, Sheridan MJ et al. "Identification of pioneer viridans streptococci in the oral cavity of human neonates." *J Med Microbiol* 42 (1995): 67-72.

Petit MDA, van Steenbergen TJM, Scholte LMH, van der Velden U, De Graaff J. "Epidemiology and transmission of *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* among children and their family members. *Journal of Clinical Periodontology* 20 (1993): 641-650.

Petit MDA, van Steenbergen TJM, Timmerman MF, De Graaff J, van der Velden U. "Prevalence of periodontitis and suspected periodontal pathogens in families of adult periodontitis patients. *Journal of Clinical Periodontology* 20 (1994): 76-85.

Root, Richard. Clinical Infectious Diseases. New York: Oxford University Press, 1999.

Sacchetti R, Baldissarri A, De Luca G, Lucca P, Stampi S, Zanetti F. "Microbial contamination in dental unit waterlines: comparison between Er:YAG laser and turbine lines. *Am Agric Environ Med.* 13 (2006): 275-279

Savitt ED, Socransky SS. "Distribution of certain subgingival microbial species in selected periodontal conditions. *Journal of Periodontal Research* 19 (1984): 111-123.

Slots J. "Microflora of the healthy gingival sulcus in man." *Scand J Dent Res* 85 (1977): 247-254.

Slots, J., Reynolds, H.S. & Genco, R.J. "Actinobacillus Actinomycetemcomitans in human periodontal disease: A cross-sectional microbiological investigation." *Infection and Immunity* 29 (1980):1013-1020.

Smith-Vaughan H, Byun R, Nadkarni M, Jacques NA, Hunter N, Halpin S, Morris P, Leach A. "Measuring nasal bacterial load and its association with otitis media." *BMC Ear, Nose and Throat Disorders* 6 (10) (2006)

Socransky S, Haffajee A. "Evidence of bacterial etiology: a historical perspective." *Periodontology 2000* 5 (1994): 7-25.

Spendlove JC, Fannin KF. "Source, Significance and Control of Indoor Microbial Aerosols: Human Health Aspects." *JSTOR* 98 (1984): 229-244.

Szymanska J. "Dental Bioaerosol as an occupational hazard in a dentist's workplace." *Ann Agric Environ Med.* 14 (2007): 203-207

Szymanska J. "Dental Bioaerosol as an occupational hazard in a dentist's workplace." *Ann Agric Environ Med.* 12 (2005): 153-155

Tanner A., Stillman N. "Oral and Dental Infections with Anaerobic Bacteria: Clinical Features, Predominant Pathogens, and Treatment." *Clinical Infectious Diseases* 16 (1993): S304-S309.

Toroglu MS, Bayramoglu O, Yarkin F, Tuli A. "Possibility of blood and Hepatitis B contamination through aerosols generated during debonding procedures." *Angle Orthodontist* 73 (2003): 571-578.

Toroglu MS, Haytac MC, Koksal F. "Evaluation of aerosol contamination during debonding procedures." *Angle Orthodontist* 71 (2001): 299-306.

Watt B., Jack E. "What are anaerobic cocci." *Journal of Medical Microbiology* 10 (1977): 461-468.

Williams REO. " Healthy carriage of *Staphylococcus aureus*: its prevalence and importance." *Bacteriology Review* 27 (1963): 56-71.

Wilson ME, Burstein R, Jonak-Urbanczyk JT, Genco R. "Sensitivity of *Capnocytophaga* Species to Bactericidal Properties of Human Serum." *Infection and Immunity* 50 (1985): 123-129.

Yu, V. "Could aspiration be the major mode of transmission for legionella." *American Journal of Medicine* 95 (1993):13-15.

Zambion, J. "Actinobacillus actinomycetemcomitans in human periodontal disease." *Journal of Clinical Periodontology* 12 (1985): 1-20.

