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Exploring the Maternal and Infant Oral Microbiomes

A Pilot Study

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ABSTRACT

Setting the stage for good oral health early in life is critical to long-term oral and overall health. This exploratory study aimed to characterize and compare maternal and newborn oral microbiota among mother-infant pairs. Oral samples were collected from 34 pregnant African American women and their infants at 1 to 3 months of age. Extracted 16Sr-RNA genes were matched to the Human Oral Microbiome Database. Alpha and beta diversity differed significantly between overall maternal and infant microbiomes. Maternal or infant alpha diversity, however, was not differentiated by maternal gingival status. Several demographic and behavioral variables were associated with, but not predictive of, maternal oral microbiome alpha diversity. There was no association, however, among birth mode, feeding mode, and the infant oral microbiome. *Megasphaera micronuciformis* was the only periodontal pathogen detected among the infants. Notably, maternal gingival status was not associated with the presence/absence of most periodontal pathogens. This study provides an initial description of the maternal and infant oral microbiomes, laying the groundwork for future studies. The perinatal period presents an important opportunity where perinatal nurses and providers can provide oral assessment, education, and referral to quality dental care.

Key Words: infant care, microbiota, oral health, perinatal care

Oral health is critical to overall well-being.[1] Not only does good oral health improve the ability to speak, chew, and swallow but it also affects an individual's self-confidence, self-esteem, and ability to communicate with others.[1] Furthermore, there is increasing evidence of an oral-systemic connection, with studies demonstrating associations between poor oral health and a myriad of extraoral conditions including adverse pregnancy outcomes,[2] cognitive decline,[3–5] rheumatoid arthritis,[6] and heart and lung diseases.[1] Despite the clear evidence pointing to the importance of good oral health, oral disease abounds in certain populations related to low oral health literacy,[7] multiple barriers to oral healthcare access,[8] and lack of integration between dental care and medical care.[9]

Although largely preventable, dental caries and periodontal disease are among the most common chronic diseases in the United States[10] and are particularly rampant among vulnerable populations.[11] Almost 40% of all children aged 2 to 8 years have experienced dental caries in their primary teeth.[12] That proportion increases to more than 67% among adolescents aged 16 to 19 years.[12] While many may consider cavities to be

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a "normal" part of childhood, consequences can be significant, including lost school hours and pain, leading to problems eating, speaking, and learning. For adults, the most common cause of tooth loss is periodontal disease.[10] Periodontal disease is a progressive chronic disease of the gingiva that begins as a direct immune response to microorganisms that inhabit the subgingival space. A survey of the US population suggests that 47% of American adults have a more progressive form of periodontal disease called periodontitis.[13] Gingivitis is an even more common, milder form of the disease that presents as red, swollen, and inflamed gums. Estimates of overall prevalence of gingivitis are challenging due to lack of comprehensive data but are thought to be extremely high.[14] Gingivitis is the most common oral disease in pregnancy and has a prevalence of 50% to 70%.[15]

The etiology of both dental caries and periodontal disease is polymicrobial, that is, caused by various combinations of microorganisms, and occur when there is a shift in the overall ecological balance of microbes in the oral cavity.[16] For caries, this imbalance is initiated when oral bacteria are exposed to and metabolize high concentrations of carbohydrate (sugars and refined starches), producing an acidic environment. This acidic environment stimulates a shift in the overall community toward a higher prevalence of acid-loving organisms, further perpetuating sustained acidity and leading to the demineralization and breakdown of the hard structures (enamel, dentin, and cementum) of the teeth.[16,17] Known acid-producing organisms include *Streptococcus mutans* and species belonging to the genera *Actinomyces, Lactobacillus, Bifidobacterium, Propinonibacterium, and Scardovia*.[16,18] Other taxa that are overrepresented in carious lesions compared with healthy tooth surfaces include *Selenomonas* spp,[19] *Veillonella parvula*, *Streptococcus cristatus,*[20] *Streptococcus sobrinus,* and *Lactobacillus acidophilus*.[21]

Reversible symptoms of gingivitis, the earliest stage of periodontal disease, appear in response to the undisturbed development of a biofilm (layer of microorganisms embedded in an extracellular matrix—more commonly known as "plaque") shifted toward gram negative and anaerobic taxa, such as species belonging to the genera *Fusobacterium* or *Treponema,* and members of the phylum Synergistetes.[14] Without treatment, gingivitis progresses to the irreversible stage called periodontitis, marked by the loss of periodontal attachment, further colonization of anaerobic bacteria, eventual recession of the gingiva, bone loss, tooth mobility, and ultimately tooth loss. Several species associated with periodontitis have been identified including *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia,*[22] *Anaeroglobus geminatus, Eubacterium saphenum, Filifactor alocis, Porphyromonas endodontalis,* and unnamed taxa belonging to the phylum Bacteroidetes and the genus *Fretibacterium*.[23]

Oral diseases are progressive, cumulative, and grow in complexity over time, which is why it is vital for good oral health to be established early in life. The origin of the neonatal oral microbiome remains poorly understood. However, there is some evidence that it appears to be influenced by exposures such as birth mode (vaginal vs cesarean delivery),[24,25] feeding mode (breast milk vs formula),[17,26] and various horizontal transmission routes (siblings, and other friends and family)[26] typically leading to a rapid colonization of the infant oral microbiome by *Streptococcus* species and taxa from the phylum Fusobacteria.[21] The transmission of the maternal oral microbiome may be particularly salient in establishing a child's oral microbiome.[27] Because the establishment of the early oral microbiota creates a foundation for future oral health,[28] this exploratory study aims to characterize and compare the maternal and newborn oral microbiota among mother-infant pairs.

