MATURATION OF THE ORAL MICROBIOME IN CARIES-FREE TODDLERS: A LONGITUDINAL STUDY

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APPENDIX

MATERIALS AND METHODS

ETHICAL CONSENT AND STUDY DESIGN

Approval for this study was obtained from the Medical Ethics Committee of the University of Michigan Medical School [Institutional Review Board (IRBMED) approval no. HUM00071519. Date of approval: 14-03-2013], Duke University [Duke Medicine Institutional Review Board approval no. PRO00044905. Date of approval: 13-06-2013], Indiana University [Indiana University Institutional Review Board approval no. 1303010908. Date of approval: 15-04-2013] and University of Iowa [University of Iowa Institutional Review Board approval no. 201302810. Date of approval: 5-03-2013].

CLINICAL EXAMINATION

Children underwent clinical oral examination conducted by calibrated dentists or dental hygienists at Duke University, Indiana University and University of Iowa. After collection of saliva and plaque samples, the teeth and mucosal tissue were assessed. Teeth were cleaned with a toothbrush, air or gauze dried, and assessed under light (Orascoptic Endeavour headlamps, Middleton, WI), without magnification, using the International Caries Detection and Assessment
System (ICDAS II) criteria for scoring (ICDAS Coordinating Committee 2012). No dental radiographs were obtained. Primary caregivers were informed of any conditions requiring treatment.

QUESTIONNAIRES

A self-reported 53-item questionnaire (DCR-007-Primary Caregiver Questionnaire) was used for the parental study. Only general questions (demographics, delivery mode, Medicaid) about the child and the caregiver were included in the current study (Daly et al. 2016; Eckert et al. 2010; Fontana et al. 2019; Fontana et al. 2011). Medicaid is a United States state and federal program that provides health coverage for some low-income families and children, pregnant women, the elderly, and people with disabilities. The ethnicity terms “White” and “Black” which were used in the questionnaire, have been replaced with “Caucasian” and “African American” in the current text.

CURRENT STUDY POPULATION

In total, 266 children completed the longitudinal collection (T1, T2, and T3) of salivary and dental plaque samples. Among the 266 participants at the third visit (T3), 127 (47.7%) children had dental caries (ICDAS≥1), 20 (7.5%) children had experienced remineralization of dental caries (ICDAS=1 at T2 and ICDAS=0 at T3), while 119 (44.7%) children maintained caries-free status during all time points (ICDAS=0). Only children who did not present with clinical signs of dental caries by visual observation until the age of 4 years (n=119) and their caregivers (n=116) were included in the current study (Appendix Fig. 1). Among these were three twin pairs and their caregivers. None of the caries-free (n=119) participants had fillings during the 3 years of the study. Five children had received sealants at some point during the study.
SAMPLE COLLECTION AND STORAGE

Sample collection in children

Unstimulated saliva and pooled dental plaque samples were collected from children at three time points: at baseline or T1, when children were 1-year-old, at T2 (2.5 years of age) and at T3 (4 years of age). The collection of the samples varied in time of the day. No recommendation was given for food intake or tooth brushing before sampling. The participants received recommendation to use Fluoride toothpaste in their daily oral hygiene after each visit. From all children, saliva samples were collected at each visit prior to the dental examination, in an OM-505 collection tube (OMNiGene® ORAL, USA). The mouth of the child was swabbed holding two sponges (Puritan PurFlock Ultra, Guilford, Maine, USA) together in the cheek pouch, by gently moving sponges around, sampling in one slow sweep the left pouch, the floor of the mouth and right pouch during 30 sec. From this point onwards saliva swab sample is referred to as saliva sample. The sponges where placed in the OM-505 tubes after breaking off the handle (at the breaking point close to the sponge). If teeth were present, a pooled plaque sample was taken prior to the ICDAS exam by swabbing all buccal surfaces of the child teeth with a sterile microbrush (Microtip micro-applicator fine size; Microbrush International, Grafton, Wisconsin, USA). After the sample was collected the entire microbrush with plaque sample was dropped into Liquid dental transport medium (LDT) vial (Anaerobe Systems, Morgan Hill, California, USA).

Sample collection in caregivers
Unstimulated saliva was collected at T1 from all primary caregivers, while caregivers from the University of Iowa (n=66) were sampled at all three visits. Saliva collection was performed by drooling 1 mL saliva into the funnel (OM-505 tube) without active spiting.

All samples (from children and their caregivers) were placed on ice immediately after collection. Samples were transported on dry ice and stored at -80°C.

SAMPLE PROCESSING

DNA isolation

Samples were subjected to DNA isolation in batches of 84 samples per sample type. All samples belonging to the same child and caregiver were processed in one batch as they were compared longitudinally. Additionally, each isolation batch contained samples originating from the three recruitment sites (Duke, Indiana and Iowa Universities). This was done to minimize the possible differences in isolation between batches of the same kit, buffers, and daily variations. To control for potential contaminations, the isolation blanks (kit chemicals) and the following separate sample blank controls were added to each of the batch: 1) unused brushes; 2) LDT fluid; 3) swabs; 4) stabilizing fluid from unused OM-505 tubes.

For plaque, the LDT vials were thawed, the microbrush was removed using sterile forceps and, together with 100 μL of the solution, transferred to an assigned well in a 1.1 mL deep-well plate (Axygen scientific Inc., CA, USA). For adult saliva samples, the vials were thawed and vortexed extensively and 200 μL of the solution was transferred to an assigned well. For child saliva samples, the vials were thawed and one swab was transferred using sterile forceps; additionally, 200 μL of the solution was added to the same well as the swab. Each 1.1 mL deep-well plate
contained 250 μL 0.1-mm Zirconia beads, 200 μL of phenol (Rotiphenol, Carl Roth GMBH&Co. KG, Germany) and 200 μL of lysis buffer (MagMini DNA isolation kit, LGC Genomics Ltd, UK). The deep well plate was sealed with a silicone lid and placed in a Mini-BeadBeater-96 (BioSpec Products, Bartlesville, OK, USA) for 2 min at 2,100 oscillations/min.

DNA was extracted and purified using the MagMini DNA Isolation Kit (MagMini DNA isolation kit, LGC Genomics Ltd, UK). Bacterial DNA concentration in the samples after purification was determined by qPCR, with universal primers specific to the bacterial 16S rRNA gene (Ciric et al. 2010).

**PCR amplification & sequencing**

The V4 hypervariable region of the 16S rRNA gene was amplified using 1 ng DNA with 1 μM of each barcoded forward and reverse primer used and performing 30 amplification cycles (Kozich et al. 2013). Samples were mixed into a pool in an equimolar fashion; PCR products of isolation blanks (kit chemicals only), sample blanks (kit chemicals and unused brushes (plaque) or LDT fluid (plaque) or swabs (child saliva) or stabilizing fluid (child and caregiver saliva) and negative PCRs (PCR mix with DNA free water) were included. Furthermore, PCR products of three designated samples and a bacterial mock community sample (HM-782D, BEI Resources, NIAID, NIH as part of the Human Microbiome Project: Genomic DNA from Microbial Mock Community B (Even, Low Concentration), v5.1L, for 16S rRNA Gene Sequencing) were added to each sample pool to serve as run-to-run controls. This was done in order to assess the potential batch effects of sequencing. Each sequencing run included replicate templates of one sample from different individuals.
Paired-end sequencing of the DNA was conducted on the MiSeq platform (Illumina, San Diego, CA, USA) in 6 runs, using a V3 kit and 2x251 nt at the AUMC Cancer Center Amsterdam (Amsterdam, the Netherlands). For each run, the flow cell was loaded with 12 pmol DNA containing 25% PhiX. The data are available in the NCBI BioProject database under accession number PRJNA575641.

DATA PROCESSING

All samples, from all runs were processed together. The 16S rDNA reads were merged, quality-filtered and checked for possibly remaining PhiX reads as previously described (Koopman et al. 2016) with the following exception: a maximum of 25 mismatches (still 10%) was used in the overlap region during read merging. Next, the quality-filtered sequences (max. expected error 0.5) were denoised using UNOISE3 (usearch v10.0.240, 32-bit; (Edgar 2016)). Mapping of the sequences to the “zero-radius OTUs” (zOTUs) was carried out using usearch_global with –maxaccepts 128 –maxrejects 1024 –maxhits 1, for higher sensitivity during mapping. The representative most abundant zOTU sequences were assigned a taxonomy as previously described (Koopman et al. 2016), however, a trimmed version of HOMD v14.51 (Chen et al. 2010) was used as taxonomic reference database for the RDP naïve Bayesian classifier with minimum confidence of 80% (Wang et al. 2007). The zOTU-table was subsampled at a depth of 7,000 reads per sample, using the single_rarefaction.py script of QIIME v1.8.0, in order to allow comparisons among the different samples.

QUANTIFICATION OF FUNGAL DNA

After DNA extraction, the fungal abundance in the samples was determined using quantitative PCR (qPCR), as previously described (Vollmer et al. 2008). These primers cover the following
fungi: *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans*, *Candida dublieniensis*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*, *Microsporum canis*, *Mucor flavus*, *Trichophyton rubrum*, *Cryptococcus neoformans*, and *Aureobasidium pullulans*. The fungal load was calculated as the relative percentage of fungal DNA to the concentration of bacterial (16S) DNA. Detection limit for fungal qPCR was 0.00188 ng/µl and for 16S qPCR: 0.041649 ng/µl.

**STATISTICAL ANALYSES**

*Microbial profile analyses of unrelated samples*

For multivariate analyses of microbial profile data and assessment of the clustering of samples, the zOTU-table was log-2 transformed and ordinated into principal components using Principal Component Analysis (PCA). To preclude negative values, log-2 transformation was performed on read counts+1. Differences in microbial profiles among the sample groups (beta diversity) were assessed with One-way Permutational Multivariate Analysis of Variance (PERMANOVA; 9999 permutations) using the Bray-Curtis similarity. The *P*-values were corrected for multiple testing using Bonferroni correction. These analyses were performed using PAST software version 3.18 (Hammer et al. 2001).

*Microbial profile analyses of related samples*

In case of dependent samples, such as samples of the same subject over time, PERMANOVA was performed using adonis (vegan v.2.4-6 (Oksanen et al. 2018); R v.3.4.3 (R-Core 2017) with a restriction on the permutations within the subject. *R*² values in adonis gives the effect size showing the variation in distances is explained by the variables being tested. Due to relatively
large sample size, Bonferroni correction was used when overall microbial profiles were compared, to rule out sporadic differences.

**Similarity and microbial diversity analyses**

Similarity in microbiome profiles between different types of sample or different time points of the same child and between the child and his/her caregiver was assessed using Bray-Curtis similarity. Alpha diversity was assessed using Shannon Diversity Index and Species richness (number of taxa/sample). All analyses were performed using PAST software version 3.18 (Hammer et al. 2001).

