Journal of Current Research in Oral Surgery
2024, Volume 4, Page No: 72-85

Available online at: www.tsdp.net

ISSN: 3062-3480

Original Article

Early-Life Establishment of the Infant Oral Microbiome: A 15-Month Longitudinal Study of Microbial Succession and Mother-to-Infant Transmission Patterns

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Received: 17 October 2023; **Revised:** 29 January 2024; **Accepted:** 04 February 2024

ABSTRACT

The oral cavity contains one of the most intricate bacterial ecosystems in humans. Yet, the processes through which newborns first obtain these microorganisms are still not well understood. This work tracked shifts in oral microbial communities among healthy infants and assessed how the maternal oral flora contributes to microbial acquisition in early life. We proposed that oral microbial diversity in infants would rise as they age. A total of 116 whole-saliva samples were obtained from 32 healthy infant–mother pairs at postpartum, 9 months, and 15 months. Bacterial DNA was isolated and analyzed using Human Oral Microbe Identification through Next Generation Sequencing (HOMINGS). Microbial diversity within each dyad (alpha diversity) was quantified with the Shannon index. Differences between infant and maternal microbiomes (beta diversity) were evaluated using weighted Bray–Curtis distances in QIIME 1.9.1. Core microbiome profiles were examined via MicrobiomeAnalyst, and linear discriminant analysis with effect size was employed to detect taxa distinguishing the two groups. Sequencing generated 6,870,571 16S rRNA reads from matched saliva samples. Infant and maternal microbial communities differed significantly ($p < 0.001$). Infant salivary microbial diversity increased over time, whereas mothers displayed a largely consistent core microbiome throughout. Neither breastfeeding nor infant sex influenced diversity patterns. Infants exhibited higher proportions of Firmicutes and lower levels of Actinobacteria, Bacteroidetes, Fusobacteria, and Proteobacteria compared with mothers. SparCC analysis revealed ongoing shifts in infants' microbial interaction networks ($p < 0.05$). Findings indicate that infants begin life with an oral microbiota distinct from that of adults. During the first year, microbial composition and diversity undergo substantial change, and by the period preceding age two, infant oral communities may resemble those of their mothers.

Keywords: Oral microbiota, Microbial diversity, Infant–mother dyads, Postpartum, Initial microbial acquisition

How to Cite This Article: Rizzi FL, Romano LF, Farouk AS. Early-Life Establishment of the Infant Oral Microbiome: A 15-Month Longitudinal Study of Microbial Succession and Mother-to-Infant Transmission Patterns. J Curr Res Oral Surg. 2024;4:72-85. https://doi.org/10.51847/NpV9lui2oj

Introduction

The human mouth hosts one of the richest bacterial communities, with more than 700 identified species [1, 2].

However, the earliest stages of microbial establishment in infants remain insufficiently described, and the factors shaping oral colonization during the first year of life are still not fully clarified. Additionally, the long-term health implications of early oral microbial formation are not yet well defined.

Although the oral microbiome plays important roles in growth and immune maturation, the timing of initial bacterial exposure in infants is debated. Under typical conditions, the fetus develops in a sterile environment. Nevertheless, recent molecular studies have detected bacterial DNA in placental and amniotic samples from healthy pregnancies [3]. The placental microbiome appears more similar to oral bacterial communities than to those of the skin, nasal passages, vagina, or gut [4, 5]. Most early colonizers in infants—largely

members of the indigenous oral flora—are acquired during birth or soon after [6]. Emerging research suggests that microbial activity in the placenta may be linked with increased likelihood of preterm delivery [7, 8]. Previous investigations report that the newborn oral cavity is rapidly dominated by Bifidobacterium species, particularly Streptococcus, Gemella, Veillonella, Granulicatella, and Rothia, followed by Haemophilus, Actinomyces, Porphyromonas, Prevotella, and Neisseria shortly after birth [6-10]. These organisms may contribute to early immune protection and maturation [11, 12]. A range of variables—maternal microbiota, delivery mode, feeding practices, dietary exposures, caregiver interactions, and antibiotic use—can influence microbial acquisition [10, 13-18].

This study analyzed changes in oral microbial communities in healthy infants from the postpartum period through 15 months and explored time-related microbial patterns between infants and mothers. Culture-based detection of Streptococcus mutans and 16S rRNA HOMINGS sequencing were used. We posited that newborns harbor a distinctive bacterial community at birth, differing from that of their mothers, and that infant oral microbial diversity increases with age while being shaped by maternal oral microbiota.

Materials and Methods

Study population

This exploratory project was carried out in parallel with a broader intervention study aimed at reducing coercive interactions in couples and parent–child relationships to support healthier daily habits [ClinicalTrials.gov ID: NCT03163082] [19]. From that dataset, 32 family units were chosen at random. Each family was tracked from the child's birth through 15 months of age. Data collection involved structured questionnaires addressing family dynamics, infant feeding, and oral health behaviors at postpartum baseline and again at 9 and 15 months. Follow-up visits took place at the Bellevue Hospital Pediatric Clinic, the Gouverneur Hospital Pediatric Clinic, and at New York University College of Dentistry. Recruitment procedures and family characteristics are documented elsewhere [20].

Ethics statement

Approval for all study activities was granted by the Institutional Review Boards of the New York University School of Medicine, the New York University College of Dentistry (Research Proposal Oversight Committee), and the New York City Health and Hospital Corporation. Written informed consent was obtained from all participating parents, and permission was provided for their infants' involvement.