METHODS

Design

Institutional review board approval was obtained. Thirty-four pregnant African American women were enrolled for a pilot study that had a primary aim of characterizing the subgingival microbiome of pregnant African American women and their infants between 1 and 3 months of age.[29]

Setting and sample

Participants were recruited from an ongoing larger investigation of the associations among a woman's oral, vaginal, and gut microbiota during pregnancy and preterm birth.[30] Inclusion criteria for the larger parent study included self-identification as African American, 18 to 40 years of age, ability to speak and read English, singleton gestation, no chronic medical problems, and no use of prescription medications. For the current study, 34 pregnant women were recruited from 2 prenatal clinics located in Atlanta, Georgia, between December 2016 and February 2017. Women agreed to allow inspection of their gingival tissue, collection of subgingival microbiome samples, and oral swabs of their infant at 1 to 3 months of age. To be included in the study, women had to have a minimum of 20 natural teeth and no professional dental cleaning in the past 3 months. Because of attrition, only 21 infants had oral swab samples collected. This article presents the data for these 21 infants and their mothers. Because of

the exploratory nature of this aim, sample calculations were not calculated.

Procedures

During the third trimester of pregnancy, each maternal participant's mouth was assessed for visual signs of gingival inflammation using the Modified Gingival Index (MGI)[31] by a single trained examiner. Scores ranged from "0" (absence of inflammation) to "4" (severe inflammation).[31] Participants with mean MGI scores less than or equal to "1" (mild inflammation—partial unit) were placed in the healthy group; those with scores greater than 1 were assigned to the gingivitis group.[31] Subgingival plaque samples were collected from participants in both groups using the sterile paper point method.[32] Supragingival plaque was first removed with sterile gauze, and each tooth site was held dry using cotton rolls while one sterile paper point was inserted into the pocket of a tooth for 20 seconds. This was repeated for 3 teeth. The 3 paper points were pooled and immediately placed in 750 mL of MoBio buffer contained in sterile MoBio bead tubes (MoBio Laboratories, Incorporate, Carlsbad, California). Saliva and plaque samples were placed on ice and transported for storage at −80°C until ready for analysis. More details on the collection method for the maternal subgingival samples can be found in the report of the parent pilot study.[29]

Maternal participants were then recontacted 1 to 3 months after giving birth at which time a home visit was conducted. Infant soft-tissue oral swabs were collected for oral microbiome analysis using a sterile HydraFlock swab (Puritan). Swabs were placed in a standard PowerBead tube (Qiagen) following the protocol of the Human Microbiome Project.[33]

DNA isolation and 16S ribosomal RNA gene library preparation and sequencing

Specimens were sent to Microbiome Insights (Vancouver, British Columbia, Canada) for extraction and sequencing. DNA was isolated using the MoBio PowerMag Soil DNA Isolation Kit. The highly conserved 16S ribosomal RNA (16SrRNA) gene, which is widely used to characterize taxonomic diversity in microbial communities, was polymerase chain reaction amplified with dual-barcoded primers targeting the V4 hypervariable region according to the protocol outlined by Kozich and colleagues.[34] Normalized library concentrations of 1 to 2 ng/mL (as per the specifications of the Sequal Prep normalization kit; Thermo Fisher Scientific, Waltham, Massachusetts) were used. Amplicons were sequenced with an Illumina MiSeq using the 250-base pair paired-end kit (version 2; Illumina, Inc, San Diego, California).

The Bioconductor workflow[35] was used to analyze the microbiome sequencing data. Forward reads were truncated at 225 and reverse reads at 160. Low-quality reads were determined on the basis of the quality scores incorporated into the FASTA files from the Illumina sequencer. The reads were subsequently de-replicated, and true sample sequences were inferred from error-prone raw reads using the Divisive Amplicon Denoising Algorithm (dada2).[36] After merging both strands and eliminating chimeras, a high-quality database was obtained and taxonomies were assigned using the Human Oral Microbiome Database.[37]

Additional maternal data collected included demographic variables (age, income, and education), oral health behavior variables, and mode of birth. Additional infant data included feeding method, gestational age at birth, and age at oral sample collection.

Analysis

Analysis included alpha diversity, a measure of species diversity within a particular ecosystem, and was performed using phyloseq, an open-source software package that imports, stores, analyzes, and graphically displays microbiome census data.[38] The simplest alpha diversity measure is richness, the number of taxa (or amplicon sequence variants [ASVs]) observed in the sample, which was calculated after rarefying samples to a sampling depth of 8287. The Shannon index is another commonly used alpha diversity metric that describes both richness and evenness.[39] Communities numerically dominated by 1 or a few species exhibit a low Shannon score, whereas communities in which abundance is distributed equally among species will exhibit high evenness. The significance of alpha diversity differences was tested using the Welch 2-sample t test. Associations among maternal and infant alpha diversity scores and maternal and infant variables were investigated using Pearson product-moment correlation coefficients. To explore beta diversity, Bray-Curtis indices were computed and then visualized on an ordination plot. Variation in community structure in relationship to maternal and infant variables was assessed with permutational multivariate analysis of variance (ANOVA) using 999 permutations for significance testing. Alpha and beta diversity was also stratified for maternal gingival status.

For both members of the dyad, ASVs were aggregated into each taxonomic rank (phylum, class, order, family, genus, and species). Additional filtering was performed as recommended by the Bioconductor workflow,[35] including the removal of unnamed phyla (14 features removed) and the removal of the Gracilibacteria (GN02) phylum, which was observed only

once. Agglomeration, or grouping of like taxa, was performed at each taxonomic level before plotting, according to relative abundance.

Frequencies of the presence of cariogenic and periodontal pathogens as defined by dental literature, as well as commensals, were listed for both groups and for matching maternal-infant pairs and stratified by maternal gingival status. Chi-square for independence tested the association between maternal gingival status and presence/absence of particular taxa. Wilcoxon's rank-sum test was used to test for differences in the mean relative abundance of specific species within each group according to maternal gingival status.