**Assessment of shared taxa**

The Venn diagram layout from Venny (Oliveros 2007) was used to illustrate number of shared zOTUs between time points or between saliva and plaque samples. The calculations were processed either per group or per child. For the pairwise Venn analysis, a threshold of 70 reads per zOTU accounting for 0.1% of the reads per sample was used. For unpaired group-wise Venn analysis of zOTUs, the reads were not filtered for abundance, in order to allow comparisons with previously published work on so called ‘core taxa’. Next, the unique zOTUs and overlaps were calculated for each group or sample (separately, child saliva and plaque over time, child and caregiver saliva, also plaque-saliva comparison) and summarized per set in a median value and range (min-max).

**Assessment of differentially abundant zOTUs**

To assess which zOTUs had a statistically different relative abundance among the sample groups, the linear discriminant analysis effect size (LEfSe) biomarker discovery tool (Segata et
al. 2011) was used. For LEfSe analysis, a 100-read cut-off per zOTU in the respective subset of samples was used to allow consistency while working with different time points and sample types.

*Univariate statistical tests*

The distribution of the univariate continuous variables was tested using the Kolmogorov-Smirnov test for normality. The Friedman test and Wilcoxon rank-sum test was applied for non-normally distributed variables of related samples. For normally distributed variables of related samples General Linear Model Repeated Measures test was used. To compare unrelated samples, Kruskal-Wallis test followed by Mann-Whitney test were used. All tests above were performed in SPSS version 25. False Discovery Rate (FDR) correction of $P$-values for multiple comparisons was performed in R v. 3.4.3. The FDR was set to 5%.

**RESULTS**

**STUDY POPULATION**

In total, 119 children who were caries-free at all study time points (T1: 1 year; median: 11.3, range 9-15.9 months, T2: 2.5 years; 29 (25.1-35.7) months and T3: 4 years; 47 (42.9-53.8) months) were included in the study (Table). Each child was paired with their own primary caregiver forming a child-caregiver pair. In case of twins ($n=3$ pairs), one caregiver was paired with each of his or her twin child separately, creating two child-caregiver pairs. Among the caregivers, 110 (92%) were mothers.

**OVERALL SEQUENCING OUTPUT**
The entire dataset consisting of 266 children and their caregivers resulted in 2,412 zOTUs. This table, including all samples and controls, was used to check for possible contaminants. zOTUs which had a relatively higher presence in controls vs samples, in total 42, were removed from the dataset. The vast majority (33 zOTUs) of these zOTUs had a very low total abundance of less than 400 reads (in all samples and all controls).

In total, 22,910,789 reads (average 24,373 reads per sample, SD 6,216, range 5-42,617), obtained from the clinical sample sequencing, passed quality filtering and were assigned to 2,345 zOTUs. After subsampling, 2,320 zOTUs remained in the dataset.

In order to assess potential batch effects of sequencing, run-to-run control samples were analyzed using PCA and PERMANOVA. These control samples clustered together according to their sample origin as three designated samples ($P=0.0001$), but not according to their run number ($P=0.99$).

From 940 samples, 925 samples passed the subsampling depth to 7,000 reads per sample. These included 246 unstimulated saliva samples from the caregivers (115 - at T1, 66 - at T2 and 65 - at T3), 323 pooled dental plaque samples (93 - at T1, 114 - at T2 and 116 - at T3) and 356 saliva samples from the children (119 - at T1, 118 - at T2 and 119 - at T3) (Appendix Fig. 1).

Saliva samples from the caregivers collected at T1 were used for all analyses below, while the caregiver samples from all three time points (T1, T2, and T3) were only used for taxonomy summary and to assess the overtime stability of salivary microbial profiles.

**TAXONOMY AND CHANGES AT PHYLUM AND GENUS LEVEL OVER TIME**
The zOTUs of the subsampled dataset were classified in 12 phyla (Fig. 1, Appendix Fig. 2) and in 163 genera or higher taxa (Appendix Table 1A, B). Of all zOTUs, 32 zOTUs (1,142 reads or 0.02% of all reads) could only be assigned to kingdom Bacteria.

The most dominant genera

Child saliva samples were dominated by genus *Streptococcus* (T1: 40%, T2: 36%, T3: 33%), followed by *Haemophilus* (T1: 11%, T2: 11%, T3: 13%) and *Neisseria* (T1: 8%, T2: 9%, T3: 9%), while most reads in saliva of the caregivers were classified as *Streptococcus* (33%), *Prevotella* (21%), *Veillonella* (8%), *Haemophilus* (7%) and *Neisseria* (6%). The top three genera in child plaque samples were *Streptococcus* (T1: 25%, T2: 22%, T3: 21%), *Neisseria* (T1: 18%, T2: 12%, T3: 13%) and *Actinomyces* (T1: 8%, T2: 11%, T3: 12%) (Appendix Table 1B). The predominance of the genera mentioned above was consistent among all three time points.

Changes in bacterial phyla over time

At the phylum level, salivary microbiome of children changed the most from T1 (1 year of age) to T2 (2.5 years), with significant decrease in relative abundance of Firmicutes and Bacteroidetes, and increase in Proteobacteria, Fusobacteria, Actinobacteria and Saccharibacteria (TM7) (Appendix Fig. 3). In plaque, only Actinobacteria increased significantly with time, while Bacteroidetes and Proteobacteria decreased (Appendix Fig. 3).

Changes in bacterial genera over time

At the genus level, *Leptotrichia*, *Fusobacterium*, *Actinomyces*, *Corynebacterium* and *Rothia* increased significantly with time both in saliva and in plaque (Fig. 2) of children. Genus *Streptococcus*, *Veillonella*, *Granulicatella*, *Porphyromonas* and *Alloprevotella* decreased
significantly with time in saliva of children, while in plaque decrease was observed with *Capnocytophaga* and *Neisseria* (Fig. 2).

CHANGES IN MICROBIAL PROFILES OVER TIME

Microbial composition of children over time

Microbial profile analyses on saliva (Fig. 3A) and dental plaque samples (Fig. 3B) of children showed that samples collected at the earliest time point (T1: 1 year of child age) formed a separate cluster from the other samples. Although no clear separation was discernible between the later time points - T2 (2.5 years) and T3 (4 years), the differences were significant among all three time points ($P=0.0001$, $R^2=0.1$ for saliva, and $P=0.0001$, $R^2=0.07$ for plaque, restricted PERMANOVA), also between T2 and T3 in saliva ($P=0.006$, $R^2=0.009$, Bonferroni corrected restricted PERMANOVA) and in plaque ($P=0.006$, $R^2=0.007$, Bonferroni corrected restricted PERMANOVA).

Microbial composition of caregivers over time

Microbial profiles of the caregivers from whom saliva was collected at all three time points ($n=66$), changed significantly ($P=0.01$, restricted PERMANOVA) across the three years (Appendix Fig. 4A). Explained variation of changes over time in salivary composition of the caregivers was 0.08% ($R^2=0.008$).

Microbial composition of children over time and their caregivers at T1

Comparison of the salivary microbiome profiles of children and their caregivers showed that samples of the caregivers ($n=115$) clustered clearly separately from those of children collected at
Similarity of the microbial composition

Although microbial composition of children and adults remained significantly different throughout the three years of the study, the similarity in salivary microbiome between each child and his or her caregiver (child-caregiver pair) increased significantly from the age of 1 (T1) until 2.5 years (T2) ($P<0.01$, FDR corrected general linear model repeated measures test).

Comparisons of similarity of the samples within individual child, collected at two different time points, showed that microbial composition of samples collected at T2 and T3 were more similar to each other than composition of samples between the first and the later time points of the same child both for saliva and for plaque samples. In other words, microbial composition changed the most between the first time point and the rest (Fig. 3D).

Microbial diversity

Alpha Diversity (Shannon Diversity index and Species Richness) of both saliva and plaque of children collected at T1 (1-year-old) was significantly lower than at the later time points (T2, T3), while there was no change in diversity from T2 (2.5 years) to T3 (4 years) (Appendix Fig. 5A, B).

Salivary microbiome of the caregivers was significantly more diverse (median 400 (244-641) zOTUs/sample) than that of children at any time point (at T1: 235 (112-457), T2: 369 (141-518), T3: 359 (161-573) zOTUs/sample) (Appendix Fig. 5B).
CHANGES OF INDIVIDUAL TAXA (zOTUs) OF CHILDREN OVER TIME AND IN COMPARISON WITH THE CAREGIVERS

Proportion of the shared taxa (zOTUs)

Of all 2,320 zOTUs, the zOTU1, classified as *Streptococcus* (*S. dentisani /infantis /mitis /oralis /tigurinus /several unclassified oral taxons*) was present in all saliva and plaque samples (*n*=925) of the study (16% of the reads).

All saliva samples of children and their caregivers (*n*=471) shared three zOTUs: zOTU1, zOTU32 (*Streptococcus*) and zOTU9 (*Gemella haemolisans /morbillorum /sanguinis*) with 22%, 0.7% and 3% of the reads, respectively (Appendix Table 1C). There was a 76% overlap in zOTUs (zOTUs > 0 reads included) in the overall child saliva composition at the three time points, and a 60% overlap between the overall child and caregiver saliva (Appendix Fig. 6A). A large variation in the proportion of the shared zOTUs was observed within an individual child over time (2-42% of the zOTUs≥70 reads) or within a child-caregiver pair throughout all time points (2-17%) (Appendix Fig. 6B).

In plaque, there was a 69% overlap in zOTUs in the overall composition at the three time points (Appendix Fig. 6A). All plaque samples collected at T1 (1 year of age, *n*=93) contained reads of zOTU1 and zOTU32 (streptococci) and zOTU7 (*Neisseria flavescens /subflava*) with 10%, 0.5% and 1.2% of reads, respectively. At the T2 (2.5 years of age), the zOTU7 was found in 112 (98%) of the 114 samples, while zOTU1 and zOTU32, together with three other zOTUs also classified as streptococci, zOTU3 (*Haemophilus parainfluenzae*), zOTU5 (*Actinomyces*) and zOTU9 (*Gemella*) were found in all plaque samples of the time point. At the T3 (4 years of age), all plaque samples (*n*=116) contained the same two zOTUs classified as streptococci (zOTU1,
zOTU4) and single *Neisseria* zOTU (zOTU2), with all but one sample sharing zOTU32 (*Streptococcus*), zOTU20 (*Lautropia mirabilis*), zOTU36 (*Abiotrophia defectiva*), three zOTUs classified as *Neisseriaceae* (zOTU22), *Haemophilus parainfluenzae* (zOTU3) and *Gemella* (zOTU9) (Appendix Table 1C). At the individual subject level, again, as in saliva, large inter-individual variation (2.5-38%) in the proportion of the shared zOTUs among the time points was observed (Appendix Fig. 6B).

*Changes of individual taxa (zOTUs)*

Next, we assessed which zOTUs changed significantly in their relative abundance both in time and in relation to the caregivers. To identify potentially differential zOTUs we applied a biomarker discovery tool LEfSe, followed by pairwise comparisons.

Of zOTUs that were included in LEfSe analyses, the relative abundance of over 200 zOTUs was significantly higher in saliva of the caregivers compared to the children, while the number of zOTUs that were significantly higher in children in comparison to the caregivers increased with time: 113 zOTUs at T1, 183 zOTUs at T2 and 252 zOTUs at T3 (Appendix Table 2A).

