The Natural Acquisition of the Oral Microbiome in Childhood: A Cross-Sectional Analysis

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

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Abstract

This cross-sectional study explored the development of the oral microbiome throughout childhood. Our previous studies of infants up to 1 year of age have shown early presence of exogenous species not commonly found in the oral cavity followed by rapid replacement with a small, shared core set of oral bacterial species. Following this initial colonization, we hypothesize that the complexity of the microbial community will steadily increase with advancing age as the oral cavity develops more intricate environmental niches for bacterial growth, and as children are exposed to new strains of bacteria and novel foods.

We sampled 116 children and adolescents ranging from age 1 to 14 years and collected salivary, supragingival and subgingival samples. Bacterial community composition was analyzed at the level of species using rRNA gene amplicon sequencing. This data allowed us to determine commonality among core species and the relationship of age to microbial complexity and community composition. Understanding when the establishment of bacterial communities will occur will help us determine if species are acquired in a specific order and will provide clues as to whether some species require the presence of others to colonize. Taken together, insight will be provided into the reconstruction of the natural acquisition of the human oral microbiome from birth through the establishment of the permanent dentition.
Changes in species complexity and the establishment of shared order of the oral microbiota have been examined in relation to age and site-specific samples. More specifically, our analyses suggest that the overall oral microbiome remains fairly stable after the first year of life, with very little influence from age, particular oral niches, and caries status.
Acknowledgments

I would like to thank my thesis committee for their mentorship and guidance on this research project. Their dedication in helping me and encouraging intellectual discussions for my research has been invaluable. I would also like to thank Rosalyn Sulyanto, Zach Thompson, Cliff Beall, Karmeil Stepter, Priyanka Iyer, and Rami Mikati for their assistance with sample scheduling, study organization and preparation, and statistical analyses.
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Fields of Study

Major Field: Dentistry
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Introduction

For over a decade, research in genome sciences has aimed to identify a core of similar species within the human microbiome. If present, a common core set of species would provide insight into identifying key organisms through sequencing studies, and their roles in metabolic activities, immune development, and disease initiation and progression [1]. Complex communities of microbiota exist among specialized niches in the human body such as the gut, skin, vaginal cavity and the oral cavity. The nature of complexity, diversity, and resilience of these bacterial communities are influenced by individual genetics, environment, diet, and microbial exposure. While these communities aid in maintaining equilibrium among biological processes, these complex habitats continue to remain unexplained [2]. Community structure variation exists among each specialized niche with core taxa dominating these habitats [3-6]. Literature has shown that the oral cavity has distinct communities that are markedly diverse among bacterial members and highly variable among individuals with various states of oral and physical health, and there is limited data on how the microbiota evolves from infant communities to adult communities in a healthy individual [7].

Based on infant gut colonization studies, we can hypothesize that bacterial communities colonize the oral cavity in a similar process. The acquisition of the gut microbiome of an infant is initially considered “chaotic.” Based on 16S rRNA gene analysis, phylogenetic diversity of the gut microbiome has slowly increased over time. While dominant taxa groups experienced shifts in quantity due to external factors such as
diet and disease, assembly of the bacterial community has shown to be non-chaotic and distinct stages of bacterial succession have been observed [8]. The initial chaos evident among the gut bacterial community may be due to presence of opportunistic colonizers, which the infant is exposed to from external factors such as the environment or diet. Eventually as the infant grows during the first year, a core set of taxa dominates due to a fitness advantage over changes in the gut environment, diet, and states of health and disease. By the end of the first year, the idiosyncratic microbial ecosystems converge toward a profile characteristic of the adult gastrointestinal tract [9]. In comparison to the oral cavity, colonization occurs similarly where specific bacterial colonies are established within a couple of days of life. By the first year of life, colonization resembles the adult oral cavity [16]. Our own unpublished studies using 454 sequencing of the 16S rRNA gene have shown that the initial acquisition of the oral microbiome among infants has a specific and orderly assembly of core microbial communities with *Streptococcus mitis* group showing 100% prevalence within the first 3 months of life. Additional early colonizers are acquired and by the first year of life, 100% prevalence was noted among *Streptococcus mitis group, Rothia mucilaginosa,* and *Veillonella atypica* [Figure 1]. In another unpublished study completed by Dr. Ann Griffen and Dr. Roslayn Sulyanto, prior to age 1, many different minor bacterial communities are acquired possibly under the influence from external factors such as diet and environment. By the first year of life, a core set of taxa dominates due to a fitness advantage and results in a distinct succession of core species [Figure 2].
Initial bacteria that colonize the oral cavity in a newborn are likely of maternal origin. The type of microorganisms the newborn is first exposed to is based on the type of maternal delivery, vaginal or cesarean. Within a few minutes after birth, bacterial communities present in the oral cavity, nasopharyngeal area, epidermis, and intestines are very similar to each other [10]. Over the next few hours and days, the newborn will be exposed to a vast array of bacterial species from breathing, breastfeeding, and human contact. At this point, the process of colonization within the oral cavity ensues.