RESULTS

The mean age of maternal participants was 26.19 ± 5.65 years. Other sociodemographic, oral health, birth mode, and feeding mode characteristics of the participants can be found in Table 1.

The 6 most abundant phyla in the maternal oral microbiome were Firmicutes, Actinobacteria, Bacteroidetes, Fusobacteria, Proteobacteria, and Spirochaetes. The oral microbiome of the infants was primarily dominated by taxa belonging to the phylum Firmicutes. At the level of the family, the most abundant taxa in the maternal oral microbiome are fairly well-distributed across several families including Streptococcaceae, Prevotellaceae, Actinomycetaceae, Veillonellaceae, and Fusobacteriaceae. In the infant samples, however, taxa are dominated by Streptococcaceae. These patterns remained consistent stratified for maternal gingival status in terms of phylum and family, that is, the distribution within the healthy/gingivitis maternal infant groups remained similar to the overall maternal/infant sample.

Significant differences in measures of alpha diversity were identified between maternal and infant oral microbiomes in terms of both richness and evenness as noted in Figure 1.

Specifically, the maternal oral microbiome had a higher number of observed features ($p < 2 \times 10^{-8}$) and a significantly higher Shannon index ($p < 2 \times 10^{-11}$) than the infant oral microbiome. Within the maternal and infant groups, there was no alpha diversity difference when looking across maternal gingival status. Alpha diversity in maternal samples was associated with several maternal factors. Increased age ($r = -0.522$, $p < .05$), education ($r = -0.562$, $p < .001$), and income ($r = -0.536$, $p < .05$) were all associated with decreased richness. Increased age ($r = -0.483$, $p < .05$), education ($r = -0.501$, $p < .05$), and income ($r = -0.507$, $p < .05$) were similarly associated with lower Shannon diversity scores. In addition, having seen a dentist in the last 3 months was associated with a lower maternal Shannon score ($r = -0.478$, $p < .05$). There was no association between infant oral microbiome alpha diversity scores and delivery mode or feeding mode. Infant Shannon diversity, however, was inversely associated with maternal flossing ($r = -0.566$, $p < .05$).

Table 1. **Descriptive statistics (*N* = 42)**

Characteristic	Frequency, *n* (%)
Mothers (n = 21)[a]	
Education	
Less than high school	11 (52.4)
High school or higher	9 (42.9)
Missing	1 (4.8)
Income	
<100% federal poverty level	11 (52.4)
≥100% federal poverty level	9 (42.9)
Missing	1 (4.8)
History of mouth/gum infection	
Yes	5 (23.8)
No	14 (66.7)
Missing	2 (9.5)
Brushed teeth in the last 2 d	
Yes	16 (76.2)
No	1 (4.8)
Missing	4 (19.0)
Flossed in the last month	
Yes	7 (33.3)
No	12 (57.0)
Missing	2 (9.5)
Visited the dentist in the last month	
Yes	1 (4.8)
No	18 (85.7)
Missing	2 (9.5)
Smoked cigarettes in the last month	
Yes	1 (4.8)
No	18 (85.7)
Missing	2 (9.5)
Gingivitis assessed with MGI[b]	
Yes	6 (28.6)
No	15 (71.4)
Infants (n = 21)[c]	
Sex	
Male	8 (38.1)
Female	13 (61.9)
Mode of birth	
Vaginal	20 (95.2)
Cesarean delivery	1 (4.8)
Feeding mode	
Breast	3 (14.3)
Bottle	8 (38.1)
Breast and bottle	6 (28.6)
Missing	4 (19.0)

Abbreviation: MGI, Modified Gingival Index.
[a]Maternal samples collected during the third trimester.
[b]MGI > 1 = gingivitis.
[c]Infant samples collected between 4 and 12 weeks.

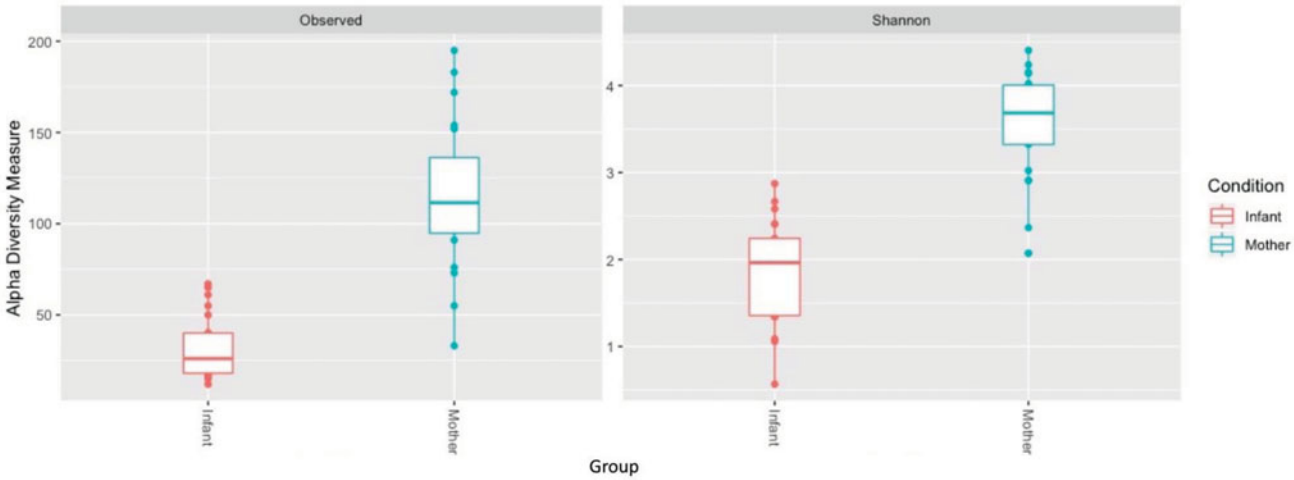

Figure 1. For the overall sample of mothers and infants, the maternal oral microbiome displays higher alpha diversity (richness and Shannon indices). This figure is available in color online (www.jpnnjournal.com).