In children, over 180 and 130 taxa increased in their relative abundance with time in saliva and plaque, respectively, including *Fusobacterium* (zOTU70), *Actinomyces* (zOTU61) and *Corynebacterium* (zOTU17) (Fig. 4AB), while over 40 taxa in saliva and over 20 taxa in plaque showed significant decrease over time (Appendix Table 2A). For instance, at T1 (1 year of age) children had the highest proportion of *Alloprevotella* (zOTU11) in their saliva and *Capnocytophaga* (zOTU29) in their plaque, compared to a later age (Fig. 4A, B).

zOTUs classified as *Streptococcus Mutans* and *Porphyromonas Gingivalis*
To address the question if specific microbial taxa – *Streptococcus mutans* and *Porphyromonas gingivalis*, traditionally associated with dental caries and periodontal diseases, respectively, - are present in orally-healthy children between 1 and 4 years of age, we assessed the presence and relative abundance of these two taxa in both child and caregiver samples. In the entire dataset (925 samples), 7 zOTUs were classified as *S. mutans*, while only single zOTU (zOTU 209) was classified as *P. gingivalis*. The latter was found at a very low prevalence (1-2 reads or 0.01-0.02%) in only 2 saliva samples (both at the age of 4 years) and in two plaque samples (at T1 and T3) in four different children. In caregivers, *P. gingivalis* zOTU was present in 13 saliva samples at a 0.01-2.4% relative abundance. None of these caregivers matched the four children with *P. gingivalis*. On the other hand, reads classified as *S. mutans* zOTUs were found in saliva of 12 child-caregiver pairs, of which 11 pairs shared the same zOTUs, suggesting transmission from caregiver to the child. In total, *S. mutans* zOTUs were identified in saliva of 40 (34.8%) caregivers, in saliva of 18 children and in plaque of 21 children. In 11 children *S. mutans* was present in both sample types (saliva and plaque) and in 9 cases *S. mutans* was found at two time points - T2 and T3 - of the same child. Of four children who had *S. mutans* in their samples at the age of 1 year, all presented it at a very low relative abundance (0.01%), and none of them remained positive for this taxon at the later time points.

DIFFERENCES BETWEEN SALIVARY AND PLAQUE MICROBIAL COMPOSITION OF CHILDREN

*Differences in the microbial profiles*

Next, we compared the microbial composition of two types of samples, unstimulated saliva and pooled dental plaque, over time. As expected, microbial profiles of saliva were significantly
different from those of plaque (T1: \( P=0.0001, R^2=0.23; \) T2: \( P=0.0001, R^2=0.13; \) T3: \( P=0.0001, R^2=0.15, \) restricted PERMANOVA) (Appendix Fig. 7A).

**Similarity and diversity of microbial composition**

The composition of saliva and plaque was less similar (Bray-Curtis similarity) at 1 year of age than at the later time points (\( P<0.0001, \) FDR corrected Wilcoxon test). Only at T1, saliva samples had significantly higher species richness (more zOTUs/sample) than plaque (\( P<0.0001, \) FDR corrected Friedman test), while Shannon Diversity index did not differ between two types of samples at any of the time points.

**Proportion of the shared taxa (zOTUs)**

There was a 72%, 84% and 83% overlap in zOTUs between the overall child saliva and plaque composition at T1, T2 and T3, respectively (Appendix Fig. 7B). A large inter-individual variation in the proportion of the shared zOTUs was observed within a child between the two types of samples: 10% (range 2.6-65%) of the zOTUs at T1, 25% (0-60%) – at T2 and 18% (2.7-56%) – at T3 (Appendix Fig. 7C).

**Changes of individual taxa (zOTUs)**

Of the zOTUs ≥ 100 reads, 349, 373 and 373 were significantly different between two sample types at T1, T2 and T3, respectively (Appendix Table 2B). Among the 349 zOTUs from T1, 214 zOTUs were at a significantly higher proportion in saliva compared to plaque. For example, zOTU1 (Streptococcus), zOTU11 (Alloprevotella), zOTU7 (Neisseria), Haemophilus (zOTU13, zOTU3) and zOTU9 (Gemella) differed the most (had the highest LDA scores by the LEfSe
biomarker discovery tool) (Appendix Fig. 8A). These taxa remained in top ten most discriminatory zOTUs also at later time points.

In plaque at T1, 135 zOTUs were significantly higher in their relative abundance than in saliva at T1. Among these, *Neisseria* (zOTU2), *Streptococcus sanguinis* (zOTU4), *Actinomyces* (zOTU5), *Capnocytophaga sputigena* (zOTU29), *Rothia aeria* (zOTU21) and *Corynebacterium durum* (zOTU14) differed the most between two sample types (Appendix Fig. 8B; Appendix Table 2B). These taxa remained in top ten most discriminatory zOTUs also at later time points (Appendix Fig. 8).

MICROBIOME DIFFERENCES BY AGE AND TOOTH ERUPTION STATUS OF THE CHILDREN AT T1

The age of the children at the start of the study (T1) varied from 8.97 to 15.9 months and included both predentate (*n*=18) and dentate (*n*=101) children (median 4 teeth, range 0-12) (Table, Fig. 1). Since the age at T1 correlated significantly with the number of the erupted teeth (*P*<0.0001, Spearman’s Rho=0.6), we assessed the relation of age and the teeth eruption status of the children at T1 with their salivary and plaque microbiome composition. For this, we stratified the children into three age subgroups: 1) below 11 months old (*n*=54), 2) 11-13.9 months old (*n*=40) and 3) 14 months old or older (*n*=25). Both, salivary and plaque microbiome of the youngest age subgroup differed significantly from the two older subgroups, but there were no significant differences between middle and the oldest subgroups (Appendix Fig. 9A, B). The species richness of the youngest children was significantly lower than in the older children, both in saliva and plaque, while Shannon Diversity index was lower only in plaque and not in saliva of the youngest children (Appendix Fig. 9E, F).
Children also were divided in three subgroups based on their teeth eruption status: 1) predentate ($n=18$); 2) 1-4 teeth erupted ($n=56$) and 3) 5-12 teeth erupted ($n=45$). Saliva of the predentate children and children with 1-4 teeth erupted differed significantly from the microbial profile of saliva from children with 5-12 teeth erupted (Appendix Fig. 9C). These differences were reflected in the diversity data (Appendix Fig. 9G, H). Microbial profiles and diversity of plaque from children with 1-4 teeth differed significantly from those with 5 or more teeth erupted (Appendix Fig. 9D, G, H).

**SALIVARY AND PLAQUE MICROBIOME BY DEMOGRAPHIC DATA AND EXPOSURE TO ANTIBIOTICS**

We found no differences in the composition of both salivary and plaque microbiome by delivery mode, while plaque at T2 differed by gender ($P=0.007$, PERMANOVA), and at T3 by race/ethnicity (Caucasian vs African American, $P=0.002$, Bonferroni corrected PERMANOVA). Microbial profiles of saliva collected at T2 (results not shown) and plaque collected at all three time points from children who were part of the Medicaid program differed from those who were not ($P<0.01$, PERMANOVA). Plaque samples collected at T3 from the Medicaid group showed higher diversity (Shannon Diversity index, $P=0.007$, Kruskal-Wallis test) than plaque from the non-Medicaid group. At all time points, multiple zOTUs significantly discriminated between the two groups (Appendix Table 2C). Microbial composition of both saliva and plaque samples of children who were exposed to antibiotics within 4 weeks of sample collection differed significantly from those who were not ($P<0.05$, Bonferroni corrected PERMANOVA).

**TOTAL BACTERIAL AND FUNGAL LOAD IN SALIVA AND PLAQUE SAMPLES**
Microbial DNA and fungal DNA in the samples was quantified using universal qPCR for 16S rRNA gene (thus bacterial DNA) and for 28S region, a specific for large group of fungi, including multiple *Candida* species (further called fungal DNA). Saliva samples collected at T1 (1-year-old) and those collected from caregivers had higher bacterial DNA concentration than saliva from the older children (Appendix Fig. 10A).

At T1, fungal DNA was found in saliva of 98% of the caregivers and in 98% of the children, while at T2 and T3 92% and 89% of the children, respectively, had detectable fungi in their saliva. Over time, the youngest children had the highest and the oldest – the lowest fungal DNA concentration in their saliva (Appendix Fig. 10B). Salivary fungal load, calculated as a relative abundance of fungal DNA over bacterial DNA in a sample, was also the highest at the youngest age (median: 0.013%, range: 0-11%) (Appendix Fig. 10C). There was no difference in fungal DNA concentration or in fungal load between saliva of the caregivers and their children at the age of 1 year, while the caregivers had significantly higher fungal concentration and fungal load than their children at 2.5 years and 4 years of age.

In plaque, only 66% of the samples had detectable fungi at T1, while at T2 fungi were present in 90% of the samples and at T3 in 68% (Appendix Fig. 10B, C). The fungal load in plaque at T3 was significantly lower in comparison with T2 (T1: median 0.007%; T2: 0.009%; T3: 0.003%), and it remained significantly lower than in saliva at T1 and T3.
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APPENDIX FIGURES:

| Participants | Children | Caregivers |
|--------------|----------|------------|
|              | 119      | 116        |

| Samples collected at each time points | T1 | T2 | T3 |
|--------------------------------------|----|----|----|
|                                      |    |    |    |
| Saliva                               | 119| 118| 119|
| Dental plaque                        | 93 | 114| 116|
| Saliva                               | 115| 66 | 65 |

Total samples collected

| Saliva | Dental plaque | Saliva |
|--------|---------------|--------|
| 356    | 323           | 246    |

Appendix Figure 1: Current study population.
Appendix Figure 2: Taxonomic distribution of the relative abundance of reads (%) of major bacterial phyla in salivary (left panel) and plaque samples (right panel) of children per time point (T1 - 1, T2 - 2.5, T3 - 4-year-old) and in salivary samples of their caregivers at the start of the study (T1). The samples are ordered according to the relative abundance of phylum Firmicutes in salivary profiles of children at T1. All remaining plots follow the same child ID order.
Appendix Figure 3: The relative abundance of major bacterial phyla (%) in salivary (black boxes) and plaque (grey boxes) samples over time. The boxplots are plotted using Tukey’s method. Significant differences over time within the respective sample type are indicated by
asterisks: *$P<0.05$, **$P<0.01$, and ***$P<0.001$ (paired samples Friedman test followed by Wilcoxon rank-sum test with FDR correction). Lines connect the time points with the respective difference. The pairwise comparisons of the two types of samples (saliva vs plaque) were significant ($P<0.05$) in all but those time points indicated with $\$. 