Within twenty-four hours of life, pioneer microorganisms, such as *Streptococcus* and *Staphylococcus*, which are Gram-positive cocci, have already been established in the oral cavity [11-12]. *Streptococcus species, S. mitis* and *S. salivarius*, both have the ability to adhere to mucosal cells on the first day of life, and *S. sanguis* after the eruption of teeth during the first year, which explains the natural distribution of streptococci during infancy [13, 21, 25]. High-sucrose diets appear to cause dominance of *S. salivarius* suggesting the influence of diet on the intra-oral levels of *S. salivarius* among infants [26]. *S. mutans*, a species most strongly associated with dental caries, may potentially be acquired from mothers through horizontal as well as vertical transmission [23]. The primary route of transmission is through mother-child saliva, which results in the initial acquisition of *S. mutans* at a time between 6 and 30 months of the child's life, with a higher risk between 18 and 30 months of age, often considered the “window of infectivity” [24, 27, 28]. As the newborn ages, the oral microbiota evolves and by five months of age, a distinct oral microbiota is present from the mother due to various environmental exposures.
With early childhood approaching, *Actinomyces* species are found in higher proportions in the oral cavity. These species have been known to be associated with early plaque development on tooth surfaces [14-15], adhering capabilities [16], and co-aggregating properties with other species [17]. Based on older longitudinal studies, *A. odontolyticus*, has been the primary and frequent colonizer among the *Actinomyces* genus and has also remained the most prominent on oral mucosal surfaces in infants up to 2 years of age [18, 33]. *Lactobacilli* are present in low numbers and under 2 years of age, the species is mostly transient in infants [25]. Other species that have been observed in infants are *Veillonella, Rothia, Gemella, Granulicatella, Leptotrichia, Neisseria, Prevotella melaninogenica* and *Porphyromonas catoniae* [19-20].

As teeth begin to erupt, there are greater areas of potential niches and attachment sites on hard tooth surfaces and in gingival crevices for further microbial colonization. Saliva and gingival crevicular fluid affect the oral surfaces, which further alter the oral microbiota [29]. With a stable core bacterial community already established in a predentate infant, newer species colonizing oral surfaces leads to an environment of multiform gram-negative anaerobic microflora in children with primary dentition [31]. The influence of cell-to-cell interactions causing stimulatory or inhibitory affects among bacterial species, allows for the formation of communities where bacterial inter-relationships affect composition and stability. *Viridans streptococci* and *Fusobacterium nucleatum*, are both influential in this environment [30]. While *Porphyromonas gingivalis* colonizes the oral cavity early in childhood possibly through parental salivary transmission, the species appears to become more stable in the late teenage years as...
deeper pockets develop [32]. This could potentially indicate a long-term risk factor for periodontal disease [34].

Throughout the transitional phases of primary, mixed, and permanent dentitions, the oral microbiota is continuously changing. Based on 454 pyrosequencing, attempts to define an overall healthy oral microbiome composed of key core bacterial species have been made. The predominant taxa have been generally described to consist of *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Fusobacteria* with an average of 266 "species-level" phylotypes [22]. Other studies have indicated the potential role of *P. catoniae* and *N. flavescens* in association with healthy, caries-free dentitions in young children [35]. However, due to a small sample sizes and lack of comprehensive microbial community analyses at the species-level, further research is warranted to understand the overall acquisition of the microbiome from infancy to adolescents.