Saliva streptococcus mutans assessment

At each time point, saliva was obtained from mothers and infants. Mothers first remained quiet for 5 min, rinsed with sterile water, chewed paraffin for 30 s, and then collected stimulated saliva into chilled 50-ml tubes. Infant samples were gathered using sterile cotton swabs rotated along the oral mucosa and dental ridges for 10–30 s until saturated. Each swab was placed into 2 ml of pre-reduced transport fluid [RTF; [21]] in a labeled vial, and the swab tip was snapped off before sealing. Samples were transported on ice to the New York University College of Dentistry microbiology laboratory. To generate reliable colony-forming unit (CFU) counts, 10 serial 10-fold dilutions (10^{-1} to 10^{-3}) were prepared. A 50-µl aliquot of each dilution was dispensed onto mitis salivarius agar supplemented with potassium tellurite and bacitracin (MSB; Difco Laboratories, Detroit, MI, United States) using an Autoplate Spiral Plating System (Advanced Instruments, Norwood, MA, United States). Plates were incubated anaerobically for 72 h at 37°C under a gas mixture of 85% N_2, 10% CO_2, and 5% H_2, after which S. mutans CFUs were quantified.

Bacterial genomic DNA extraction

Genomic DNA was isolated from 1 ml of saliva (mothers) or from infant swab material using a modified Epicenter DNA purification protocol (Madison, WI, United States) following earlier descriptions [22]. Each sample was treated with 10 µl proteinase K (10 mg/ml in TES buffer: 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 100 mM NaCl), 10 µl lysozyme (100 mg/ml in TES), and 2 µl mutanolysin (5,000 U/ml in PBS), then processed using phenol/chloroform/isoamyl alcohol extraction. DNA quantity and purity were evaluated with a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, United States). Extracts were stored at −20°C until sequencing.

16S rDNA sequencing and data processing

Libraries targeting the V1–V3 region of the 16S gene were prepared for sequencing using the HOMINGS protocol at the Forsyth Institute Sequencing Facility (Cambridge, MA, United States) as adapted from earlier work [23, 24]. Between 10 and 50 ng of purified DNA served as template for PCR using forward primer 341F (5′-AATGATACGGCGACCACCGAGATCTACACTA

TGGTAATTGTCCTACGGGAGGCAGCAG-3′) and reverse primer 806R (5′-CAAGCAGAAGACGGCATACGAGATNNNNNN NNNNNNAGTCAGTCAGCCGGACTACHVGGGT WTCTAAT-3′). Amplicons were cleaned with AMPure beads, and a 12 pM denatured library containing 20% PhiX spike-in was loaded onto an Illumina MiSeq (San Diego, CA, United States).

Across samples, sequencing generated more than 50,000 reads on average, with mean read lengths near 441 bp. Any dataset containing fewer than 2,000 bp of usable reads or showing evidence of chimeras was removed. Sequences were screened using QIIME 1.91 "split_libraries.py" with default filters, including a minimum read length of 200 and a minimum quality score of 25, and then joined with "join_paired_ends.py" [25]. Taxonomic assignment relied on 660 species-specific probes and an additional 107 genus-level probes from the Human Oral Microbiome Database (HOMD). ProbeSeq software performed exact-sequence matching [23]. Only quality-approved samples were included for open-reference OTU assignment. OTUs were kept for downstream work if at least 20% of values contained ≥4 counts and displayed ≥10% variability (inter-quantile range). Final OTU tables were converted to relative abundance profiles for each taxonomic rank [23].

Statistical analysis

Comparisons of relative bacterial abundance were carried out across mother–infant groups, sampling times, infant sex, and breastfeeding categories. Alpha diversity was quantified using the Shannon index through the phyloseq interface in the R vegan package [26]. Diversity outcomes were visualized as multi-group boxplots reflecting the study variables. Group differences were assessed with Wilcoxon–Mann–Whitney tests and the Kruskal–Wallis test. Beta diversity was evaluated using non-phylogenetic Bray–Curtis dissimilarities generated in the QIIME workflow [25]. A heatmap was used to display the key genera that contributed to clustering patterns between maternal and infant saliva samples at each visit.

Principal coordinates analysis (PCoA) was applied to the beta diversity matrices to create two-dimensional ordination plots reflecting sample separation by age point, sex, and feeding mode. Differences in microbial community structure were tested through PERMANOVA, with adjustments for sequencing depth.

To illustrate taxonomic differences between groups, relative abundance profiles at phylum, genus, and species resolution were graphed for mothers versus infants and for breastfed versus non-breastfed infants. Species-level comparisons across postpartum, 9-month, and 15-month assessments were conducted using MaAsLin2 [27]. Core microbiome determination was performed with MicrobiomeAnalyst [28, 29], and the outputs are shown as heatmaps including taxa detected in more than 20% of participants with relative abundance greater than 0.01%. In these heatmaps, the y-axis represents taxon prevalence, while the x-axis corresponds to the detection threshold. Core communities of mothers and infants were compared across all three sampling occasions.

Differentially enriched features were identified using linear discriminant analysis (LDA) with effect size estimation (LEfSe) [30], complemented by nonparametric Kruskal–Wallis testing. Features were considered significant when the FDR-corrected p-value was below 0.1 and the Log LDA score exceeded 2.0. LDA plots reported the discriminating taxa on the y-axis and corresponding LDA values on the x-axis; greater LDA scores indicated higher abundance in mothers. To examine relationships among microbial taxa and developmental trends in infants, SparCC correlation networks were generated with a threshold of 0.5 and $p < 0.05$, where correlated taxa were linked graphically.