![Graphs showing principal component analysis results](image-url)
Appendix Figure 4: Principal Component Analysis (PCA) plots of salivary microbiome profiles of (A) the caregivers at three time points (T1, T2, T3) and (B-D) the children at different time points (B – child at T1; C – child at T2 and D – child at T3) in comparison with the salivary microbiome profiles of their caregivers at the start of the study (T1). Blue dots – samples collected from caregivers at T1 (A) and children at T1 (1 year of child age) (B), red dots – samples collected from caregivers at T2 (A) and children at T2 (2.5 years of age) (C), and green dots – samples collected from caregivers at T3 (A) and children at T3 (4 years of age) (D); orange dots – saliva samples of caregivers at T1 (BCD). PCA was performed on subsampled and log2 transformed zOTU data. Axis shows the first two greatest principal components (PCs) explaining the highest intersample variation (% of variance). The \( P \)- and F-values indicate the output of PERMANOVA analyses, using Bray-Curtis similarity.

Appendix Figure 5: Alpha diversity presented as (A) Shannon Diversity Index and as (B) Species richness of the microbial composition in both salivary (black boxes) and plaque (grey boxes) samples over time. The boxplots are plotted using Tukey’s method. Different letters indicate statistically significant differences (\( P<0.001 \), paired samples Friedman test followed by Wilcoxon rank-sum test with FDR correction) within each sample type over time. Saliva (black)
and plaque (grey) samples were tested separately and plotted together as they showed the same pattern of changes. The pairwise comparisons of two types of samples (saliva vs plaque) were significant ($P<0.05$) in all but those time points indicated with $.}
Appendix Figure 6: Venn diagrams of number and proportion (%) of the shared zOTUs (A) groupwise among saliva and plaque samples of the children over time and saliva samples of the children and the caregivers. All zOTUs were included. (B) The median of the number and of the relative abundance and the range (minimum – maximum) of the shared zOTUs within an individual child over time in saliva and in plaque samples and the shared zOTUs between the respective child at the three time points and his or her caregiver. Only zOTUs ≥ 70 reads in the subsampled dataset were included in these analyses.
Appendix Figure 7: (A) Principal Component Analysis (PCA) plots of microbiome profiles of children by sample type (saliva – dot, plaque – circle) over time (blue - T1, red - T2, green - T3). (B) Number and % of the shared zOTUs in saliva and plaque samples, groupwise. (C) The median of the number and of the relative abundance and the range (minimum – maximum) of the shared zOTUs in two types of sample (saliva and plaque) within an individual child over time. Top 50 list of significantly different zOTUs is shown in Appendix Table 2B.
Appendix Figure 8: The top ten most discriminatory zOTUs between saliva (black boxes) and plaque (grey boxes) samples that were higher in (A) saliva or in (B) plaque samples over time. T1 – samples collected at 1 year, T2 – at 2.5 years, T3 – at 4 years of child age. The boxplots are plotted using Tukey’s method. All zOTUs except those indicated with $ differed significantly between saliva and plaque samples at all time points ($P<0.001$, paired samples Friedman test).
Appendix Figure 9: Principal Component Analysis (PCA) plots of microbiome profiles of samples collected at T1 (AB) by age (blue dots – below 11 months; red – 11–13.9 months, green – 14 months or older) of the children and (CD) the eruption of teeth (blue – predentate; red – 1–4 teeth erupted; green – 5–12 teeth erupted). Both saliva (A) and plaque (B) of children younger than 11 months (blue dots) differed significantly from the older groups. Salivary (C) and plaque (D) microbiome profiles of children, who were predentate or had 1–4 teeth erupted differed from those with 5 or more teeth present.

Alpha diversity of microbial profiles of saliva (black boxes) and plaque (grey boxes) of children at T1 as Shannon Diversity Index (EG) and Species richness (FH) by the child age (EF) and number of the teeth erupted (GH). The boxplots are plotted using Tukey’s method. Significant differences within the respective sample type are indicated by asterisks: \( *P<0.05 \), \( **P<0.01 \), and \( ***P<0.001 \) (Kruskal Wallis test and Mann-Whitney test). Lines connect the subgroups with the respective difference (black – for saliva, grey – for plaque).
Appendix Figure 10: Concentration of bacterial (A) and fungal DNA (B) and fungal load (C) in salivary (black boxes) and plaque (grey boxes) samples. The boxplots are plotted using Tukey’s method. Significant differences over time within the respective sample type are indicated by asterisks: *$P<0.05$, **$P<0.01$, and ***$P<0.001$ (paired samples Friedman test followed by Wilcoxon rank-sum test with FDR correction). Lines connect the time points with the respective difference. The pairwise comparisons of the two types of samples (saliva vs plaque) were significant ($P<0.05$) in all but those time points indicated with $\$. 
### Appendix Table 1A: Relative abundance (%) of the reads at the phylum level per sample type and timepoint.

Phyla ordered from the most to the least abundant in child saliva at T1.

| Phyla                        | Child saliva T1; N=119 | Child saliva T2; N=118 | Child saliva T3; N=119 | Child plaque T1; N=93 | Child plaque T2; N=114 | Child plaque T3; N=116 | Caregiver saliva T1; N=115 | Caregiver saliva T2; N=66 | Caregiver saliva T3; N=65 |
|------------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|---------------------------|---------------------------|---------------------------|
| Firmicutes                  | 53.9                   | 47.7                   | 44.7                   | 32.6                   | 31.6                   | 29                     | 47.5                      | 47.9                      | 49                        |
| Proteobacteria              | 20.2                   | 25.3                   | 25.7                   | 33.0                   | 25.7                   | 25.6                   | 14.3                      | 15.3                      | 14.6                      |
| Bacteroidetes               | 17.2                   | 13.1                   | 14.7                   | 9.9                    | 9.1                    | 8                      | 25.3                      | 23.2                      | 22.8                      |
| Actinobacteria              | 2.3                    | 5.2                    | 4.7                    | 16.6                   | 22.6                   | 26.7                   | 7.3                       | 7.3                       | 7.1                       |
| Fusobacteria                | 6.3                    | 8.4                    | 9.9                    | 7.2                    | 10.5                   | 10.1                   | 5                         | 5.8                       | 5.9                       |
| Saccharibacteria (TM7)      | 0.1                    | 0.2                    | 0.2                    | 0.6                    | 0.4                    | 0.5                    | 0.4                       | 0.3                       | 0.4                       |
| SR1                         | 0.04                   | 0.1                    | 0.05                   | 0.1                    | 0.1                    | 0.04                   | 0.1                       | 0.1                       | 0.1                       |
| Spirochaetes                | 0.001                  | 0.01                   | 0.03                   | 0.002                  | 0.01                   | 0.02                   | 0.2                       | 0.1                       | 0.1                       |
| Granulibacteria             | 0.003                  | 0.02                   | 0.02                   | 0.1                    | 0.1                    | 0.1                    | 0.01                      | 0.01                      | 0.01                      |
| Unclassified Bacteria       | 0.02                   | 0.03                   | 0.02                   | 0.005                  | 0.008                  | 0.01                   | 0.02                      | 0.03                      | 0.03                      |
| Synergistetes               | 0                      | 0                      | 0                      | 0.0002                 | 0                      | 0.0001                 | 0.02                      | 0.01                      | 0.01                      |
| Chloroflexi                 | 0                      | 0                      | 0                      | 0                      | 0                      | 0                      | 0                         | 0                         | 0.0002                    |
### Appendix Table 1B: Relative abundance of the reads at the genus level per sample type and timepoint.
From 163 genera or higher taxa top 30 are shown.

| Genera               | Child saliva T1; N=119 | Child saliva T2; N=118 | Child saliva T3; N=119 | Child plaque T1; N=93 | Child plaque T2; N=114 | Child plaque T3; N=116 | Caregiver saliva T1; N=115 | Caregiver saliva T2; N=66 | Caregiver saliva T3; N=65 |
|----------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|---------------------------|---------------------------|---------------------------|
| Streptococcus        | 39.86                  | 35.48                  | 32.73                  | 25.15                  | 22.31                  | 21.07                  | 32.69                     | 32.94                     | 33.81                     |
| Haemophilus          | 10.75                  | 11.43                  | 12.46                  | 2.53                   | 2.84                   | 3.70                   | 6.98                      | 6.10                      | 5.99                      |
| Neisseria            | 8.08                   | 9.27                   | 8.96                   | 17.88                  | 12.23                  | 12.93                  | 5.46                      | 7.42                      | 6.66                      |
| Alloprevotella       | 6.36                   | 2.99                   | 2.42                   | 0.69                   | 0.61                   | 0.38                   | 2.02                      | 1.91                      | 1.69                      |
| Veillonella          | 6.07                   | 4.38                   | 3.76                   | 3.38                   | 4.84                   | 3.47                   | 8.27                      | 7.93                      | 8.61                      |
| Porphyromonas        | 5.21                   | 3.63                   | 4.45                   | 0.91                   | 2.11                   | 1.90                   | 1.62                      | 1.64                      | 1.31                      |
| Leptotrichia         | 4.04                   | 5.45                   | 6.08                   | 6.00                   | 8.13                   | 7.70                   | 2.61                      | 2.95                      | 3.33                      |
| Gemella              | 3.97                   | 4.05                   | 3.79                   | 0.76                   | 0.88                   | 0.80                   | 2.63                      | 2.39                      | 2.29                      |
| Granulicatella       | 2.96                   | 2.23                   | 2.67                   | 0.75                   | 0.82                   | 0.76                   | 0.95                      | 0.84                      | 0.83                      |
| Prevotella           | 2.93                   | 3.35                   | 3.82                   | 0.54                   | 1.97                   | 1.68                   | 21.04                     | 19.07                     | 19.15                     |
| Bergeyella           | 2.06                   | 1.30                   | 2.02                   | 0.87                   | 0.42                   | 0.43                   | 0.21                      | 0.15                      | 0.17                      |
| Fusobacterium        | 1.73                   | 2.63                   | 3.61                   | 1.08                   | 2.34                   | 2.35                   | 2.40                      | 2.78                      | 2.48                      |
| Rothia               | 1.69                   | 2.62                   | 2.46                   | 4.73                   | 3.58                   | 5.11                   | 4.58                      | 4.89                      | 4.45                      |
| Capnocytophaga       | 0.58                   | 1.69                   | 1.78                   | 6.88                   | 3.81                   | 3.43                   | 0.31                      | 0.36                      | 0.37                      |
| Sneathia             | 0.46                   | 0.27                   | 0.15                   | 0.07                   | 0.04                   | 0.02                   | 0.03                      | 0.02                      | 0.07                      |
| Actinomyces          | 0.43                   | 1.43                   | 1.10                   | 8.00                   | 10.77                  | 11.59                  | 2.18                      | 1.87                      | 1.96                      |
| uncl. Neisseriaceae  | 0.42                   | 1.28                   | 0.81                   | 5.08                   | 2.91                   | 2.38                   | 0.27                      | 0.001                     | 0.001                     |
| Aggregatibacter      | 0.30                   | 1.04                   | 1.51                   | 1.06                   | 1.50                   | 1.33                   | 0.48                      | 0.37                      | 0.45                      |
| uncl. Bacilli        | 0.29                   | 0.29                   | 0.27                   | 0.03                   | 0.05                   | 0.04                   | 0.16                      | 0.001                     | 0.01                      |
| uncl. Lactobacillales| 0.22                   | 0.18                   | 0.21                   | 0.08                   | 0.11                   | 0.11                   | 0.19                      | 0.03                      | 0.03                      |
| Kingella             | 0.17                   | 0.47                   | 0.43                   | 1.72                   | 1.33                   | 1.28                   | 0.07                      | 0.08                      | 0.07                      |
| Corynebacterium      | 0.15                   | 1.01                   | 0.95                   | 3.79                   | 7.30                   | 9.33                   | 0.17                      | 0.13                      | 0.18                      |
| uncl. Pasteurellaceae| 0.12                   | 0.27                   | 0.41                   | 0.02                   | 0.08                   | 0.08                   | 0.30                      | 0.0                      | 0                         |
| Lachnosaerobaculum   | 0.11                   | 0.16                   | 0.21                   | 0.13                   | 0.30                   | 0.41                   | 0.25                      | 0.28                      | 0.31                      |
| Abiotrophia          | 0.10                   | 0.46                   | 0.38                   | 2.01                   | 1.25                   | 1.30                   | 0.11                      | 0.14                      | 0.10                      |
| Lautropia            | 0.10                   | 0.80                   | 0.50                   | 2.31                   | 3.02                   | 2.44                   | 0.18                      | 0.28                      | 0.36                      |
| uncl. Leptotrichiaceae| 0.09                | 0.05                   | 0.03                   | 0.003                  | 0.004                  | 0.003                  | 0.002                     | 0.006                     | 0.004                     |
| Cardiobacterium      | 0.09                   | 0.29                   | 0.30                   | 1.19                   | 1.07                   | 0.87                   | 0.04                      | 0.04                      | 0.06                      |
| Campylobacter        | 0.08                   | 0.19                   | 0.24                   | 0.18                   | 0.32                   | 0.25                   | 0.39                      | 0.46                      | 0.48                      |
| Ruminococcaceae[G-1] | 0.06                   | 0.08                   | 0.06                   | 0.03                   | 0.04                   | 0.04                   | 0.07                      | 0.04                      | 0.06                      |
Appendix Table 1C: Prevalence (%) of the microbial taxa (zOTUs) in the respective samples type and timepoints.