The objective of this research is to conduct a cross-sectional study to reveal the overall prevalence of oral bacterial communities and their natural path of acquisition from after the first year of life up until the age of fourteen. This will allow us to understand community stability, determine how microbiota manipulation can cause or prevent oral disease such as caries, and create an integrative map of the transitioning oral microbiota from primary dentition to permanent dentition. By understanding the oral microbiome in its most comprehensive state, bacterial agents that contribute to disease susceptibility may be identified and therapeutic strategies in controlling caries may further be explored.
Hypothesis

The establishment of the oral microbial communities has been studied for years however previous research has focused on limited numbers of intra-oral bacterial species colonization. With the availability of next generation sequencing, large-scale whole-genome sequencing has become faster and allows for comprehensive microbial community profiling. This novel technology has allowed us to determine the order of acquisition of the oral microbiota. In conjunction, 16S rRNA gene pyrosequencing was used to determine microbial community composition and diversity at the level of species.

These methods have allowed us to understand the core set of oral microbial species among children, its evolution throughout adolescence, and the role in bacterial community inter-relationships. We expect to find an orderly acquisition of the oral microbiota, composed of key core bacterial communities, from infancy to adolescence. With advancing age leading to tooth eruption, and eventually transitioning from primary to permanent dentition, we expect to see increasing complexity of the oral microbial communities, perhaps due to the availability of different niches for bacterial adherence, exposure to salivary enzymes, environmental factors, and dietary habits.

Hypothesis: The community composition of the oral microbiome among the supragingival, subgingival, and salivary environments will show increase in species diversity with advancing age (beginning after age 1).
Materials and Methods

Clinical Methods

Subject Recruitment

116 subjects between the ages of 1 through 14 were recruited from the Dental Clinic at Nationwide Children’s Hospital by the study hygienist under the supervision of Dr. Ann Griffen. Written permission from parents and an additional assent from children 8 yrs of age or older were both obtained. Subjects were examined and sampled for baseline data. Participants with missing data for a single timepoint were included in the analysis, but subjects were dropped from the study if two consecutive visits were missed. Our electronic medical record (EPIC with a custom in-house developed pediatric dental module) facilitated tracking and review of dental and medical records and also retaining up-to-date contact information since records are updated if a patient returns to any NCH facility for any services. The hospital provides a million outpatient visits per year with over 33,000 patient visits in the dental clinic, thus providing an ample pool of subjects for our study.

Inclusion/exclusion criteria

Inclusion criteria were ASA status I or II (no serious medical problems) and an English-speaking primary caregiver who accompanied the child to dental visits. Exclusion criteria were ASA status III or greater, chronic disease affecting the immune system or requiring chronic use of antibiotics (including requirement for antibiotic prophylaxis for infective
endocarditis), early onset periodontitis (rare and expected to show an altered microbial profile), and diabetes. Sampling was not conducted within 30 days of any antibiotic therapy, and at times, delayed if necessary. Each age cohort was relatively balanced on gender and race.

Data Collection

At each visit tooth presence, caries, gingivitis, plaque levels, presence of visible tongue biofilm, restorations, sealants, orthodontic appliances, and soft tissue lesions were scored and recorded by the study hygienist under the supervision of Dr. Griffen or the attending dentist at the clinic. History of antibiotic use, oral hygiene practices, number of persons residing in household, daycare or school arrangements, fluoride exposure (water and topical), a simple diet survey focusing on carbohydrate frequency, and tobacco exposure/use history were collected by interview. Information on breast-feeding and delivery mode was obtained. For subjects younger than 3 years of age, information on pacifier, bottle or sippy cup use was recorded as well.

Sampling

Subjects were sampled at least 1 hr after home oral hygiene or consuming food or drink, and prior to any dental prophylaxis or other dental procedure. Saliva sampling was conducted with a technique that can be uniformly used for children of all ages so that samples were comparable. Saliva was collected by placing a sterile, soft, flocked Copan swab in the right lingual vestibule for a minimum of 30 seconds and then swabbing into
both buccal vestibules and finally across the dorsal surface of the tongue. The size of the swab was adjusted to size of child. The swab was placed in ATL buffer and stored under refrigeration until being transported to the lab for storage at -20° C and until DNA was isolated. In addition, both supragingival and subgingival samples were collected. Sterile microbrushes were used to collect the supragingival plaque from the buccal surfaces of all teeth in the mandibular right quadrant, and sterile paper points were then inserted into the mesial sulcus of each of these teeth to obtain subgingival samples. Supra- and subgingival samples were each pooled separately and stored at -80°.