For S. mutans culture data, CFU counts from MSB agar were converted to log10 values. Mean differences between mothers and infants, as well as comparisons by infant sex and visit time, were evaluated with Wilcoxon–Mann–Whitney tests and the Kruskal–Wallis one-way ANOVA. All analyses were performed using Stata version 17.0 (StataCorp LLC, College Station, TX, United States).

All statistical evaluations were two-tailed, with significance set at $p < 0.05$. Benjamini–Hochberg FDR correction was applied, with $q < 0.05$ indicating statistical significance.

Results and Discussion

At the postpartum baseline visit, 32 mother–infant pairs were enrolled. Maternal ages ranged from 18 to 34 years, with a mean of 24.2 ± 4.2. All infants were full-term newborns; 22 were male and 10 were female. Reported infant racial distribution included: 15 Latino, 5 African American, 3 Asian, and 9 of mixed background. By the 9- and 15-month visits, 13 dyads returned for follow-up. In total, 116 saliva samples were used for culture-based S. mutans evaluation, 16S rDNA sequencing, and HOMINGS profiling.

S. mutans colonization

Based on culture results, S. mutans was found in 96.8% of maternal saliva samples at baseline and in 100% of samples at both follow-up assessments. Maternal mean log10 CFU values increased slightly from 4.67 ± 1.23 at postpartum to 4.88 ± 0.99 at 15 months. No infants carried S. mutans at birth. Only 2 infants (15.4%) tested positive at 9 months (mean 1.84 ± 0.34, log10) and again at 15 months (mean 3.47 ± 1.03, log10) **(Table 1)**. Infant colonization patterns were not related to sex, age, or breastfeeding history.

HOMINGS and 16S rDNA sequencing indicated that the 20 most abundant genera or species accounted for roughly 86.0% of sequences in newborn samples and 88.6% in maternal samples. As summarized in **Table 2**, Streptococcus was the dominant genus in infants, comprising more than 72.5% of all identified taxa. At the species level **(Table 3)**, S. mutans represented 0.0088% of newborn salivary reads and appeared alongside S. sanguinis and other oral Streptococcus species.

Table 1. Colonization of S. mutans in mother–infant dyads.

Time Point	Postpartum Visit	9-Month Well-Child Visit	15-Month Well-Child Visit
Number of mother–infant pairs	N = 32	N = 13	N = 13
	Positive (%)	Mean ± SD	Positive (%)
Infant	0 (0%)	0	2 (15.4%)
Biological Mother	31 (96.8%)	4.67 ± 1.23	13 (100%)

Mean values reflect CFU counts from MSB medium converted to log10 scale.

Table 2. Comparison of the percentage of top 20 16S rDNA genus probes present in the saliva of the mother–infant dyads.

Probe ID	Genus / Species	Relative Abundance in Infants (%)	Relative Abundance in Mothers (%)
GP-081	Streptococcus (Genus probe 4)	71.77%	39.58%
RO-03	Rothia mucilaginosa	10.08%	24.26%
PR-14	Prevotella melaninogenica	0.30%	3.62%
GP-110	Granulicatella (Genus probe)	0.38%	3.00%
ST-20	Streptococcus sanguinis	0.11%	1.82%
GE-04	Gemella sanguinis	0.02%	1.64%
GP-126	Streptococcus (Genus probe 1)	0.14%	1.61%
GE-02	Gemella haemolysans	4.43%	1.32%
PR-09	Prevotella histicola	0.06%	1.19%
GP-060	Neisseria (Genus probe 2)	0.07%	1.17%
GP-004	Actinomyces (Genus probe 4)	0.12%	1.15%
GP-073	Rothia (Genus probe)	0.26%	0.99%
HA-05	Haemophilus parainfluenzae	0.19%	0.91%
GP-089	Veillonella (Genus probe 2)	0.44%	0.63%
PO-09	Porphyromonas sp. oral taxon 279	0.14%	0.60%
RO-02	Rothia dentocariosa	0.01%	0.59%
OR-01	Oribacterium sinus	0.01%	0.55%
SO-01	Solobacterium moorei	0.04%	0.47%
FU-10	Fusobacterium periodonticum	0.07%	0.47%
GP-063	Parvimonas (Genus probe)	0.01%	0.43%

Table 3. Comparison of the percentage of top genus and species of Streptococcus 16S rDNA probes present in the saliva of the mother–infant dyads."

Probe ID	Target Taxon / Probe Description	Relative Abundance in Infants (%)	Relative Abundance in Mothers (%)
GP-081	Streptococcus (Genus probe 4)	71.7651%	39.5773%
ST-20	Streptococcus sanguinis	0.1148%	1.8175%
GP-126	Streptococcus (Genus probe 1)	0.1411%	1.6107%
ST-22	Streptococcus sp. oral taxon 064	0.1317%	0.0785%
ST-26	Streptococcus sp. oral taxon 431	0.1049%	0.0535%