A visualization of beta diversity with an ordination plot demonstrated that the maternal and infant oral samples also clustered separately. Permutational multivariate ANOVA of Bray-Curtis distances confirmed the dissimilarity of the 2 groups (Pr [>*F*] < .001; see Figure 2).

The presence of known caries-associated organisms, periodontal pathogens, and commensals in the mother and the infant is listed in Table 2.

All were identified as present to some degree in maternal samples. Cariogenic or periodontal pathogens were largely absent from the infant oral cavity. *Megasphaera micronuciformis* was the only periodontal pathogen that was detected among the infant samples. Although the presence of this organism was detected more frequently among infants with mothers who had visual signs of gingivitis (66.7%) than mothers with

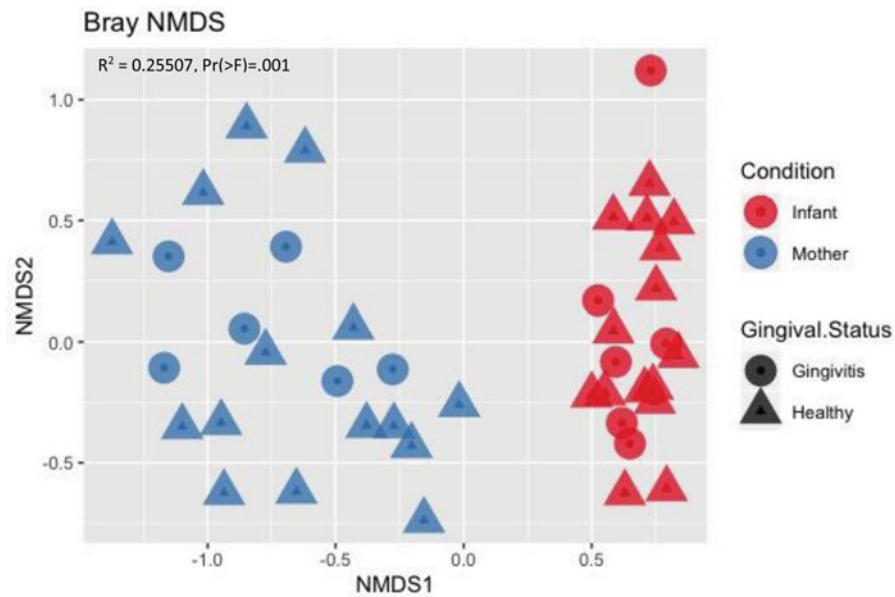

Figure 2. This ordination plot reflects a clear separation between maternal and infant samples. Each point represents 1 microbiome sample. Red reflects infant samples, and the blue represents infant samples. Shapes differentiate maternal gingival status; gingival status does not appear to cluster separately. Points that cluster together reflect similarity. The separation between the maternal and infant groups was confirmed statistically with the R^2 value, which showed that the percentage of variation between groups was significant. This figure is available in color online (www.jpnnjournal.com).

Table 2. **Percentage of mothers, infants, and mother-infant pairs harboring various organisms according to maternal gingivitis status**

		Mother ($n = 21$)		Infant ($n = 21$)		Mother-infant pair[a] ($n = 21$)	
Identified oral species	**Phylum**	**Healthy ($n = 15$)**	**Gingivitis ($n = 6$)**	**Healthy ($n = 15$)**	**Gingivitis ($n = 6$)**	**Healthy ($n = 15$)**	**Gingivitis ($n = 6$)**
Caries-associated organisms							
Streptococcus mutans	Firmicutes	53.3%	16.7%	0[b]	0[b]	0[b]	0[b]
Veillonella parvula	Firmicutes	100%[b]	100%[b]	26.7%	0	30.8%	0
Scardovia wiggsiae	Actinobacteria	13.3%	16.7%	6.7%	0	0[b]	0[b]
Periodontal pathogens							
Fusobacterium nucleatum (sp vincentii)	Fusobacteria	73.3%	100%	0[b]	0[b]	0[b]	0[b]
Fusobacterium nucleatum (sp animalis)	Fusobacteria	26.7%	66.7%	0[b]	0[b]	0[b]	0[b]
Porphyromonas gingivalis	Bacteroidetes	26.7%	16.7%	0[b]	0[b]	0[b]	0[b]
Tannerella forsythia	Bacteroidetes	46.7%	33.3%	0[b]	0[b]	0[b]	0[b]
Prevotella intermedia	Bacteroidetes	20.0%	66.7%	0[b]	0[b]	0[b]	0[b]
Parvimonas micra[c]	Firmicutes	73.3%	100%	0[b]	0[b]	0[b]	0[b]
Treponema denticola	Spirochaetes	40.0%	50.0%	0[b]	0[b]	0[b]	0[b]
Prevotella nicrescens	Bacteroidetes	73.3%	100.0%	0[b]	0[b]	0[b]	0[b]
Megasphaera micronuciformis	Firmicutes	20.0%	16.7%	20%	66.7%	0	12.5%
Anaeroglobus geminatus	Firmicutes	13.3%	50.0%	0[b]	0[b]	0[b]	0[b]
Filifactor alocis	Firmicutes	40.0%	50.0%	0[b]	0[b]	0[b]	0[b]
Porphyromonas endodontalis	Bacteroidetes	53.3%	100.0	0[b]	0[b]	0[b]	0[b]
Commensals							
Streptococcus salivarius	Firmicutes	80.0%	66.7%	86.7%	100%	84.6%	50.0%
Lactobacillus gasseri	Firmicutes	6.7%	0	26.7%	50.0%	0[b]	0[b]
Escherichia coli	Proteobacteria	26.7%	16.7%	20.0%	16.7%	15.4%	12.5%