**Laboratory Methods**

**New Generation Sampling**

The relative levels of bacterial species were analyzed using a novel technique. DNA was isolated followed by 16S rRNA gene amplification and Illumina MiSeq sequencing. 16S metagenomic sequence library prep was modified to work on the U1 to U3 region of the 16S gene. Sequences were identified at the level of species by blast of the CORE database with >98% sequence identity (36). Mothur program was used to make contigs and filter sequence (37). The relationship of age to microbial community composition was determined using Bray Curtis multidimensional scaling and the EnvFit test, and the relationship of microbial community complexity to age was analyzed.

Information derived from the following thesis “Sulyanto, Rosalyn. "The Natural History of Oral Bacteria Acquisition in the Developing Infant." Electronic Thesis or Dissertation. Ohio State University, OhioLINK Electronic Theses and Dissertations Center. 17 Jul
2016.”

**Bioinformatics Methods**

Sequences were separated by barcodes, trimmed for primers and low quality and filtered for length using mothur [37]. The sequence reads were used as queries for a blastn search [38] (parameters: -dust no -gapopen 0 -gapextend 0 -reward 1 -penalty -2 –word_size 10) of an extended version of the CORE database [39]. The sequence reads were assigned to species-level OTUs if they matched database sequences at over 98% identity for a region of greater than 350 bp. Some previously named species have been difficult to disambiguate based on 16S analysis and those have been indicated with combined names. Sequences without matches were used to expand the coverage of the database. They were first clustered at 99.5% identity using uclust [40], and non-chimeric clusters were identified using uchime [41]. Clusters with over 25 members were judged likely to be error free and to represent permanent residents of the oral cavity. Such clusters of pyrosequences were used to search Genbank to identify longer sequences, preferably near full length to add to the database. If there were no long matches \( \geq 98\% \) identity, a representative pyrosequence from the cluster was added to the database.

Statistical Analysis

NMDS of Bray-Curtis dissimilarities between samples and species was performed with the `metaMDS` function of the `vegan` package in R [42]. Dispersion ellipses were calculated using the `ordiellipse` function of `vegan`, and PERMANOVA tests of differences between groups were calculated with the `adonis` function [42]. The natural logarithm base with the `diversity` function of `vegan` was calculated using the Shannon diversity index. Shannon diversity was calculated using R. Measure of levels of bacterial species over time were analyzed using a linear mixed effects model. False discovery rate correction for multiple comparisons was made using the Benjamini and Hochberg procedure. Wilcoxon signed-rank test was used to compare related samples among sites for each species. In our secondary analysis focused on the effect of caries on overall bacterial composition, caries status was categorized in the following manner: “No caries” was defined as no clinical caries detected during clinical and radiographic exams, “ECC” also known as early childhood caries was defined as caries present in participants under the age of 5, and “Non-ECC” or late-onset caries was defined as caries present in participants over the age of 5. Decayed and filled surfaces (dfs/+DFS) scores were calculated based on the numbers of cavitated lesions and completed restorations.
Ethical Considerations

This study was approved by the Institutional Review Boards of Nationwide Children’s Hospital and the Ohio State University. Signed informed consent from all parents and an additional assent from children 8 yrs of age or older were obtained for the study prior to enrollment.
Results

Study Participants

One hundred and sixteen participants were recruited for this study. Age distribution varied among the participants with the most participants being around 8 years of age (12.1%) [Figure 1]. All participants varied among race and were relatively evenly divided among gender (54.3% females, 45.7% males). About 54.3% of the participants were Black or African American with the remaining being White (44.8%) or Asian (0.9%). 90.5% were of non-Hispanic or Latino origin [Table 2]. Among the participants, 294 samples were collected with the following site-specific sample distribution: saliva (89.7%), supragingival (89.7%), and subgingival (74.1%). Lower percentage values for subgingival samples are due to samples not being sequenced [Table 3].