ST-16	Streptococcus parasanguinis II	0.0789%	0.0448%
ST-23	Streptococcus sp. oral taxon 066	0.0707%	0.0385%
GP-128	Streptococcus (Genus probe 3)	0.0411%	0.0269%
ST-27	Streptococcus sp. oral taxon 486	0.0256%	0.0152%
ST-15	Streptococcus mutans	0.0088%	0.0782%
ST-24	Streptococcus sp. oral taxon 068	0.0064%	0.0063%
GP-127	Streptococcus (Genus probe 2)	0.0039%	0.0024%
ST-09	Streptococcus anginosus	0.0036%	0.0986%
ST-10	Streptococcus constellatus	0.0032%	0.1042%
ST-11	Streptococcus cristatus	0.0031%	0.0016%
ST-14	Streptococcus intermedius	0.0023%	0.0634%
ST-21	Streptococcus sobrinus	0.0007%	0.0671%
ST-12	Streptococcus downei	0.0000%	0.0004%
ST-28	Streptococcus sp. oral taxon 487	0.0000%	0.0004%

Comparison of microbiome diversity between mothers and infants

Sequencing of 116 purified bacterial DNA samples on the MiSeq platform generated a total of 6,870,571 reads, with individual fragments averaging 460 bp (spanning 330–591 bp). Samples contained a mean of 65,270 reads (range 120–151,900; median 47,330). Four samples yielding <1,500 bp were removed before analysis. Out of 767 probes, 600 (78.2%) were positive: 484 (63.5%) in infants and 561 (73.1%) in mothers. Of all assigned sequences, 3,819,592 (55.6%) aligned uniquely to genus-level probes, 2,088,913 (30.4%) matched only one species probe, while 960,108 (14.0%) could not be assigned.

Marked distinctions in community structure and taxonomic distribution were observed between maternal and infant saliva (Wilcoxon; $p < 0.001$). Maternal samples consistently exhibited greater microbial richness at postpartum, 9 months, and 15 months, as indicated by the Shannon index (**Figure 1a**; Kruskal–Wallis, $p < 0.001$). Infant alpha diversity rose steadily at the 9- and 15-month visits compared with the postpartum period, whereas maternal diversity showed minimal temporal change. Beta-diversity analyses (Bray–Curtis distances), visualized via PCoA, demonstrated clear separation between the two groups at every visit **(Figure 1b)**. Maternal samples clustered more tightly than infant samples, reflecting lower within-group variation.

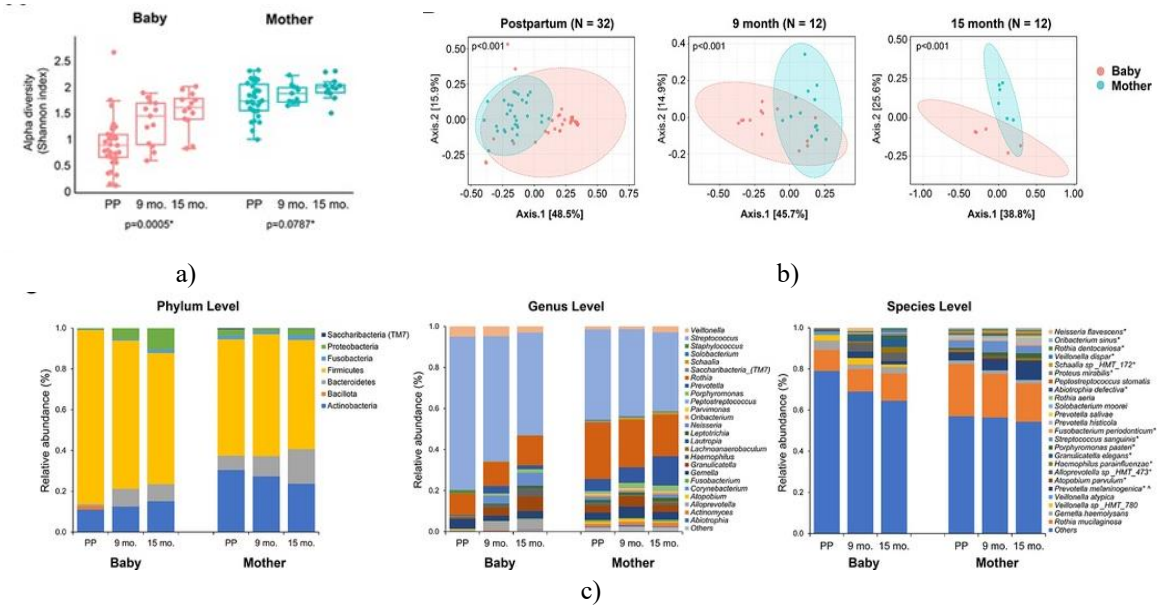

Figure 1. (a) Differences in Shannon diversity across the three infant time points are statistically significant (Kruskal–Wallis). (b) Bray–Curtis comparisons show persistent separation between mothers and infants at all visits ($p < 0.001$, Wilcoxon–Mann–Whitney). (c) Relative percent composition of phyla, genera, and species, displaying the 25 most abundant taxa. Symbols (*) and (^) denote taxa with significant temporal shifts within infants and mothers, respectively (adjusted $p < 0.05$).