From 2320 top 50 zOTUs are shown. zOTUs are ordered from the most to the least prevalent.

The data on saliva of caregivers at T2 and T3 is not shown.

| zOTUs                                      | Child saliva T1; N=119 | Child saliva T2; N=118 | Child saliva T3; N=119 | Child plaque T1; N=93 | Child plaque T2; N=114 | Child plaque T3; N=116 | Caregiver saliva T1; N=115 |
|--------------------------------------------|------------------------|------------------------|------------------------|-----------------------|------------------------|------------------------|---------------------------|
| zOTU 1  Streptococcus; (s dentisani / s infantis / s mitis / s oralis / s HOT 058/ 061/ 064/ 070/ 423/ 431/ s tigurinus) | 100                    | 100                    | 100                    | 100                   | 100                    | 100                    | 100                       |
| zOTU 9  Gemella; (s haemolytica / s morbillorum / s sanguinis)  | 100                    | 100                    | 100                    | 96.77                 | 100                    | 99.14                  | 100                       |
| zOTU 2336 unclassified Streptococcus       | 100                    | 99.15                  | 100                    | 78.49                 | 78.07                  | 81.90                  | 100                       |
| zOTU 32 unclassified Streptococcus         | 100                    | 100                    | 100                    | 100                   | 100                    | 99.14                  | 100                       |
| zOTU 309 Streptococcus; (several species)  | 100                    | 98.31                  | 94.96                  | 62.37                 | 83.33                  | 76.72                  | 99.13                     |
| zOTU 75 unclassified Streptococcus         | 100                    | 100                    | 99.16                  | 78.49                 | 94.74                  | 87.07                  | 99.13                     |
| zOTU 13 unclassified Haemophilus           | 100                    | 100                    | 97.48                  | 84.95                 | 91.23                  | 86.21                  | 93.04                     |
| zOTU 2100 Streptococcus; (several species) | 100                    | 98.31                  | 94.12                  | 30.11                 | 48.25                  | 39.66                  | 87.83                     |
| zOTU 7 Neisseria; (s flavescens / s subflava) | 99.16                  | 97.46                  | 100                    | 100                   | 98.25                  | 97.41                  | 97.39                     |
| zOTU 2398 Streptococcus; (several species) | 99.16                  | 95.76                  | 95.80                  | 20.43                 | 48.25                  | 40.52                  | 91.30                     |
| zOTU 1468 unclassified Streptococcus       | 99.16                  | 100                    | 99.16                  | 87.10                 | 98.25                  | 94.83                  | 88.70                     |
| zOTU 33 Granulicatella; s adiacens         | 98.32                  | 100                    | 99.16                  | 94.62                 | 99.12                  | 98.28                  | 100                       |
| zOTU 2322 Gemella; (s haemolytica / s morbillorum / s sanguinis) | 98.32                  | 96.61                  | 100                    | 47.31                 | 59.65                  | 57.76                  | 96.52                     |
| zOTU 27 Granulicatella; s elegans          | 98.32                  | 98.31                  | 96.64                  | 94.62                 | 90.35                  | 86.21                  | 59.13                     |
| zOTU 47 unclassified Streptococcus         | 97.48                  | 98.31                  | 99.16                  | 82.80                 | 95.61                  | 89.66                  | 100                       |
| zOTU 52 unclassified Streptococcus         | 97.48                  | 99.15                  | 100                    | 97.85                 | 100                    | 97.41                  | 99.13                     |
| zOTU 2276 Gemella; (s haemolytica / s morbillorum / s sanguinis) | 97.48                  | 94.92                  | 93.28                  | 34.41                 | 52.63                  | 56.90                  | 90.43                     |
| zOTU 200 unclassified Streptococcus        | 97.48                  | 96.61                  | 96.64                  | 70.97                 | 84.21                  | 70.69                  | 80.00                     |
| zOTU 28 Veillonella; HOT 780               | 97.48                  | 91.53                  | 85.71                  | 87.10                 | 85.96                  | 65.52                  | 58.26                     |
| zOTU 122 Granulicatella; s elegans         | 97.48                  | 92.37                  | 90.76                  | 58.06                 | 57.02                  | 40.52                  | 36.52                     |
| zOTU 1942 Gemella; (s haemolytica / s morbillorum / s sanguinis) | 96.64                  | 96.61                  | 95.80                  | 56.99                  | 64.04                  | 68.10                  | 86.09                     |
| OTU     | Species/Description                                    | 96.64 | 98.31 | 96.64 | 24.73 | 41.23 | 31.03 | 79.13 |
|---------|--------------------------------------------------------|-------|-------|-------|-------|-------|-------|-------|
| OTU 1725 | Streptococcus; (several species)                      |       |       |       |       |       |       |       |
| OTU 870  | unclassified Streptococcus                           | 96.64 | 92.37 | 92.44 | 81.72 | 81.58 | 75.00 | 72.17 |
| OTU 12   | Streptococcus; (several species)                      | 95.80 | 98.31 | 98.32 | 87.10 | 77.19 | 88.79 | 100   |
| OTU 6    | Streptococcus; (s. salivarius/s vestibularis)         | 95.80 | 96.61 | 93.28 | 68.82 | 68.42 | 72.41 | 99.13 |
| OTU 1944 | Streptococcus; (several species)                      | 95.80 | 98.31 | 99.16 | 43.01 | 78.07 | 66.38 | 92.17 |
| OTU 1746 | unclassified Bacilli                                  | 95.80 | 96.61 | 93.28 | 33.33 | 56.14 | 49.14 | 87.83 |
| OTU 1820 | unclassified Bacilli                                  | 95.80 | 94.92 | 99.16 | 32.26 | 48.25 | 40.52 | 80.87 |
| OTU 38   | Porphyromonas; HOT930                                | 95.80 | 90.68 | 89.08 | 59.14 | 57.02 | 47.41 | 34.78 |
| OTU 26   | unclassified Porphyromonas                            | 94.96 | 99.15 | 99.16 | 97.85 | 100   | 98.28 | 100   |
| OTU 140  | Gramalactobacter; elegans                           | 94.96 | 87.29 | 85.71 | 51.61 | 45.61 | 31.03 | 26.96 |
| OTU 11   | Alloprevotella; s HOT473                             | 94.12 | 90.68 | 84.03 | 83.87 | 85.09 | 68.97 | 76.52 |
| OTU 3    | Haemophilus; s parainfluenza                          | 93.28 | 100    | 100   | 93.55 | 100   | 99.14 | 100   |
| OTU 1458 | Streptococcus; (several species)                      | 93.28 | 83.90 | 80.67 | 27.96 | 56.14 | 47.41 | 88.70 |
| OTU 10   | unclassified Veillonella                             | 91.60 | 94.07 | 89.92 | 63.44 | 70.18 | 72.41 | 99.13 |
| OTU 566  | unclassified Gramalactobacter                         | 91.60 | 92.37 | 93.28 | 41.94 | 53.51 | 54.31 | 76.52 |
| OTU 1490 | Streptococcus; (several species)                      | 90.76 | 87.29 | 77.31 | 45.16 | 64.91 | 56.03 | 93.91 |
| OTU 663  | Haemophilus; s parainfluenza                          | 90.76 | 90.68 | 96.64 | 43.01 | 65.79 | 58.62 | 88.70 |
| OTU 1665 | unclassified Bacilli                                  | 90.76 | 94.07 | 94.12 | 23.66 | 44.74 | 40.52 | 80.00 |
| OTU 60   | Streptococcus; (s. salivarius/s vestibularis)         | 89.92 | 88.98 | 86.55 | 26.88 | 23.68 | 18.10 | 98.26 |
| OTU 1958 | unclassified Streptococcus                            | 89.92 | 98.31 | 97.48 | 86.02 | 92.11 | 88.79 | 81.74 |
| OTU 1809 | Gemella; (s haemolytic/s morbillum/s sanguinis)      | 89.92 | 88.98 | 84.87 | 18.28 | 28.95 | 27.59 | 73.04 |
| OTU 1962 | Gemella; (s haemolytic/s morbillum/s sanguinis)      | 89.92 | 86.44 | 84.03 | 15.05 | 29.82 | 17.24 | 55.65 |
| OTU 80   | unclassified Streptococcus                            | 89.08 | 94.07 | 93.28 | 38.71 | 40.35 | 42.24 | 100   |
| OTU 8    | unclassified Prevotella                               | 89.08 | 91.53 | 91.60 | 54.84 | 50.88 | 44.83 | 98.26 |
| OTU 1231 | Neisseria; (s flavescens/s subflava)                 | 89.08 | 81.36 | 84.87 | 41.94 | 48.25 | 43.10 | 79.13 |
| OTU 411  | unclassified Veillonella                              | 88.24 | 77.97 | 80.67 | 46.24 | 73.68 | 64.66 | 99.13 |
| OTU 106  | unclassified Haemophilus                              | 87.39 | 96.61 | 97.48 | 25.81 | 47.37 | 36.21 | 79.13 |
| OTU 62   | unclassified Porphyromonas                            | 87.39 | 83.05 | 76.47 | 54.84 | 65.79 | 43.97 | 32.17 |
| OTU 46   | Porphyromonas; HOT930                                | 87.39 | 94.07 | 81.51 | 60.22 | 53.51 | 45.69 | 26.96 |
### Appendix Table 2A: zOTUs by their relative abundances in timepoints and in relation to the caregiver: Output from LEfSe.