Effects of Age

Based on the Shannon Diversity Index/Species Richness linear mixed effects model, age had very little effect on species diversity and species richness in the subgingival, supragingival, and salivary samples [Figures 3-4]. Site-specific MDS plots showed that age had no effect on community composition. A very small effect was detected in the supragingival sample (p=0.015), however due to the small sample size of this study, the effect was not highly significant [Figure 5]. Multiple bacterial communities were also
detected in the samples, however as participants aged these colonies continued to decline over time [Figure 6]. *Streptococcus intermedius, Kingella denitrificans,* and *Porphyromonas HF001* showed reduce number of communities in the subgingival samples. Of note, *Streptococcus mutans* decreased in the supragingival samples, which may be due to our small sample size of participants younger than 4 years of age, and *Granulicatella elegans* and *Porphyromonas HF001* decreased in the salivary samples. There were no samples that showed significant increase in specific bacterial communities within the three sites.

*Effects of Site*

Differences in terms of community composition were detected through site-specific MDS analysis. Bacterial composition among the supragingival and subgingival niches were closely related, with communities clustering near each other [Figure 7]. Communities in the salivary sample were relatively distant from the other two sites. This analysis was driven by species, genus and class of the detected species listed in [Figure 8].

*Effects of Caries*

A secondary analysis focused on caries status (no caries, early childhood caries (ECC), and non-ECC) and dfs/+DFS (decayed and filled surfaces in primary and mixed dentition) was completed. Site-specific MDS plots showed that decayed surfaces in
primary, mixed and permanent dentitions did not effect overall community composition [Figure 9 and Figure 10].

Discussion

This cross-sectional study focuses on exploring the complexity of the oral microbiome from infancy to adolescence utilizing a novel technique, Illumina MiSeq sequencing. Our findings support our older studies on the infant microbiome complexity as well as highlight prevalence of bacterial communities as children approach adolescence. Understanding the natural acquisition of the oral microbiome can aid in our ability to develop caries preventive strategies.

Our study participants varied over race and ethnicity while gender remained balanced. All subjects were recruited during hygiene appointments to ensure random distribution of low caries risk and high caries risk patients. Patients on recent antibacterial therapy (<30 days of sampling appointment) were not sampled due to alterations of the oral microbiome. To determine variability in microbial complexity, sampling was obtained from three environmental niches: saliva, subgingival and supragingival. In particular, we were interested in seeing if tooth eruption and further development of subgingival and supragingival areas altered oral microbial composition over time.

Our analysis showed that age (after the first year of life through 14 years) appeared to have little effect on species diversity and community composition in salivary,
supragingival, and subgingival samples. Particular site-specific bacterial taxa decreased over time. Within the subgingival environments, gram-positive *Streptococcus intermedius* and gram-negative *Kingella denitrificans* and *Porphyromonas HF001* communities decreased over time. Studies have shown that *Streptococcus intermedius* is the dominant species found in subgingival plaque and known to cause abscesses [43]. In the supragingival environment, gram-positive *Streptococcus mutans* decreased over time, which was not a significant finding. Based on our MDS plot analysis, a very small effect was detected in the supragingival samples in terms of community composition however this was also not significant. This is mainly due to our small sample size of participants younger than 4 years of age. The salivary environment showed decreased communities of gram-positive *Granulicatella elegans* and *Porphyromonas HF001*. It is important to note that the decline of *Porphyromonas HF001* colonies is unusual as over time, the species becomes more stable and may possibly be indicative as a risk factor for periodontal disease. [32, 34].

In addition, site-specific community composition differences were driven by rank. Based on differences in species, genus and class, supragingival and subgingival communities were similar. This is likely due to the presence of plaque surrounding these environments. The composition of the oral microbiome among these sites may be influenced by type and frequency of food intake, as well as capabilities of bacterial adherence at teeth erupt [20, 23].

Our secondary analysis involving caries status and dfs/+DFS showed no impact on community composition or age. We sampled directly into three specific niches:
salivary biofilm against the buccal mucosa, supragingival margin, and subgingival sulcus. While these areas harbor small communities of *Streptococcus mutans*, sufficient quantities of colonies were not detected in the overall microbiome due to sampling not being performed in large cavitated lesions, which contain larger quantities.