APPENDIX

Table 6: Relative Abundance & Normalized data for *A. comm* Pre/Post Nasal Samples

Case No.	Universal		*A. comm*			
	$10^{10}.2^{-CT\ pre}$	$10^{10}.2^{-CT\ post}$	$10^{10}.2^{-CT\ pre}$	$10^{10}.2^{-CT\ post}$	N-pre	N-post
2	1.115	0.786	0.000	0.180	0.000	0.229
3	1.655	1.121				
4	3.554	1.773	2.098	0.092	0.590	0.052
5	0.669	0.829				
6	0.507	0.218				
7	4.635	3.342				
8	2.509	1.511	0.197	0.089	0.079	0.059
9	0.964	0.271				
12	0.420	3.619				
15	0.024	0.124				
16	0.207	2.226				
17	0.357	0.614	0.092	0.441	0.258	0.718
18	0.147	2.058	0.000	0.082	0.000	0.040
20	2.224	31.815				
22	0.116	3.993				
24	0.157	1.620				
25	3.653	3.698				
26	9.758	3.440				
27	0.033	0.160				
28	0.039	0.071				

Table 7: Relative Abundance & Normalized data for *C. Sputa* Pre/Post Nasal Samples

Case No.	Universal		C. sputa			
	$10^{10}.2^{-CT\ pre}$	$10^{10}.2^{-CT\ post}$	$10^{10}.2^{-CT\ pre}$	$10^{10}.2^{-CT\ post}$	N-pre	N-post
2	1.115	0.786				
3	1.655	1.121				
4	3.554	1.773				
5	0.669	0.829				
6	0.507	0.218				
7	4.635	3.342				
8	2.509	1.511				
9	0.964	0.271				
12	0.420	3.619				
15	0.024	0.124				
16	0.207	2.226				
17	0.357	0.614				
18	0.147	2.058				
20	2.224	31.815	0.601	0.060	0.270	0.002
22	0.116	3.993	0.033	0.028	0.288	0.007
24	0.157	1.620				
25	3.653	3.698				
26	9.758	3.440	0.065	0.115	0.007	0.033
27	0.033	0.160				
28	0.039	0.071				

Table 8: Relative Abundance & Normalized data for *E.Corr* Pre/Post Nasal Samples

Case No.	Universal		E. corro			
	$10^{10}.2^{-CT\ pre}$	$10^{10}.2^{-CT\ post}$	$10^{10}.2^{-CT\ pre}$	$10^{10}.2^{-CT\ post}$	N-pre	N-post
2	1.115	0.786				
3	1.655	1.121				
4	3.554	1.773				
5	0.669	0.829				
6	0.507	0.218				
7	4.635	3.342	0.517	0.023	0.112	0.007
8	2.509	1.511				
9	0.964	0.271				
12	0.420	3.619				
15	0.024	0.124				
16	0.207	2.226				
17	0.357	0.614				
18	0.147	2.058				
20	2.224	31.815				
22	0.116	3.993				
24	0.157	1.620				
25	3.653	3.698				
26	9.758	3.440	0.144	0.142	0.015	0.041
27	0.033	0.160				
28	0.039	0.071				

Table 9: Relative Abundance & Normalized data for *F. Nucl* Pre/Post Nasal Samples

Case No.	Universal		F. nucl			
	$10^{10}.2^{-CT\ pre}$	$10^{10}.2^{-CT\ post}$	$10^{10}.2^{-CT\ pre}$	$10^{10}.2^{-CT\ post}$	N-pre	N-post
2	1.115	0.786	0.206	0.755	0.185	0.961
3	1.655	1.121	20.674	15.117	12.491	13.489
4	3.554	1.773				
5	0.669	0.829				
6	0.507	0.218	0.077	0.514	0.152	2.356
7	4.635	3.342	24.156	2.165	5.212	0.648
8	2.509	1.511	0.294	0.028	0.117	0.018
9	0.964	0.271	3.517	0.258	3.650	0.950
12	0.420	3.619	0.125	1.121	0.298	0.310
15	0.024	0.124	0.000	0.084	0.000	
16	0.207	2.226	1.017	1.152	4.925	0.518
17	0.357	0.614				
18	0.147	2.058				
20	2.224	31.815	42.644	30.575	19.177	0.961
22	0.116	3.993	10.086	9.059	87.226	2.268
24	0.157	1.620	1.704	84.406	10.867	52.096
25	3.653	3.698	7.058	7.058	1.932	1.908
26	9.758	3.440	34.879	28.232	3.574	8.207
27	0.033	0.160	2.530	6.677	76.967	41.784
28	0.039	0.071	14.973	16.671	381.882	233.631