The top 10 zOTUs by their LDA score are shown.

| zOTUs | Time points | LDA score | p value | zOTUs | Time points | LDA score | p value | zOTUs | Time points | LDA score | p value |
|-------|-------------|-----------|---------|-------|-------------|-----------|---------|-------|-------------|-----------|---------|
| Streptococcus | T1 | 4.3 | 1.25E-02 | Streptococcus | T1 | 4.4 | 8.28E-04 | Neisseria | T2 | 3.9 | 4.29E-01 |
| Allوبرvotella HOT 473 | T1 | 4.2 | 2.87E-04 | Allوبرvotella HOT 473 | T1 | 4.3 | 1.26E-09 | Alloprevotella HOT 473 | T2 | 3.5 | 1.19E-09 |
| N. flavevosus/subflava | T1 | 4.0 | 6.51E-02 | Veillonella HOT 780 | T1 | 4.0 | 1.58E-11 | Streptococcus sanguinis | T2 | 3.4 | 1.93E-04 |
| Veillonella HOT 780 | T1 | 4.0 | 1.93E-07 | Veillonella HOT 780 | T1 | 4.0 | 5.19E-09 | Actinomyces | T2 | 3.3 | 2.30E-02 |
| Streptococcus | T1 | 4.0 | 6.78E-11 | Haemophilus | T1 | 3.7 | 1.39E-02 | Rothia sarsi | T2 | 3.2 | 3.66E-03 |
| Porphyromonas HOT 930 | T1 | 3.7 | 1.68E-03 | Veillonella | T1 | 3.6 | 2.99E-05 | Neisseria | T2 | 3.2 | 3.54E-03 |
| Porphyromonas HOT 930 | T1 | 3.7 | 2.16E-02 | Porphyromonas HOT 930 | T1 | 3.6 | 1.08E-04 | Lautropia mirabilis | T2 | 3.2 | 6.10E-04 |
| Veillonella | T1 | 3.5 | 3.75E-05 | Porphyromonas HOT 930 | T1 | 3.5 | 1.58E-02 | Veillonella HOT 780 | T2 | 3.0 | 2.68E-02 |
| Granulicatella elegans | T1 | 3.4 | 1.15E-04 | Streptococcus salivarius/ventilularis | T1 | 3.5 | 4.96E-02 | Streptococcus | T2 | 2.9 | 3.91E-02 |
| Neisseria s. flavevosus/subflava | T1 | 3.3 | 1.20E-03 | Granulicatella elegans | T1 | 3.2 | 1.45E-03 | Corynebacterium dorum | T2 | 2.9 | 1.33E-02 |
| Neisseria | T2 | 4.2 | 1.28E-21 | Haemophilus parainfluenzae | T3 | 4.0 | 5.21E-03 | Neisseria | T3 | 3.8 | 4.31E-02 |
| Streptococcus sanguinis | T2 | 3.9 | 3.60E-26 | Neisseria | T3 | 3.9 | 4.99E-14 | Fusobacterium periodonticum | T3 | 3.1 | 4.01E-02 |
| Haemophilus parainfluenzae | T2 | 3.8 | 2.74E-02 | Streptococcus sanguinis | T3 | 3.8 | 8.21E-16 | Rothia mucilaginosa | T3 | 3.1 | 1.28E-02 |
| Veillonella dispar | T2 | 3.7 | 1.74E-20 | Fusobacterium | T3 | 3.6 | 2.47E-20 | Peptostreptococcus nigerescens | T3 | 2.9 | 3.92E-03 |
| Actinomyces | T2 | 3.6 | 3.60E-26 | Aggregatibacter segnis/HOT 458_512 | T3 | 3.6 | 3.12E-20 | Granulactobacterales adiacens | T3 | 2.8 | 2.87E-02 |
| Rothia sarsi | T2 | 3.5 | 5.76E-25 | Porphyromonas | T3 | 3.5 | 8.74E-08 | Porphyromonas | T3 | 2.8 | 4.23E-02 |
| Fusobacterium | T2 | 3.5 | 2.53E-24 | Veillonella dispar | T3 | 3.5 | 2.60E-14 | Leptotrichia | T3 | 2.7 | 4.96E-02 |
| Lautropia mirabilis | T2 | 3.5 | 3.41E-29 | Haemophilus | T3 | 3.4 | 1.22E-05 | Rothia mucilaginosa | T3 | 2.7 | 1.35E-02 |
| Leptotrichia hongkongensis | T2 | 3.5 | 1.32E-14 | Fusobacterium | T3 | 3.4 | 1.28E-20 | Fusobacterium | T3 | 2.7 | 3.23E-02 |
| Neisseria | T2 | 3.4 | 3.16E-16 | Actinomyces | T3 | 3.4 | 4.93E-20 | Leptotrichia | T3 | 2.6 | 4.99E-02 |
| Saliva samples                                                                 | Child T1 and caregiver (total 471 significantly discriminative zOTU) | Child T2 and caregiver (total 488 significantly discriminative zOTUs) | Child T3 and caregiver (total 498 significantly discriminative zOTUs) |
|-------------------------------------------------------------------------------|---------------------------------------------------------------------|---------------------------------------------------------------------|---------------------------------------------------------------------|
| | zOTUs | Groups | LDA score | p value | zOTUs | Groups | LDA score | p value | zOTUs | Groups | LDA score | p value |
| zOTU 6 Streptococcus salivarius/ventilbularis | care-giver | 4.4 | 2.43E-21 | zOTU 6 Streptococcus salivarius/ventilbularis | care-giver | 4.5 | 1.73E-23 | zOTU 6 Streptococcus salivarius/ventilbularis | care-giver | 4.5 | 1.96E-26 |
| zOTU 8 Prevotella | care-giver | 4.4 | 1.73E-24 | zOTU 8 Prevotella | care-giver | 4.4 | 2.13E-26 | zOTU 8 Prevotella | care-giver | 4.4 | 3.94E-26 |
| zOTU 12 Streptococcus several species | care-giver | 4.2 | 2.13E-32 | zOTU 10 Veillonella | care-giver | 4.2 | 6.27E-19 | zOTU 10 Veillonella | care-giver | 4.3 | 1.23E-20 |
| zOTU 10 Veillonella | care-giver | 4.1 | 3.54E-12 | zOTU 112 Streptococcus several species | care-giver | 4.2 | 3.91E-31 | zOTU 102 Streptococcus several species | care-giver | 4.2 | 5.65E-34 |
| zOTU 112 Veillonella | care-giver | 4.1 | 1.17E-24 | zOTU 235 Pseudomonas | care-giver | 4.0 | 2.07E-20 | zOTU 235 Pseudomonas | care-giver | 4.0 | 8.48E-21 |
| zOTU 235 Veillonella | care-giver | 4.0 | 3.36E-23 | zOTU 236 Strepotoccus | care-giver | 3.9 | 2.13E-21 | zOTU 236 Strepotoccus | care-giver | 3.8 | 2.17E-19 |
| zOTU 235 Prevotella | care-giver | 3.8 | 2.03E-30 | zOTU 239 Prevotella | care-giver | 3.8 | 2.87E-23 | zOTU 239 Prevotella | care-giver | 3.8 | 1.26E-23 |
| zOTU 40 Leptotrichia | care-giver | 3.7 | 5.40E-21 | zOTU 42 Rothia mucilaginosa | care-giver | 3.7 | 6.83E-17 | zOTU 40 Leptotrichia | care-giver | 3.7 | 5.73E-16 |
| zOTU 50 Leptotrichia | care-giver | 3.7 | 1.39E-21 | zOTU 40 Leptotrichia | care-giver | 3.7 | 8.50E-15 | zOTU 110 Prevotella | care-giver | 3.7 | 3.79E-21 |
| zOTU 3 Haemophilus parainfluenzae | care-giver | 3.7 | 6.59E-08 | zOTU 110 Prevotella | care-giver | 3.7 | 5.25E-22 | zOTU 51 Prevotella | care-giver | 3.6 | 1.71E-18 |
| zOTU 11 Streptococcus several species | care-giver | 5.0 | 7.99E-26 | zOTU 11 Streptococcus several species | care-giver | 4.9 | 1.16E-19 | zOTU 1 Streptococcus several species | care-giver | 4.8 | 1.29E-16 |
| zOTU 11 Allolpveotella HOT 473 | care-giver | 4.4 | 4.82E-18 | zOTU 2 Neisseria | care-giver | 4.2 | 2.31E-23 | zOTU 13 Haemophilus | care-giver | 4.1 | 4.24E-19 |
| zOTU 13 Haemophilus | care-giver | 4.3 | 5.21E-23 | zOTU 13 Haemophilus | care-giver | 4.2 | 1.62E-24 | zOTU 2 Neisseria | care-giver | 4.0 | 1.06E-14 |
| zOTU 28 Veillonella HOT 780 | care-giver | 4.2 | 2.62E-31 | zOTU 11 Allolpveotella HOT 473 | care-giver | 4.0 | 2.43E-09 | zOTU 11 Allolpveotella HOT 473 | care-giver | 3.9 | 1.22E-02 |
| zOTU 38 Porphyromonas HOT 930 | care-giver | 4.0 | 6.12E-30 | zOTU 4 Strptococcus sanguinis | care-giver | 3.8 | 2.04E-13 | zOTU 3 Strptococcus sanguinis | care-giver | 3.9 | 7.21E-12 |
| zOTU 27 Granulicatella elegans | care-giver | 3.9 | 6.57E-36 | zOTU 9 Granulicatella elegans | care-giver | 3.8 | 2.16E-66 | zOTU 27 Granulicatella elegans | care-giver | 3.9 | 1.83E-27 |
| zOTU 46 Porphyromonas HOT 930 | care-giver | 3.9 | 1.15E-27 | zOTU 127 Granulicatella elegans | care-giver | 3.8 | 1.03E-30 | zOTU 128 Porphyromonas HOT 930 | care-giver | 3.8 | 4.79E-27 |
| zOTU 31 Porphyromonas HOT 930 | care-giver | 3.9 | 7.73E-08 | zOTU 38 Porphyromonas HOT 930 | care-giver | 3.7 | 1.57E-28 | zOTU 9 Granulicatella elegans | care-giver | 3.7 | 1.16E-04 |
| zOTU 7 Neisseria | care-giver | 3.9 | 1.34E-02 | zOTU 28 Veillonella HOT 780 | care-giver | 3.7 | 1.24E-20 | zOTU 4 Strptococcus sanguinis | care-giver | 3.7 | 4.68E-03 |
| zOTU 43 Leptotrichia | care-giver | 3.9 | 6.85E-16 | zOTU 31 Porphyromonas HOT 930 | care-giver | 3.6 | 1.35E-08 | zOTU 37 Fusobacterium | care-giver | 3.6 | 2.94E-11 |
| OTU    | Time points | LDA score | p value | | OTU | Time points | LDA score | p value |
|--------|-------------|-----------|---------|--------|--------|-----------|-----------|---------|
| Neisseria | T1 | 4.5 | 4.67E-04 | | Neisseria | T1 | 4.4 | 3.20E-03 |
| Streptococcus sanguinis | T1 | 4.3 | 5.34E-05 | | Neisseria | T1 | 4.0 | 6.82E-03 |
| Capnocytophaga | T1 | 4.0 | 2.54E-02 | | Neisseria | T1 | 4.0 | 5.00E-03 |
| Capnocytophaga sp. | T1 | 4.0 | 1.47E-00 | | Rothia aeria | T1 | 3.5 | 2.31E-02 |
| Bergeyella HOT 322 | T1 | 3.2 | 4.38E-02 | | Neisseria | T1 | 3.4 | 1.54E-03 |
| Streptococcus | T1 | 3.1 | 6.00E-02 | | Veillonella | T1 | 3.3 | 1.33E-02 |
| Veillonella HOT 780 | T1 | 3.1 | 3.02E-02 | | Veillonella | T1 | 3.3 | 1.54E-05 |
| Cardio bacterium hominis | T1 | 3.1 | 1.57E-02 | | Veillonella | T1 | 3.2 | 3.94E-02 |
| Veillonella HOT 780 | T1 | 3.0 | 1.20E-02 | | Veillonella | T1 | 3.2 | 1.53E-02 |
| Bergeyella HOT 322 | T1 | 2.9 | 9.38E-03 | | Veillonella | T1 | 2.8 | 3.13E-04 |
| Corynebacterium macrinioti | T1 | 8.31E-15 | | Streptococcus | T1 | 3.1 | 1.34E-02 |
| Leptotrichia shahii | T2 | 3.9 | 9.98E-11 | | Leptotrichia HOT 225 | T2 | 2.7 | 2.63E-02 |
| Veillonella dispar | T2 | 3.8 | 7.94E-07 | | Leptotrichia | T3 | 4.1 | 4.23E-14 |
| Streptococcus | T2 | 3.8 | 2.79E-05 | | Leptotrichia | T3 | 4.1 | 9.75E-08 |
| Leptotrichia shahii | T3 | 3.8 | 2.79E-05 | | Actinomyces | T3 | 4.0 | 2.00E-05 |
| Actinomyces masillanensis | T2 | 3.7 | 3.72E-20 | | Actinomyces | T3 | 3.7 | 2.79E-07 |
| Porphyromonas | T2 | 3.6 | 1.15E-14 | | Actinomyces | T3 | 3.7 | 1.87E-25 |
| Porphyromonas | T2 | 3.6 | 9.97E-13 | | Porphyromonas | T3 | 3.7 | 2.50E-12 |
| Veillonella | T2 | 3.6 | 2.21E-06 | | Veillonella | T3 | 3.6 | 2.04E-06 |
| Streptococcus | T2 | 3.5 | 2.26E-15 | | Streptococcus | T3 | 3.5 | 1.95E-11 |
| Actinomyces | T2 | 3.5 | 3.80E-02 | | Actinomyces | T3 | 3.5 | 3.80E-02 |
### Appendix Table 2B: zOTUs by their relative abundances in two sample types per time points: Output from LEfSe.