Taken together, our results demonstrated that the acquisition of the oral microbiome appears to be seen during the first 12 months of life with no significant changes detected after the first year of life. The oral microbiome may not be altered until much later on possibly during the late teen years. Further long-term monitoring during will allow us to understand community stability, determine how microbiota manipulation can cause or prevent oral disease such as caries. This will allow us to create an integrative map of the transitioning oral microbiota from primary dentition to permanent dentition.

**Limitations and Future Directions**

Our primary limitation in this study was our small sample size. This limited the statistical power in many of our analyses. Another major limitation was the inability to control for confounding factors from our participants due to our study being a cross-sectional analysis. In addition, further sequencing is required on the DNA samples that were collected during subgingival sampling. Further focus on recruitment of healthy young (<4 years of age) and older (>8 years of age) patients and maintenance of balance among ethnicity, race and gender is needed. We should also consider targeting late teenagers and young adults to assess when the introduction of the adult microbiome occurs in a new study. The future direction of this cross-sectional study is focused on
longitudinal monitoring with six-month recalls over the span of 3 years and understanding the stability of the oral microbiome from after the first year of life through 14 years.
Tables
Table 1. Percent Distribution of Age Cohorts
<table>
<thead>
<tr>
<th>Factor</th>
<th>Number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>63</td>
<td>54.3</td>
</tr>
<tr>
<td>Male</td>
<td>53</td>
<td>45.7</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>52</td>
<td>44.8</td>
</tr>
<tr>
<td>Black or African America</td>
<td>63</td>
<td>54.3</td>
</tr>
<tr>
<td>Asian</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
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<td></td>
</tr>
<tr>
<td>Non-Hispanic or Latino</td>
<td>105</td>
<td>90.5</td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>11</td>
<td>9.5</td>
</tr>
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</table>

Table 2. Participant Demographic Factors
Table 3. Participant Sample Distribution

<table>
<thead>
<tr>
<th>Participants</th>
<th>116</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Samples</td>
<td>294</td>
</tr>
<tr>
<td>Participants by Site (% of participants)</td>
<td></td>
</tr>
<tr>
<td>Saliva</td>
<td>104 (89.7%)</td>
</tr>
<tr>
<td>Supragingival</td>
<td>104 (89.7%)</td>
</tr>
<tr>
<td>Subgingival</td>
<td>86 (74.1%)</td>
</tr>
</tbody>
</table>
Figures
### Figure 1. Abundance Heatmap Reflecting Prevalence of 26 Core Species

<table>
<thead>
<tr>
<th>Species</th>
<th>% prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-3 mo</td>
</tr>
<tr>
<td><strong>Early Colonizers</strong></td>
<td></td>
</tr>
<tr>
<td>Streptococcus mitis group</td>
<td>100</td>
</tr>
<tr>
<td>Rothia mucilaginosa</td>
<td>88</td>
</tr>
<tr>
<td>Veillonella atypica dispar parvula</td>
<td>71</td>
</tr>
<tr>
<td>Streptococcus salivarius group</td>
<td>94</td>
</tr>
<tr>
<td>Gemella haemolysans</td>
<td>88</td>
</tr>
<tr>
<td>Veillonella HB016</td>
<td>71</td>
</tr>
<tr>
<td>Prevotella melaninogenica</td>
<td>53</td>
</tr>
<tr>
<td>Streptococcus peroris</td>
<td>47</td>
</tr>
<tr>
<td><strong>Late Colonizers</strong></td>
<td></td>
</tr>
<tr>
<td>Streptococcus parasanguinis</td>
<td>41</td>
</tr>
<tr>
<td>Granulicatella adiacens</td>
<td>47</td>
</tr>
<tr>
<td>Granulicatella elegans</td>
<td>41</td>
</tr>
<tr>
<td>Actinomyces odontolyticus lingnae</td>
<td>41</td>
</tr>
<tr>
<td>Porphyromonas catoniae</td>
<td>35</td>
</tr>
<tr>
<td>Streptococcus australis</td>
<td>24</td>
</tr>
<tr>
<td>Porphyromonas HF001*</td>
<td>35</td>
</tr>
<tr>
<td>Neisseria flavescens</td>
<td>35</td>
</tr>
<tr>
<td>Leptotrichia FP036*</td>
<td>12</td>
</tr>
<tr>
<td>TM7 B350-25*</td>
<td>24</td>
</tr>
<tr>
<td>Gemella sanguinis</td>
<td>12</td>
</tr>
<tr>
<td>Streptococcus VG051</td>
<td>41</td>
</tr>
<tr>
<td>Streptococcus cristatus</td>
<td>41</td>
</tr>
<tr>
<td>Neisseria meningitidis polysaccharea</td>
<td>18</td>
</tr>
<tr>
<td>Prevotella oral taxon 299</td>
<td>18</td>
</tr>
<tr>
<td>Neisseria flavosa mucosa pharyngis</td>
<td>35</td>
</tr>
</tbody>
</table>
Figure 2. Resolution of Early Chaos
Figure 3. Shannon Diversity Index by Age
Figure 4. Species Richness by Age
Figure 5. Effects of Age on Community Composition
Figure 6. Presence of Site Specific Species Decreasing Over Age
Saliva vs Supragingival vs Subgingival: Mean Levels: Species
Permanova $p = 0.000999$ ***
Beta-dispersion $p = 0.798$