Comparison of core oral microbiome between mothers and infants

Figure 1c presents relative abundance distributions across taxonomic levels. Infants showed a predominance of Firmicutes ($p < 0.05$) and substantially reduced relative levels of Actinobacteria, Bacteroidetes, Fusobacteria, and Saccharibacteria compared with mothers ($p < 0.001$). Among the 25 dominant taxa, infants demonstrated significant temporal variation in Neisseria flavescens, Oribacterium sinus, Rothia dentocariosa, Veillonella dispar, Schaalia sp._HMT_172, Proteus mirabilis, Abiotrophia defectiva, Rothia aeria, Fusobacterium periodonticum, Streptococcus sanguinis, Porphyromonas pasteri, Granulicatella elegans, Haemophilus parainfluenzae, Alloprevotella sp._HMT_473, Atopobium parvulum, and Prevotella melaninogenica. Only Prevotella melaninogenica changed significantly over time in the maternal cohort. Core microbial genera were defined by ≥20% prevalence and ≥0.01% relative abundance for infants **(Figure 2a)** and mothers **(Figure 2b)** at postpartum (a-1, b-1), 9 months (a-2, b-2), and 15 months (a-3, b-3). Maternal core taxa were largely constant throughout the 15-month observation period, whereas infants showed substantial expansion of their core community with age. Mothers retained a stable set of genera—including Streptococcus, Rothia, Prevotella, Gemella, Veillonella, Neisseria, and Actinomyces—at all visits. Infant saliva initially contained relatively few core taxa but became enriched with additional genera such as Prevotella, Neisseria, Alloprevotella, and Haemophilus beginning at the 9-month visit. By 15 months, the divergence in community diversity between infants and mothers had narrowed considerably.

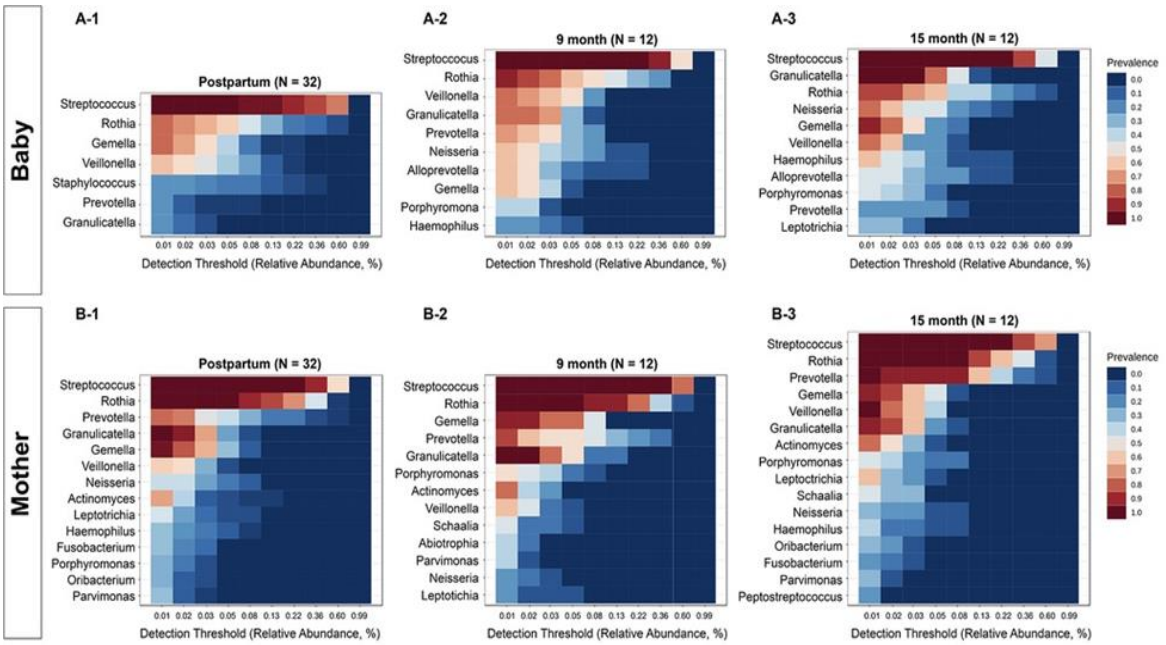

Figure 2. Core microbiome profiles for infants (a) and mothers (b), defined by 20% prevalence and 0.01% abundance thresholds. Maternal profiles remained steady, while infant core taxa broadened over time, with Neisseria, Alloprevotella, and Haemophilus becoming more prominent after 9 months.

Distinguishing taxa between mothers and infants

Genera that differed significantly between mothers and their infants were identified using the linear discriminant analysis effect size approach (LEfSe, FDR 0.05, LDA 2.0) and compared at the postpartum visit **(Figure 3a)**, 9-month visit **(Figure 3b)**, and 15-month visit **(Figure 3c)**. Overall, mothers exhibited a greater number of genera with elevated relative abundance. In early infancy, Streptococcus and Staphylococcus appeared at higher levels in infants than in mothers, though this pattern was no longer evident by 15 months. Conversely, Corynebacterium was initially more abundant in infants immediately after birth but showed higher levels in mothers at 9 months; this difference also disappeared by 15 months **(Figure 3c)**.