The top 25 zOTUs by their LDA score are shown.

| Sample vs plaque at T1 (total 349 significantly discriminative zOTUs) | Sample vs plaque at T2 (total 373 significantly discriminative zOTUs) | Sample vs plaque at T3 (total 373 significantly discriminative zOTUs) |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| zOTUs Sample type | LDA score | p value | zOTUs Sample type | LDA score | p value | zOTUs Sample type | LDA score | p value |
| Neisseria plaque | 4.8 | 5.0E-22 | Actinomyces plaque | 4.6 | 1.3E-14 | Actinomyces plaque | 4.6 | 3.5E-10 |
| Streptococcus sanguinis plaque | 4.7 | 6.2E-20 | Streptococcus sanguinis plaque | 4.4 | 1.9E-18 | Neisseria plaque | 4.5 | 2.9E-13 |
| Actinomyces plaque | 4.5 | 2.1E-25 | Neisseria plaque | 4.3 | 6.9E-07 | Streptococcus sanguinis plaque | 4.5 | 4.3E-25 |
| Neisseriaceae plaque | 4.2 | 1.0E-18 | Corynebacterium durnum plaque | 4.2 | 1.4E-23 | Corynebacterium durnum plaque | 4.4 | 6.7E-28 |
| Rothia arisa plaque | 4.1 | 1.9E-08 | Corynebacterium matnocchioti plaque | 4.1 | 3.9E-16 | Corynebacterium matnocchioti plaque | 4.2 | 1.3E-16 |
| Capnoctophaga sp. gatniga plaque | 4.1 | 1.9E-15 | Lautropia mirabilis plaque | 4.0 | 4.5E-16 | Rothia dantocrosa plaque | 4.1 | 2.8E-11 |
| Corynebacterium durnum plaque | 4.1 | 2.3E-18 | Lepotrichia shahii plaque | 3.9 | 8.5E-04 | Rothia arisa plaque | 4.0 | 4.8E-12 |
| Lautropia mirabilis plaque | 4.0 | 2.5E-17 | Veillonella dispar plaque | 3.9 | 1.8E-06 | Lautropia mirabilis plaque | 4.0 | 2.0E-18 |
| Abiotrophia deflexa plaque | 4.0 | 6.8E-23 | Rothia dantocrosa plaque | 3.8 | 4.5E-04 | Lepotrichia shahii plaque | 3.8 | 6.2E-04 |
| Lepotrichia hongongensis plaque | 4.0 | 8.5E-04 | Neisseriaceae plaque | 3.8 | 2.0E-08 | Veillonella dispar plaque | 3.8 | 4.1E-08 |
| Rothia dantocrosa plaque | 3.9 | 3.5E-08 | Rothia arisa plaque | 3.7 | 1.8E-05 | Neisseriaceae plaque | 3.8 | 2.0E-12 |
| Veillonella dispar plaque | 3.7 | 1.0E-07 | Actinomyces massiliensis plaque | 3.7 | 5.2E-13 | Actinomyces massiliensis plaque | 3.7 | 2.2E-21 |
| Cardiobacterium hominis plaque | 3.7 | 7.4E-15 | Kingella oralis plaque | 3.6 | 1.6E-07 | Abiotrophia deflexa plaque | 3.7 | 2.3E-15 |
| Capnoctophaga leadbetteri plaque | 3.7 | 4.2E-08 | Abiotrophia deflexa plaque | 3.6 | 2.7E-11 | Streptococcus plaque | 3.5 | 6.4E-13 |
| Streptococcus plaque | 3.7 | 2.5E-10 | Cardiobacterium hominis plaque | 3.5 | 1.9E-14 | Capnoctophaga sp. gatniga plaque | 3.5 | 4.0E-04 |
| Capnoctophaga plaque | 3.6 | 2.4E-06 | Streptococcus plaque | 3.5 | 1.8E-08 | Kingella oralis plaque | 3.4 | 1.9E-11 |
| Capnoctophaga gingivalis plaque | 3.6 | 1.3E-13 | Actinobacillus HOT B8 plaque | 3.5 | 6.2E-03 | Cardiobacterium hominis plaque | 3.4 | 6.8E-13 |
| Lepotrichia HOT 212 plaque | 3.4 | 1.8E-03 | Capnoctophaga leadbetteri plaque | 3.5 | 1.8E-07 | Actinomyces plaque | 3.3 | 1.2E-05 |
| Kingella oralis plaque | 3.4 | 6.0E-09 | Capnoctophaga sp. gatniga plaque | 3.4 | 2.0E-05 | Capnoctophaga leadbetteri plaque | 3.2 | 8.2E-08 |
| OTU 65 | Borrelyella HOT 322 plaque | 3.4 | 1.43E-13 | OTU 53 | Lepthotrichia HOT 225 plaque | 3.3 | 4.12E-02 | OTU 53 | Lepthotrichia HOT 225 plaque | 3.1 | 1.98E-02 |
| OTU 71 | Lepthotrichia HOT 212 plaque | 3.4 | 1.69E-04 | OTU 57 | Fusobacterium plaque | 3.2 | 1.91E-03 | OTU 26 | Streptococcus plaque | 3.1 | 1.78E-02 |
| OTU 111 | Nisseria plaque | 3.4 | 3.06E-19 | OTU 57 | Capnocytophaga plaque | 3.1 | 4.40E-04 | OTU 368 | Actinomyces plaque | 3.0 | 1.79E-24 |
| OTU 116 | Kingella HOT 012 plaque | 3.3 | 3.65E-02 | OTU 26 | Streptococcus plaque | 3.1 | 2.30E-02 | OTU 1215 | Corynebacterium durum plaque | 3.0 | 1.69E-26 |
| OTU 98 | Capnocytophaga plaque | 3.3 | 3.84E-02 | OTU 105 | Streptococcus intermedius plaque | 3.1 | 1.07E-08 | OTU 94 | Actinobaculum HOT 848 plaque | 3.0 | 1.15E-02 |
| OTU 265 | Nisseria plaque | 3.2 | 9.65E-16 | OTU 76 | Prevotella HOT 317 plaque | 3.1 | 4.72E-02 | OTU 57 | Capnocytophaga plaque | 3.0 | 1.62E-03 |
| OTU 1 | Streptococcus several species saliva | 5.0 | 1.73E-25 | OTU 1 | Streptococcus several species saliva | 4.9 | 3.47E-22 | OTU 1 | Streptococcus several species saliva | 4.9 | 2.64E-24 |
| OTU 11 | Alloprevotella HOT 473 saliva | 4.4 | 4.41E-15 | OTU 3 | Haemophilus paraflavens/saliva | 4.3 | 4.24E-13 | OTU 7 | Nisseria saliva | 4.2 | 3.28E-16 |
| OTU 7 | Nisseria flavescens/subflava saliva | 4.3 | 1.35E-12 | OTU 13 | Haemophilus saliva | 4.1 | 3.18E-28 | OTU 3 | Haemophilus saliva | 4.2 | 9.41E-12 |
| OTU 13 | Haemophilus saliva | 4.3 | 6.54E-24 | OTU 9 | Gemella haemolytica/morbillorum/sanguinis saliva | 4.1 | 1.03E-28 | OTU 13 | Haemophilus saliva | 4.2 | 1.27E-28 |
| OTU 3 | Haemophilus paraflavens/saliva | 4.2 | 2.45E-09 | OTU 7 | Nisseria flavescens/subflava saliva | 4.0 | 5.75E-11 | OTU 9 | Gemella haemolytica/morbillorum/sanguinis saliva | 4.1 | 1.09E-28 |
| OTU 9 | Gemella haemolytica/morbillorum/sanguinis saliva | 4.1 | 1.20E-26 | OTU 11 | Alloprevotella HOT 473 saliva | 4.0 | 5.55E-08 | OTU 11 | Alloprevotella HOT 473 saliva | 3.9 | 2.45E-05 |
| OTU 28 | Veillonella HOT 780 saliva | 4.1 | 5.64E-14 | OTU 6 | Streptococcus salivarius/salivarius saliva | 3.8 | 3.14E-25 | OTU 31 | Borrelyella saliva | 3.8 | 3.63E-17 |
| OTU 2336 | Streptococcus saliva | 4.0 | 4.84E-20 | OTU 8 | Prevotella saliva | 3.7 | 5.13E-22 | OTU 27 | Granulactobacillus saliva | 3.8 | 9.41E-23 |
| OTU 38 | Porphyromonas HOT 930 saliva | 4.0 | 3.81E-21 | OTU 27 | Granulactobacillus saliva | 3.7 | 6.99E-24 | OTU 38 | Porphyromonas HOT 930 saliva | 3.8 | 1.19E-23 |
| OTU 31 | Borrelyella saliva | 3.9 | 1.94E-08 | OTU 10 | Veillonella saliva | 3.7 | 1.39E-13 | OTU 8 | Prevotella saliva | 3.8 | 1.02E-23 |
| OTU 27 | Porphyromonas HOT 930 saliva | 3.9 | 1.78E-26 | OTU 35 | Haemophilus saliva | 3.7 | 1.56E-22 | OTU 35 | Haemophilus saliva | 3.7 | 6.10E-17 |
| OTU 46 | Veillonella HOT 780 saliva | 3.9 | 2.35E-17 | OTU 31 | Borrelyella saliva | 3.6 | 3.92E-15 | OTU 6 | Streptococcus salivarius/salivarius saliva | 3.7 | 7.60E-21 |
| OTU 45 | Lepthotrichia saliva | 3.9 | 5.76E-03 | OTU 38 | Porphyromonas HOT 930 saliva | 3.6 | 1.42E-20 | OTU 10 | Veillonella saliva | 3.6 | 7.72E-13 |
| OTU 6 | Streptococcus salivarius/salivarius saliva | 3.8 | 2.83E-18 | OTU 28 | Veillonella HOT 780 saliva | 3.6 | 1.30E-11 | OTU 46 | Porphyromonas HOT 930 saliva | 3.6 | 4.69E-15 |
| OTU 10 | Veillonella saliva | 3.8 | 1.40E-17 | OTU 41 | Rothia mucilaginosa saliva | 3.5 | 7.27E-15 | OTU 30 | Fusobacterium periodonticum saliva | 3.6 | 3.57E-20 |
| OTU 8 | Prevotella saliva | 3.8 | 8.15E-15 | OTU 12 | Streptococcus several species saliva | 3.5 | 5.06E-21 | OTU 28 | Veillonella HOT 780 saliva | 3.6 | 1.13E-10 |
| OTU   | Species                      | Concentration | OTU   | Species                      | Concentration | OTU   | Species                      | Concentration | OTU   | Species                      | Concentration |
|-------|-----------------------------|---------------|-------|-----------------------------|---------------|-------|-----------------------------|---------------|-------|-----------------------------|---------------|
| OTU 35 | Haemophilus                 | 3.7           | OTU 43 | Leptotrichia                | 3.5           | OTU 43 | Leptotrichia                | 3.5           | OTU 30 | Fusobacterium               | 1.0E-11        |
| OTU 30 | Fusobacterium               | 3.6           | OTU 46 | Porphyromonas HOT 309       | 3.5           | OTU 41 | Rothia mucilaginosa         | 3.5           | OTU 12 | Streptococcus several       | 3.67E-14       |
| OTU 12 | Streptococcus               | 3.5           | OTU 50 | Porphyromonas OPT 930       | 3.5           | OTU 59 | Haemophilus                 | 3.5           | OTU 62 | Porphyromonas               | 8.38E-13       |
| OTU 62 | Porphyromonas               | 3.4           | OTU 2336 | Streptococcus              | 3.4           | OTU 2336 | Streptococcus              | 3.4           | OTU 18 | Porphyromonas               | 3.13E-08       |
| OTU 18 | Porphyromonas               | 3.4           | OTU 59 | Haemophilus                 | 3.4           | OTU 12 | Streptococcus several       | 3.5           | OTU 75 | Streptococcus               | 1.94E-33       |
| OTU 75 | Streptococcus               | 3.4           | OTU 62 | Porphyromonas               | 3.3           | OTU 42 | Rothia mucilaginosa         | 3.4           | OTU 81 | Leptotrichia                | 6.78E-07       |
| OTU 81 | Leptotrichia                | 3.3           | OTU 81 | Leptotrichia                | 3.3           | OTU 62 | Porphyromonas               | 3.3           | OTU 90 | Neathia                     | 5.77E-06       |
| OTU 90 | Neathia                     | 3.3           | OTU 106 | Haemophilus                 | 3.2           | OTU 49 | Aggregatibacter              | 3.2           | OTU 42 | Rothia mucilaginosa         | 3.0E-09        |
| OTU 42 | Rothia mucilaginosa         | 3.3           | OTU 75 | Streptococcus               | 3.1           | OTU 81 | Leptotrichia                | 3.3           |
Appendix Table 2C: zOTUs by their relative abundances in children plaque samples per Medicaid over time: Output from LEfSe.