Figure 7. Effects of Site on Community Composition
Figure 8. Species, Genus and Class Abundance Profiles
Figure 9. Effects of Caries Status on Community Composition
Figure 10. Effects of dfs/+DFS on Community Composition
References


Appendix A: Exam and Sampling Form
1 Exam & Sampling Form—Acquisition of the oral microbiome

Subject #

Date

Data from EPIC record (can be completed after patient leaves)

Examiner(s)

Confirm

- [ ] No antibiotics within 30 days
- [ ] No prophylactic within 30 days

Clinical exam

1. Samples (check off)
   - [ ] Saliva & soft tissue swab
   - [ ] Supragingival membrane buccal right side
   - [ ] Subgingival papopoint mesiobuccal right side

2. Tongue biotin
   - [ ] No
   - [ ] Yes

3. Ortho appliances
   - [ ] No
   - [ ] Yes
   - Describe:

OK to prophy now or later

4. Plaque levels
   - [ ] None
   - [ ] Light
   - [ ] Moderate
   - [ ] Heavy

5. Gingivitis
   - [ ] None
   - [ ] Localized
   - [ ] Generalized

6. Calculus
   - [ ] Present
   - [ ] None noted

7. Current medications

8. Medical conditions

9. Other findings

Notes / comments

10. Tooth exam

- [ ] Caries teeth present
- [ ] Whitespots "W"
- [ ] Cavities and restorations
- [ ] Black

Rev 12/19/14
Appendix B: Contact Info and History Form
Page 2: Contact Info & History Form: Acquisition of the Oral Microbiome

Subject #  

Date  

Early history
1. How was your child delivered? □ vaginally □ C-section
2. How was your child fed? □ breast-fed □ formula fed □ combination?
3. Did your child use a bottle or sippy cup outside of meal times? □ yes □ no
   If yes, what did they usually drink in it?  
4. Did your child use a pacifier? □ yes □ no
5. Did your child suck his or her thumb? □ yes □ no
6. At what age did your child first attend daycare, school or babysitter?  

Recent history (past 6 months)
7. Did your child take any antibiotics during the past 6 months?
   
<table>
<thead>
<tr>
<th>Approximate date</th>
<th>Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8. Does your child smoke? □ yes □ no
9. Has your child been around tobacco smoke? □ yes □ no
10. How many persons reside in your household?

<table>
<thead>
<tr>
<th>Relationship to your child</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
</tbody>
</table>

11. How many pets reside in your household?

<table>
<thead>
<tr>
<th>number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs</td>
</tr>
<tr>
<td>Cats</td>
</tr>
<tr>
<td>Other</td>
</tr>
</tbody>
</table>

12. What kind of toothpaste does your child use? □ fluoride □ non-fluoride □ none
13. Does your child use any other oral care products regularly?  
   □ mouthwash  
   □ floss  
   □ other  