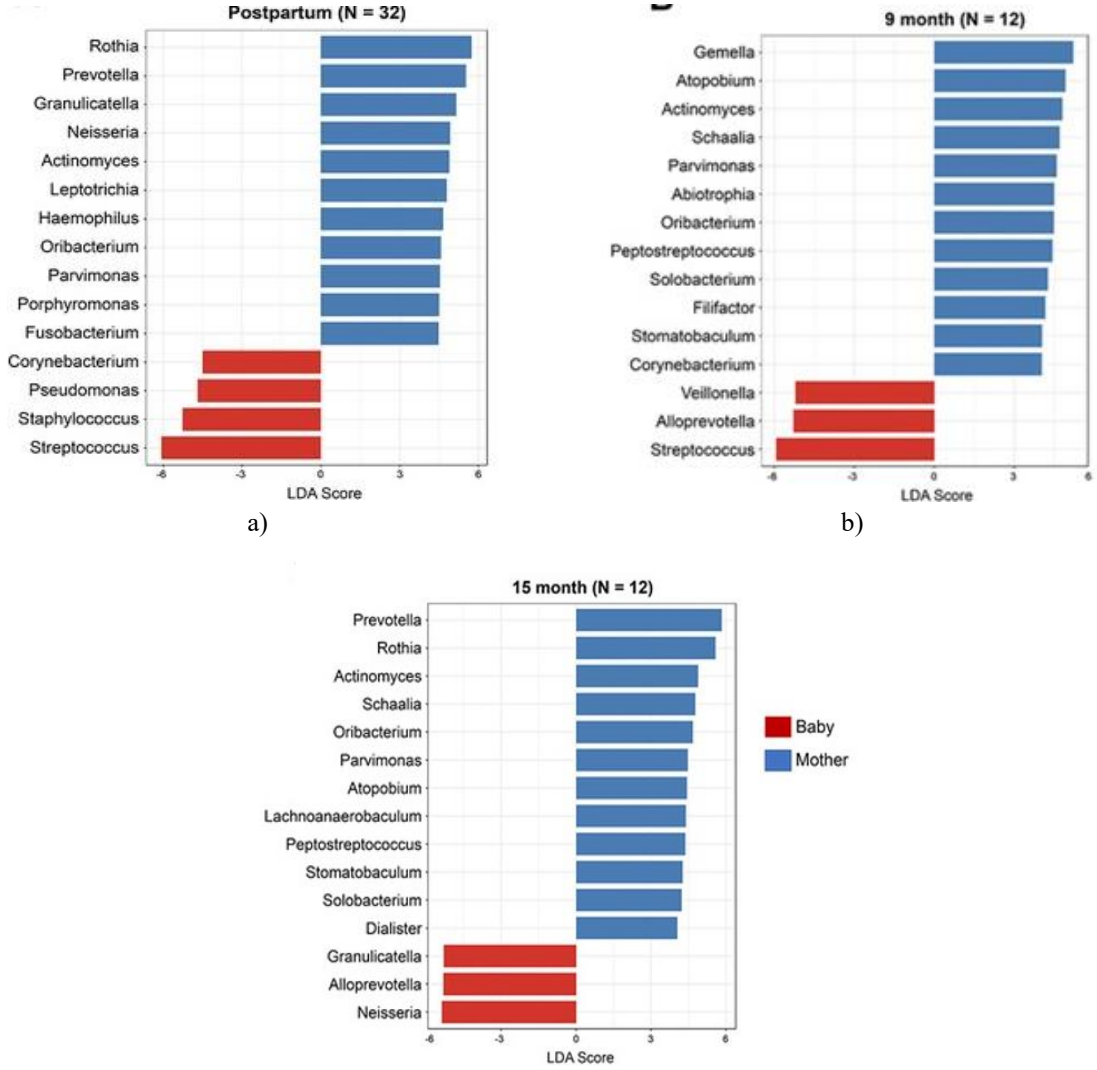

Figure 3. Differentially represented oral genera in mothers and infants during early development. Significant genera were identified by LEfSe (FDR 0.05, LDA 2.0) with Kruskal–Wallis testing across the postpartum (a), 9-month (b), and 15-month (c) assessments. Mothers consistently carried more genera with higher abundance. Differences in Corynebacterium and Staphylococcus diminished steadily over time.

Correlation analysis of oral microbial maturation in infants

Longitudinal comparison of microbial diversity showed that infants experienced a marked rise in salivary microbiome diversity after birth (Kruskal–Wallis, $p < 0.001$). SparCC modeling revealed correlation networks among salivary genera **(Figure 4)**, stratified by infant feeding mode. A correlation cutoff of 0.5 and $p < 0.05$ was applied. The structure of these microbial networks shifted substantially during early development. At the postpartum time point, Prevotella was strongly linked with Leptotrichia, Actinomyces, and Granulicatella **(Figure 4a)**. By 9 months, the network expanded to include Lautropia, Schaalia, Neisseria, Abiotrophia, Porphyromonas, and Saccharibacteria (TM7) **(Figure 4b)**. At 15 months, Lactobacillus also became integrated into the network **(Figure 4c)**.