[a]Organism present in both mother and infant pair.
[b]No statistics computed because of constant value in one category.
[c]Higher mean relative abundance among mothers with gingivitis than among those with healthy gums ($P < .05$).

healthy gums (20%), the difference in the frequency of detection was not significant. There was no association between maternal gingival status and the presence of pathogens or commensals. Commensals were present in the infant oral microbiome. *Streptococcus salivarius,* an organism known as an early colonizer of the oral cavity, was found in the majority of infant samples. *Lactobacillus gasseri* and *Escherichia coli* represent examples of next-stage colonizers.[17] There was no association, however, between maternal gingival status and the presence of commensals in the infant oral cavity.

Differences in mean relative abundance for each of these species were tested. Only *Parvimonas micra* was found to be more abundant in mothers who had symptoms of gingivitis than among those who had healthy gums ($P < .05$).

DISCUSSION

The World Health Organization defines "health" as encompassing physical, mental, and social well-being.[40] This holistic definition demands the inclusion of oral health, since the mouth is inextricably linked to the rest of the body. The association of the maternal and infant oral microbiomes may inform oral health promotion efforts, allowing health providers, families, and individuals to prioritize the promotion of good oral health from the beginning of life.

The top phyla represented in the maternal microbiome were Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria, and Spriochaetes. This is consistent with previous findings of nonpregnant populations where researchers found that 96% of the bacteria in the oral cavity belong to these 6 phyla.[41] Of the 6, Firmicutes was most highly represented within the maternal oral microbiome. The infant microbiome also largely comprised taxa from the Firmicutes, with a lesser abundance of taxa from Actinobacteria, Bacteroidetes, and Proteobacteria. In their examination of the infant oral microbiome, Tuominen and colleagues[28] similarly found that Firmicutes was the most predominant phylum in infant oral

microbiome samples, followed by Proteobacteria or Bacteroidetes.

By far, the most abundant family of organisms among this study's infant participants was Streptococcaceae. On the other hand, the neonates in the Tuominen and colleagues[28] study had a more even distribution of taxa at the family level, with only a few samples exhibiting a dominant Streptococcaceae or Lactobacillaceae profile. The difference in sampling time frame may explain this difference since the previous researchers sampled the oral microbiome for their study immediately after birth and prior to any feeding whereas study samples from this study were taken at 1 to 3 months of age, providing ample time for the bloom of taxa in the Streptococcaceae family that is common in early infanthood and associated with the oligosaccharide stimuli that comes from breast milk or formula feeding.[17]

The newborn oral microbiome is largely undifferentiated at birth[24] but becomes significantly more diverse over the first months and years of life.[42] Results from this study confirmed these findings: The infant microbiome was significantly less diverse than the maternal oral microbiome, reflecting an early stage of bacterial colonization and succession, likely related to the lack of teeth in the infant's mouth, and the lack of variety in food intake. Microbial community diversity can be described in 2 ways. Alpha diversity describes how many organisms are present in a community and how evenly these organisms are distributed. Richness (observed taxa) and evenness (Shannon index) are common ways to describe diversity within a bacterial community, as previously described. The maternal oral microbiome had a higher number of observed taxa that were more evenly distributed than the infant microbiome. For the oral microbiome, a higher alpha diversity, particularly richness, is thought to increase the risk of disease.[43] This is related to the process of biofilm formation that provides an environment for a successively more diverse and abundant bacterial community.[43] Looking within groups and across maternal gingival status, there was no difference in diversity between women who had signs of gingivitis and women who did not. Similarly, there was no difference in diversity between infants of mothers with and without symptoms of gingivitis. Factors associated with poor oral health include increased age, lower socioeconomic status, and certain behaviors such as smoking. Because of this, a positive association between alpha diversity and these factors was expected. Indeed, results confirmed that having a lower income and education level are associated with increased alpha diversity of the maternal oral microbiome. Increased education was also associated with decreased observed abundance in the infant microbiome.

Although increasing age is associated with declining oral health, results of this study suggest that maternal age is associated with decreased alpha diversity, in terms of both richness and evenness. Although the literature on the association between aging and the oral microbiome is inconclusive, studies suggest that the dominant species that constitute the adult oral microbiome do not change[44] but changes in the microbial ecosystem can occur related to age-associated deterioration in mucosal immunity and/or general health.[45]

Oral hygiene behaviors also had an association with maternal and infant alpha diversity. Toothbrushing in the past 2 days was associated with lower maternal observed abundance, which is consistent with the notion that regular toothbrushing will mitigate plaque buildup. Maternal flossing in the past month was also associated with decreased alpha diversity among the infant oral microbiome, suggesting that maternal hygiene behaviors may affect the composition of the infant oral microbiome. Having seen a dentist in the last month was also associated with a lower maternal Shannon score, suggesting that whatever procedure was performed during the visit reduced the evenness of the maternal microbiome.

Beta diversity describes the difference between microbial communities from 2 different environments. The plot of the quantitative nonphylogenetic Bray-Curtis metric clearly demonstrates the dissimilarity between the microbiota of the members of the maternal-infant dyad.

Although research suggests that the acquisition of the infant oral microbiome is influenced by maternal factors including mode of birth,[26] maternal gut,[46] skin,[47] and breast milk,[48] this study found no association between birth mode, feeding mode, and the infant oral microbiome. This inconsistency, however, may be explained by the modest sample size, infant participants 1 to 3 months age (other studies sampled the infant oral microbiome swab shortly after birth),[28] and the fact that many of the infant participants were partially or fully formula-fed, thereby diluting the impact of breastfeeding on the oral microbiome.