The top 10 zOTUs by their LDA score are shown.

| zOTUs | Medicaid status | LDA score | p value | zOTUs | Medicaid status | LDA score | p value | zOTUs | Medicaid status | LDA score | p value |
|-------|----------------|-----------|---------|-------|----------------|-----------|---------|-------|----------------|-----------|---------|
| zOTU 16 | Rothia dentocariosa | Yes | 3.9 | 1.74E-03 | zOTU 17 | Corynebacterium matruchotii | Yes | 3.9 | 7.54E-03 | zOTU 25 | Lepotrichia shahii | Yes | 3.9 | 2.27E-03 |
| zOTU 25 | Lepotrichia shahii | Yes | 3.7 | 2.85E-03 | zOTU 25 | Lepotrichia shahii | Yes | 3.9 | 3.68E-03 | zOTU 34 | Streptococcus | Yes | 3.6 | 9.27E-03 |
| zOTU 11 | Alloprevotella HOT473 | Yes | 3.6 | 7.12E-03 | zOTU 40 | Lepotrichia | Yes | 3.7 | 2.48E-03 | zOTU 70 | Fusobacterium | Yes | 3.5 | 2.94E-02 |
| zOTU 34 | Streptococcus | Yes | 3.4 | 4.98E-02 | zOTU 26 | Streptococcus | Yes | 3.3 | 3.78E-02 | zOTU 26 | Streptococcus | Yes | 3.4 | 1.61E-03 |
| zOTU 17 | Corynebacterium matruchotii | Yes | 3.4 | 3.94E-02 | zOTU 220 | Lepotrichia HOT 498 | Yes | 3.2 | 3.44E-02 | zOTU 40 | Lepotrichia | Yes | 3.2 | 2.06E-03 |
| zOTU 10 | Veillonella | Yes | 3.4 | 3.24E-02 | zOTU 143 | Lepotrichia HOT 498 | Yes | 3.1 | 3.34E-04 | zOTU 57 | Capnocytophaga | Yes | 3.2 | 8.15E-03 |
| zOTU 9 | Gemella | Yes | 3.4 | 8.18E-03 | zOTU 76 | Prevotella HOT 317 | Yes | 3.1 | 1.15E-02 | zOTU 84 | Lepotrichia | Yes | 3.2 | 1.33E-03 |
| zOTU 28 | Veillonella HOT 780 | Yes | 3.4 | 8.50E-03 | zOTU 86 | Prevotella | Yes | 3.0 | 1.65E-02 | zOTU 49 | Aggregatibacter actiniomycetemcomitans/ HOT 458/512 | Yes | 3.1 | 1.04E-02 |
| zOTU 179 | Neissericaceae | Yes | 3.1 | 2.45E-02 | zOTU 177 | Semiononas | Yes | 2.9 | 8.75E-03 | zOTU 128 | Actinomyces | Yes | 3.1 | 3.57E-04 |
| zOTU 61 | Actinomyces maffini | Yes | 3.1 | 1.18E-02 | zOTU 56 | Actinomyces odontolyticus/HOT 180 | Yes | 2.9 | 3.78E-02 | zOTU 143 | Lepotrichia HOT 498 | Yes | 3.0 | 1.26E-04 |
| zOTU 20 | Lautropia mirabilis | No | 4.1 | 9.56E-04 | zOTU 4 | Streptococcus sanguinis | No | 3.9 | 4.63E-02 | zOTU 14 | Corynebacterium dorum | No | 4.1 | 1.61E-02 |
| zOTU 56 | Abiotrophia defectiva | No | 3.6 | 2.85E-02 | zOTU 22 | Neissericaceae | No | 3.8 | 4.71E-03 | zOTU 4 | Streptococcus sanguinis | No | 4.1 | 5.01E-03 |
| zOTU 65 | Bacteroides HOT 322 | No | 3.3 | 1.74E-02 | zOTU 104 | Neisseria oralis | No | 3.6 | 3.71E-02 | zOTU 20 | Lautropia mirabilis | No | 3.9 | 2.11E-03 |
| zOTU 454 | Lautropia mirabilis | No | 2.9 | 1.06E-02 | zOTU 29 | Capnocytophaga spathula | No | 3.6 | 5.61E-03 | zOTU 58 | Neisseria oralis | No | 3.5 | 2.37E-02 |
| zOTU 704 | Bacteroides bacteriaceae | No | 2.8 | 1.15E-02 | zOTU 20 | Lautropia mirabilis | No | 3.5 | 1.19E-02 | zOTU 93 | Lepotrichia wadai | No | 3.0 | 2.11E-03 |
| zOTU 2223 | Lautropia mirabilis | No | 2.8 | 5.72E-04 | zOTU 48 | Cardio bacterium hominis | No | 3.3 | 2.56E-02 | zOTU 65 | Bacteroides HOT 322 | No | 2.9 | 1.56E-02 |
| zOTU 1426 | Bacteroides bacteriaceae | No | 2.8 | 1.39E-03 | zOTU 73 | Aggregatibacter aphrophilus/paraphrophilu s | No | 3.3 | 9.96E-03 | zOTU 454 | Lautropia mirabilis | No | 2.6 | 3.51E-02 |
| zOTU 1139 | Neisseria | No | 2.7 | 7.17E-03 | zOTU 74 | Capnocytophaga gingivalis | No | 3.0 | 1.95E-02 | zOTU 2239 | Lautropia mirabilis | No | 2.4 | 4.67E-04 |
| zOTU 936 | Neissericaceae | No | 2.7 | 1.56E-02 | zOTU 65 | Bacteroides HOT 322 | No | 2.8 | 5.74E-04 | zOTU 942 | Neisseria oralis | No | 2.4 | 7.96E-03 |
| zOTU 929 | Neisseria | No | 2.7 | 9.82E-03 | zOTU 265 | Neissericaceae | No | 2.8 | 2.04E-02 | zOTU 1426 | Bacteroides bacteriaceae | No | 2.4 | 2.44E-03 |