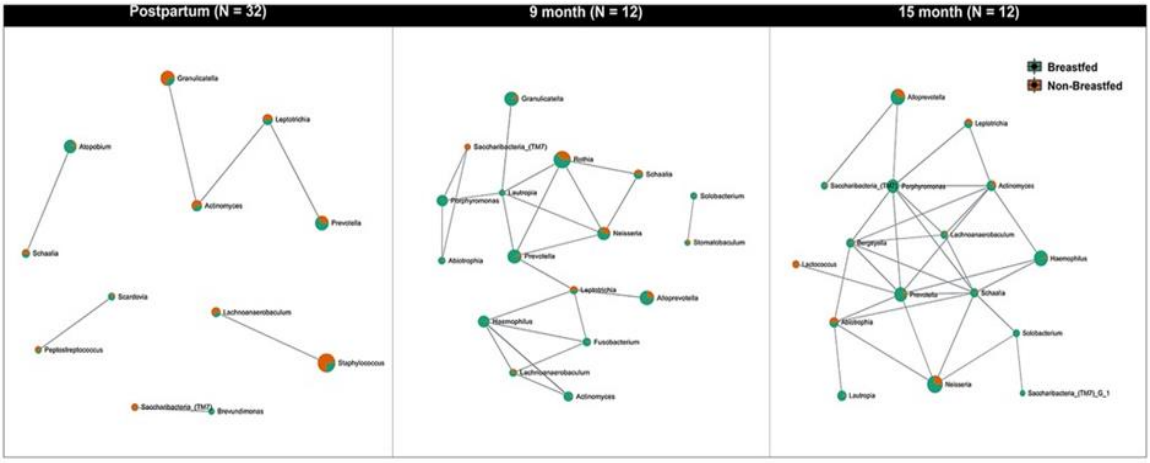

Figure 4. Network mapping of infant oral microbiome progression. Networks were constructed using SparCC correlations at the genus level, categorized by feeding type. Nodes represent genera and edges denote correlation coefficients. The results demonstrate that microbial relationships diversify and become increasingly interconnected as infants age. Threshold: $r = 0.5$, $p < 0.05$. We further evaluated the impact of sex and feeding patterns on infant microbial diversity. Differences in alpha diversity (Shannon index) and beta diversity (Bray–Curtis) between breastfed and formula-fed infants at the postpartum time point were not statistically significant. Compositional distinctions also lacked significance.

Stability of the maternal oral microbiome

We assessed the temporal stability of the salivary microbiome in mothers who completed all visits (N = 8). The Shannon diversity index indicated lower diversity at postpartum than at 9 months ($p = 0.02$) and 15 months ($p = 0.001$). However, the Chao1 metric showed no significant shifts over the 15-month period ($p > 0.05$), suggesting an overall stable maternal microbiome. Bray–Curtis beta diversity similarly revealed no significant temporal changes. When infant microbiome stability was compared with their mothers', consistent differences between paired samples were observed at all three time points.

The inclusion of S. mutans assessment served three aims: (1) to determine the timing of initial S. mutans acquisition in a healthy infant cohort, (2) to compare culture-based detection with 16S rRNA sequencing for identifying S. mutans in infant saliva, and (3) to characterize inter- and intra-species interactions between S. mutans and other early colonizers. We characterized the oral microbiome of 32 healthy infants at birth, 9 months, and 15 months, with particular emphasis on determinants shaping early microbial establishment. S. mutans is a major pathogen implicated in dental caries [31, 32], and early colonization markedly increases disease susceptibility [32-34]. As anticipated, conventional plating detected S. mutans in 96.8–100% of mothers at all time points. Infants were negative at birth but positive in 15.4% of cases at both 9 and 15 months, consistent with earlier findings [34-36]. Some studies report higher colonization rates in children under 14 months [37, 38], while others indicate a window for initial colonization spanning 7–36 months as primary teeth erupt [39-41]. Given that only two infants were S. mutans-positive in our sample, mother–infant transmission patterns could not be conclusively evaluated.

Using the HOMINGS assay, S. mutans appeared in small quantities alongside S. sanguinis and other Streptococcus taxa, supporting its role as an early colonizer and potential anchor for a Streptococcus-rich polymicrobial community [42]. Core genera in newborn saliva included Streptococcus, Rothia, Gemella, Veillonella, Staphylococcus, Prevotella, and Granulicatella, aligning with previous reports [43]. These observations reinforce the ecological plaque hypothesis [44], emphasizing that early microbial diversification may shape community development and influence lifelong oral health.

We extended our analysis to compare infant oral microbial diversity and genus-level composition at postpartum, 9 months, and 15 months with those of their mothers. Overall, mothers consistently exhibited a richer and more varied salivary microbiome than their infants. Maternal community structure, diversity indices, and dominant taxa remained largely unchanged across the study period. In contrast, infants showed a pronounced age-related rise in alpha diversity. These observations align with findings from Ramadugu *et al*. who reported that infant salivary richness and diversity increase steadily with age, regardless of maternal oral health, educational background, delivery mode, or feeding practices [45]. Although clear beta-diversity differences were present

between mothers and infants at each sampling point, we also noted substantial overlap: 85.7% of infant core genera at postpartum, 80.0% at 9 months, and 90.1% at 15 months were also detectable in the mothers' core microbiome. By 9 and 15 months, infants exhibited more interconnected taxa and denser correlation networks, indicating that their salivary microbial ecosystems undergo continuous restructuring. These patterns suggest that newborn oral cavities begin with a distinct microbial signature, and as colonization expands, the divergence between maternal and infant communities gradually narrows. It is plausible that infant microbial composition may approach maternal-like profiles before the end of the second year of life. Ramadugu *et al*. similarly found that infant salivary communities become increasingly adult-like with age [45], although their study could not identify the specific bacterial contributors. Conversely, Ferrett *et al*. provided evidence for maternal–infant microbial transmission using genomic tracing, strain-level metagenomics, and longitudinal analyses [43].