The early years are critical for acquiring certain bacteria.[21] *Streptococcus salivarius* is one of these organisms. It was present in 85.7% of this study's infant samples, confirming previous findings that this organism is the predominant microorganism in the early oral cavity.[49] In vitro human and animal studies suggest that this organism that also lives in the gut may contribute to the establishment of immune homeostasis and regulation of host responses.[50] Other reported commensal early colonizers, which were not detected among this study's maternal or infant samples, are *Streptococcus mitis, Streptococcus sanguinis*, and *Streptococcus*

gordonii.[51] The dominance of these organisms is strongly associated with good oral health.[51] The rapid domination of *Streptococcus* spp is associated with oligosaccharide stimuli; the metabolic by-products of the oligosaccharides in breast milk or formula may contribute to an oral environment for other commensals to thrive.[16] Commensal species that increase in abundance as the infant matures include *E coli, Lactobacillus crispatus,* and *L gasseri.* Although *L crispatus* was not identified among this study's identified sequences, *E coli* and *L gasseri* were present among infant samples.

Streptococcus mutans has been isolated in the mouths of infants as young as 6 months[52]; however, few studies have identified this organism among younger infants. *Streptococcus mutans* was not identified among the infant samples in this study. A strong rationale for this is that *S mutans* colonizes and thrives on the dental surface, and infants in this study were all toothless (edentulous).

Overall, the detection of periodontal pathogens was limited among the infant samples in this study. *Fusobacterium nucleatum* is an opportunistic pathogen and is ubiquitous in the oral cavity of adults.[21] It may even be transmitted hematogeneously to the uterus, inducing preterm birth.[53] This organism is also present in the genitourinary tract and may confer perinatal translocation.[21] Although it was detected in all of the mothers who had visual signs of gingivitis and most of the mothers with healthy gums, it was not identified in any of the infant samples. In contrast to our findings, Merglova and Polenik[21] found that *F nucleatum* was present in edentulous infants. *Porphyromonas gingivalis* and some of the other known periopathogens were also not detected among the infant samples. These pathogens, however, are known to live and thrive in the subgingival pocket, which these young infants do not yet have.

Megasphaera micronuciformis was the only pathogen detected among the study's infant samples, regardless of maternal gingival status. This organism was first isolated in 2003 and appears to be widely distributed in the oral cavity.[54] It has been associated with oral tumor tissues[55] and with periodontal disease.[56] This organism was found in 12.5% of the mother-infant dyads, where the mother had signs of gingivitis, suggesting the possibility of horizontal maternal-child transmission. This suggestion is not unprecedented since periodontal pathogens cluster in families and horizontal transmission of organisms is known to occur between family members.[57] Adhikari and colleagues[58] also demonstrated a correspondence between maternal and newborn *P gingivalis* levels. Therefore, acquisition of pathogens via salivary contact may be possible and this has implications for management of maternal periodontal disease and perinatal teaching to mitigate behaviors that might transmit saliva.

Notably, maternal gingival status was not associated with the presence/absence of most periodontal pathogens. This is possibly attributable to the limited range of disease in this small cohort; gingivitis in pregnancy tends to be mild. *Parvimonas micra* was the only taxa that had a higher mean relative abundance among mothers with gingivitis than among those with healthy gums.

Clinical implications

The perinatal period is a unique window of opportunity where perinatal nurses and providers can provide oral assessment, education, and referral to quality dental care. Although frequently overlooked, oral assessment and examination are important components of the perinatal examination, particularly for women in disadvantaged communities who may not have ready or regular access to professional dental care.[59]

Perinatal nurses and providers can provide education and guidance to raise awareness about the importance of good oral health for a healthy pregnancy and overall maternal and newborn health. Education promoting healthy eating habits for a healthy oral cavity includes lowering sucrose intake and reducing acidic beverage consumption in order to minimize suboptimal bacterial colonization and cavity development. In addition, the overall healthy diet recommended for pregnancy has the added benefit of promoting a healthy mouth.[60] Essential for good oral hygiene habits is routine toothbrushing and flossing, which are key to both maternal oral health and overall pregnancy health. Anticipatory guidance in the antenatal period for neonatal oral health includes the promotion of exclusive breastfeeding until the infant reaches 6 months of age, with continued breastfeeding as complementary foods are introduced through the infant's first year or longer, as desired by the mother and the infant.[61] Not only do the oligosaccharides found in breast milk encourage the healthy bloom of commensal *Streptococcus* spp in the newborn but also *Lactobacillus* spp isolated from the oral cavity of breastfed children have a suppressive effect on cariogenic *S mutans*.[17] Supplementation of fluoride may also be advised depending on infant feeding mode and community water fluoridation.[59] A perinatal woman's oral health status is an essential consideration for education. Periodontal treatment and the avoidance of activities that expose the infant to saliva, for example, kissing, and sharing or prechewing of food and sharing of utensils[58,62,63] are recommended for women with chronic periodontal disease who risk transmission of pathogens to their infants.

A health equity lens is critical when thinking about clinical implications for maternal-newborn oral health. Poor oral health disproportionately affects socioeconomically disadvantaged communities and racial/ethnic minority groups.[11] These disparities are exacerbated by lack of prevention and intervention. Access to quality dental care is a primary reason for these inequities.[8] Perinatal nurses and providers have the unique opportunity to provide pregnant women who do not have a "dental home"[59] with options for accessible quality dental care. Modeled after the medical home concept, the dental home describes a dentist who provides comprehensive and accessible care during pregnancy and beyond to both the woman and her child.[59] A streamlined and seamless referral process between the perinatal provider and dental home is critical. Furthermore, in states where Medicaid dental insurance for perinatal women is lacking, nurses and providers would do well to form coalitions to advocate for Medicaid coverage for comprehensive dental care for women throughout the perinatal period.