Table 10: Relative Abundance & Normalized data for *A. Israel* Pre/Post Nasal Samples

Case No.	Universal		A. israel			
	$10^{10}.2^{-CT\ pre}$	$10^{10}.2^{-CT\ post}$	$10^{10}.2^{-CT\ pre}$	$10^{10}.2^{-CT\ post}$	N-pre	N-post
2	1.115	0.786				
3	1.655	1.121	13.496	11.030	8.154	9.843
4	3.554	1.773				
5	0.669	0.829	0.000	0.046	0.000	0.056
6	0.507	0.218				
7	4.635	3.342				
8	2.509	1.511				
9	0.964	0.271				
12	0.420	3.619	0.371	2.345	0.882	0.648
15	0.024	0.124				
16	0.207	2.226				
17	0.357	0.614				
18	0.147	2.058				
20	2.224	31.815	90.465	10.587	40.682	0.333
22	0.116	3.993	4.498	4.054	38.900	1.015
24	0.157	1.620	1.347	44.301	8.585	27.343
25	3.653	3.698	4.360	10.587	1.193	2.863
26	9.758	3.440	8.600	13.309	0.881	3.869
27	0.033	0.160	0.763	7.257	23.202	45.408
28	0.039	0.071	5.257	3.679	134.062	51.557

Table 11: Relative Abundance & Normalized data for *P.anaer* Pre/Post Nasal Samples

Case No.	Universal		P. anaer			
	$10^{10}.2^{-CT\ pre}$	$10^{10}.2^{-CT\ post}$	$10^{10}.2^{-CT\ pre}$	$10^{10}.2^{-CT\ post}$	N-pre	N-post
2	1.115	0.786				
3	1.655	1.121	0.038	0.010	0.023	0.009
4	3.554	1.773				
5	0.669	0.829				
6	0.507	0.218				
7	4.635	3.342				
8	2.509	1.511				
9	0.964	0.271				
12	0.420	3.619				
15	0.024	0.124				
16	0.207	2.226				
17	0.357	0.614				
18	0.147	2.058				
20	2.224	31.815	0.082	0.037	0.037	0.001
22	0.116	3.993				
24	0.157	1.620	0.000	0.058	0.000	0.036
25	3.653	3.698	1.021	0.697	0.279	0.188
26	9.758	3.440	0.023	0.047	0.002	0.014
27	0.033	0.160				
28	0.039	0.071	0.040	0.077	1.012	1.085

Table 12: Relative Abundance & Normalized data for *S.mutans* Pre/Post Nasal Samples

Case No.	Universal		S. mutans			
	$10^{10}.2^{-CT\ pre}$	$10^{10}.2^{-CT\ post}$	$10^{10}.2^{-CT\ pre}$	$10^{10}.2^{-CT\ post}$	N-pre	N-post
2	1.115	0.786				
3	1.655	1.121	1.124	0.716	0.679	0.639
4	3.554	1.773				
5	0.669	0.829				
6	0.507	0.218				
7	4.635	3.342				
8	2.509	1.511				
9	0.964	0.271				
12	0.420	3.619				
15	0.024	0.124	0.000	0.015	0.000	0.118
16	0.207	2.226				
17	0.357	0.614				
18	0.147	2.058				
20	2.224	31.815	7.941	0.329	3.571	0.010
22	0.116	3.993	0.270	0.052	2.332	0.013
24	0.157	1.620	0.077	0.820	0.489	0.506
25	3.653	3.698	0.074	0.215	0.020	0.058
26	9.758	3.440				
27	0.033	0.160				
28	0.039	0.071				