The biological processes responsible for initial microbial acquisition and the gradual establishment of the infant oral community remain largely unresolved. A prevailing assumption is that bacterial diversity rises with age—a trend supported here. During the first month, Streptococcus accounted for 74.8% of the infant salivary microbiome, decreasing to 60.9% at 9 months and 50.0% at 15 months. Rothia, the second most abundant genus, increased from 10.5% postpartum to 11.7% and 14.5% at the later visits. Neisseria expanded dramatically—by 15.8-fold at 9 months and 77-fold at 15 months. Additional genera, including Alloprevotella, Granulicatella, Prevotella, and Haemophilus, also rose progressively during the 15-month window. These patterns mirror the trends documented by Ramadugu *et al*. [45]. Haemophilus comprises gram-negative bacteria, many associated with infections. Neisseria includes both pathogenic and commensal species commonly residing in the oral cavity [46]. Xu *et al*. observed that Rothia levels were significantly reduced in caries-affected preschool children, implying a possible protective association [47]. Uranga *et al*. reported enhanced interactions among Rothia, Streptococcus, and Staphylococcus during responses to Rothia-derived enterobactin biosynthesis [48]. Increases in Rothia, Alloprevotella, and Haemophilus have been linked to oral disease and cancer risk [49, 50]. Although these genera readily colonize mucosal surfaces, including the mouth, our results demonstrate that they are detectable early in infant saliva. The biological significance of their initial acquisition and early composition patterns remains an open question.

Linear discriminant analysis effect size (LEfSe) is designed to evaluate group differences in microbial abundance [30]. It quantifies the strength of associations and identifies discriminatory taxa. In our cohort, LEfSe revealed at least 15 genera with significant mother–infant differences based on Kruskal–Wallis testing. Notably, Staphylococcus comprised 1.7% of the infant salivary microbiome postpartum but disappeared from the infant core microbiome by 9 months. Divergences between mother–infant pairs were smaller by 15 months. Reports on Staphylococcus colonization in infant saliva are scarce. As a key constituent of the skin microbiome, various species are frequently found in infant saliva, skin, blood, and stool samples [51-53]. Environmental exposure and breastfeeding practices are thought to be primary contributors to early colonization. Another notable finding was the declining significance of Corynebacterium differences between mothers and infants from postpartum to 15 months; it was also absent from the infant core microbiome at 9 months. How Staphylococcus and Corynebacterium contribute to early oral ecological dynamics and how they influence long-term health is still uncertain. The present study adds evidence that may help shape future hypotheses and research directions.

Breast milk serves as a major reservoir of bacteria that can be transferred to an infant's mouth. The relationship between breastfeeding and the promotion or suppression of S. mutans in infant saliva has remained debated for many years [38, 54-56]. Much of the existing literature has concentrated on links between breastfeeding practices and the risk of early childhood caries, yet far fewer studies have examined how breastfeeding shapes microbial diversity in the infant oral cavity. Holgerson *et al*. reported in a 2013 cross-sectional study that 3-month-old infants who were breastfed showed distinctly different oral microbial profiles compared with formula-fed infants [57]. Another recent investigation found that breastfed infants exhibited lower salivary microbial diversity at two months, but this difference was no longer present at 12 months [45].

In our cohort of 32 healthy infants, only five were exclusively breastfed through 15 months. We detected compositional and diversity differences between the feeding groups, with breastfed infants showing increased levels of Streptococcus and Veillonella, whereas formula-fed infants demonstrated higher levels of Rothia. However, the differences were not

statistically significant, likely due to the small sample size. These observations should therefore be considered preliminary. As accumulating research highlights the dynamic interplay between the oral environment and microbial colonization, larger, well-powered studies are needed to clarify how feeding patterns influence early microbial acquisition and maturation.

Conclusion

The oral microbiome plays a critical role in maintaining human health [58]. Early microbial establishment contributes substantially to immune development and general well-being in infants. In this study, we utilized the MiSeq sequencing platform, the HOMINGS assay, and bioinformatic analyses to characterize the oral microbiota of 32 healthy newborns from the postpartum period through 15 months—a key window for microbial community formation. Our results indicate that:

(1) Streptococcus is the predominant genus in infant saliva, with S. mutans appearing at low levels.
(2) Noticeable differences in microbial composition persist between mothers and their infants throughout the first 15 months.
(3) Infants show increasing microbial diversity and shifts in core taxa over time, whereas maternal microbiomes remain comparatively stable.
(4) No significant differences in microbial diversity were observed based on infant sex or feeding method.

Together, these findings contribute to a clearer understanding of how a health-associated oral microbiome is seeded and develops during infancy.
This work had limitations, particularly the modest number of mother–infant pairs. While 32 dyads were enrolled at postpartum, only 13 completed all follow-up visits. A substantial proportion of sequencing reads could not be identified at the species level, restricting deeper analysis of microbial interactions. As a result, the generalizability of our findings is limited. Moreover, detailed information on maternal oral health—such as caries history or periodontal status—was unavailable. Future studies would benefit from larger cohorts and more comprehensive maternal data to better elucidate the processes governing oral microbiome establishment in infancy and its potential long-term implications for health.

Acknowledgments: None

Conflict of Interest: None

Financial Support: This study was supported by the National Institute of Dental and Craniofacial Research Grants R01 DE019455, R01 DE013937, R01 DE031025, and NSF-CCF-1934962. Data collection was supported by NIDCR Grant 1R34DE022269-01.

Ethics Statement: The study involving human participants was reviewed and approved by the Institutional Review Boards of the New York University School of Medicine, New York University College of Dentistry (Research Proposal Oversight Committee), and the New York City Health and Hospital Corporation (for the Bellevue Hospital Center) for human subjects participating in research activities. All parents provided informed consent and permitted their children to participate in the study. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

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