The results of this study contribute to the literature and lay the groundwork for future, in-depth research investigating the relationship between the maternal and infant oral microbiomes, oral health, and systemic health. Future microbiome research studies are imperative for researchers who seek to understand the biobehavioral underpinnings of maternal-newborn health and for clinicians who directly assess, educate, and care for pregnant women and newborns. Specifically, research is needed to understand factors that affect the acquisition and progression of the newborn oral microbiome from birth onward. Particular maternal factors to consider include the presence of oral disease, oral hygiene behavior, diet and nutrition, and socioeconomic factors that lead to health disparities. Newborn factors include infant nutrition (breast milk/formula), birth mode, and gestational age at birth. A deeper understanding of factors influencing the infant oral microbiome in addition to the function and diversity of the microbiome will inform perinatal education regarding the importance of oral hygiene behaviors and oral healthcare. Future research may also be instrumental in the development of new tools and diagnostics that shape prenatal oral health assessment, education, and intervention guidelines to promote healthy mouth for both the mother and the infant.

Limitations

Limitations of this study include its cross-sectional study design and modest sample size. Although the Human Oral Microbiome Database offers a well-curated and up-to-date database for 16SrRNA sequences, the sequencing technology limits the resolution with which to identify taxa. Despite these limitations, given that few studies have investigated the maternal and infant oral microbiomes, this study enhances the preliminary understanding of this topic.

CONCLUSION

Findings from this study provide an initial description of the maternal and infant oral microbiomes. Various factors from feeding, social and environmental factors, and maternal behaviors that transmit organisms likely play a role in the maturity of this microbiome. Despite the many consequences of untreated oral disease and what is known about the importance of oral health to overall health, the majority of Americans take oral health for granted. The perinatal window provides a unique opportunity for healthcare professionals to assess, educate, and intervene in the oral health of maternal-newborn populations, increasing the potential of a long-term healthy oral cavity for both mothers and infants.

References

1. Vamos CA, Thompson EL, Avendano M, Daley EM, Quinonez RB, Boggess K. Oral health promotion interventions during pregnancy: a systematic review. *Community Dent Oral Epidemiol*. 2015;43(5):385–396.
2. Han YW. Oral health and adverse pregnancy outcomes—what's next? *J Dent Res*. 2011;90(3):289–293.
3. Ide M, Harris M, Stevens A, et al. Periodontitis and cognitive decline in Alzheimer's disease. *PLoS One*. 2016;11(3):e0151081.
4. Cestari JAF, Fabri GM, Kalil J, et al. Oral infections and cytokine levels in patients with Alzheimer's disease and mild cognitive impairment compared with controls. *J Alzheimer's Dis*. 2016;52(4):1479–1485.
5. Olsen I, Singhrao SK. Can oral infection be a risk factor for Alzheimer's disease? *J Oral Microbiol*. 2015;7(1):29143.
6. Bingham CO III, Moni M. Periodontal disease and rheumatoid arthritis: the evidence accumulates for complex pathobiologic interactions. *Curr Opin Rheumatol*. 2013;25(3):345–353.
7. VanWormer JJ, Tambe SR, Acharya A. Oral health literacy and outcomes in rural Wisconsin adults. *J Rural Health*. 2019;35:12–21.
8. Vujicic M, Buchmueller T, Klein R. Dental care presents the highest level of financial barriers, compared to other types of health care services. *Health Aff (Millwood)*. 2016;35(12):2176–2182.
9. Simon L. Overcoming historical separation between oral and general health care: interprofessional collaboration for promoting health equity. *AMA J Ethics*. 2016;18(9):941–949.
10. Frencken JE, Sharma P, Stenhouse L, Green D, Laverty D, Dietrich T. Global epidemiology of dental caries and severe periodontitis: a comprehensive review. *J Clin Periodontol*. 2017;44(suppl 18):S94–S105.
11. Lee JY, Divaris K. The ethical imperative of addressing oral health disparities: a unifying framework. *J Dent Res*. 2014;93(3):224–230.
12. Dye BA, Thornton-Evans G, Li X, Iafolla TJ. Dental caries and sealant prevalence in children and adolescents in the United States, 2011-2012. *NCHS Data Brief*. 2015;(191):1–8.

13. Eke P, Dye B, Wei L, Thornton-Evans G, Genco R. Prevalence of periodontitis in adults in the United States: 2009 and 2010. *J Dent Res*. 2012;91(10):914–920.
14. Wade WG. The oral microbiome in health and disease. *Pharmacol Res*. 2013;69(1):137–143.
15. Anil S, Alrowis RM, Chalisserry EP, Chalissery VP, AlMoharib HS, Al-Sulaimani AF. Oral health and adverse pregnancy outcomes. In: Virdi M, ed. *Emerging Trends in Oral Health Sciences and Dentistry*. London, England: IntechOpen Limited; 2015:631–662.
16. Strużycka I. The oral microbiome in dental caries. *Pol J Microbiol*. 2014;63(2):127–135.
17. Gomez A, Nelson KE. The oral microbiome of children: development, disease, and implications beyond oral health. *Microb Ecol*. 2017;73(2):492–503.
18. Scannapieco FA. The oral microbiome: its role in health and in oral and systemic infections. *Clin Microbiol Newslett*. 2013;35(20):163–169.
19. Jagathrakshakan SN, Sethumadhava RJ, Mehta DT, Ramantahan A. 16S rRNA gene-based metagenomic analysis identifies a novel bacterial co-prevalence pattern in dental caries. *Eur J Dent*. 2015;9(1):127–132.
20. Tanner AC, Mathney JM, Kent RL, et al. Cultivable anaerobic microbiota of severe early childhood caries. *J Clin Microbiol*. 2011;49(4):1464–1474.
21. Merglova V, Polenik P. Early colonization of the oral cavity in 6- and 12-month-old infants by cariogenic and periodontal pathogens: a case-control study. *Folia Microbiol*. 2016;61(5):423–429.
22. Socransky S, Haffajee A, Cugini M, Smith C, Kent R. Microbial complexes in subgingival plaque. *J Clin Periodontol*. 1998;25(2):134–144.
23. Dabdoub SM, Ganesan SM, Kumar PS. Comparative metagenomics reveals taxonomically idiosyncratic yet functionally congruent communities in periodontitis. *Sci Rep*. 2016;6:38993.
24. Lif Holgerson P, Harnevik L, Hernell O, Tanner ACR, Johansson I. Mode of birth delivery affects oral microbiota in infants. *J Dent Res*. 2011;90(10):1183–1188.
25. Li H, Wang J, Wu L, et al. The impacts of delivery mode on infant's oral microflora. *Sci Rep*. 2018;8(1):11938.
26. Zaura E, Nicu EA, Krom BP, Keijser BJ. Acquiring and maintaining a normal oral microbiome: current perspective. *Front Cell Infect Microbiol*. 2014;4:85. doi:10.3389/fcimb.2014.00085.
27. Chaffee B, Gansky S, Weintraub J, Featherstone J, Ramos-Gomez F. Maternal oral bacterial levels predict early childhood caries development. *J Dent Res*. 2014;93(3):238–244.
28. Tuominen H, Collado MC, Rautava J, Syrjänen S, Rautava S. Composition and maternal origin of the neonatal oral cavity microbiota. *J Oral Microbiol*. 2019;11(1):1663084.
29. Yang I, Knight AK, Dunlop AL, Corwin EJ. Characterizing the subgingival microbiome of pregnant African American women. *J Obstet Gynecol Neonatal Nurs*. 2019;48(2):140–152.
30. Corwin EJ, Hogue CJ, Pearce B, et al. Protocol for the Emory University African American vaginal, oral, and gut microbiome in pregnancy cohort study. *BMC Pregnancy Childbirth*. 2017;17(1):161.
31. Lobene R, Weatherford T, Ross N, Lamm R, Menaker L. A Modified Gingival Index for use in clinical trials. *Clin Prev Dent*. 1985;8(1):3–6.
32. Zhou X, Li Y. *Atlas of Oral Microbiology*. Amsterdam, the Netherlands: Elsevier; 2015.
33. McInnes P, Cutting M. *Manual of* Procedures *for Human Microbiome Project: Core* Microbiome Sampling *Protocol A, HMP Protocol# 07-001*, Version Number: 12.0. Bethesda, MD: National Institutes of Health; 2009. https://www.hmpdacc.org/doc/HMP_Clinical_Protocol.pdf. Accessed December 1, 2017.
34. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl Environ Microbiol*. 2013;79(17):5112–5120.
35. Callahan BJ, Sankaran K, Fukuyama JA, McMurdie PJ, Holmes SPJF. Bioconductor workflow for microbiome data analysis: from raw reads to community analyses. *F1000Res*. 2016;5:1492. https://f1000research.com/articles/5-1492/v2. Accessed January 2, 2020.
36. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods*. 2016;13(7):581–583.
37. Escapa IF, Chen T, Huang Y, Gajare P, Dewhirst FE, Lemon KP. New insights into human nostril microbiome from the expanded Human Oral Microbiome Database (eHOMD): a resource for species-level identification of microbiome data from the aerodigestive tract. *bioRxiv*. 2018:347013. https://msystems.asm.org/content/msys/3/6/e00187-18.full.pdf. Accessed February 1, 2020.
38. McMurdie PJ, Holmes SJ. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*. 2013;8(4):e61217.
39. Magurran AE. *Measuring Biological Diversity*. Hoboken, NJ: Wiley-Blackwell; 2013.
40. Constitution. World Health Organization Web site. https://www.who.int/about/who-we-are/constitution. Updated 2020. Accessed March 31, 2020.
41. Dewhirst FE, Chen T, Izard J, et al. The human oral microbiome. *J Bacteriol*. 2010;192(19):5002–5017.
42. Holgerson PL, Öhman C, Rönnlund A, Johansson I. Maturation of oral microbiota in children with or without dental caries. *PLoS One*. 2015;10(5):e0128534.
43. Abusleme L, Dupuy AK, Dutzan N, et al. The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. *ISME J*. 2013;7(5):1016–1025.
44. Feres M, Teles F, Teles R, Figueiredo LC, Faveri M. The subgingival periodontal microbiota of the aging mouth. *Periodontol 2000*. 2016;72(1):30–53.
45. Belibasakis GN. Microbiological changes of the ageing oral cavity. *Arch Oral Biol*. 2018;96:230–232.
46. Ferretti P, Pasolli E, Tett A, et al. Mother-to-infant microbial transmission from different body sites shapes the developing infant gut microbiome. *Cell Host Microbe*. 2018;24(1):133–145.e5.
47. Hendricks-Muñoz KD, Xu J, Parikh HI, et al. Skin-to-skin care and the development of the preterm infant oral microbiome. *Am J Perinatol*. 2015;32(13):1205–1216.
48. Al-Shehri SS, Knox CL, Liley HG, et al. Breastmilk-saliva interactions boost innate immunity by regulating the oral microbiome in early infancy. *PLoS One*. 2015;10(9):e0135047.
49. Sampaio-Maia B, Monteiro-Silva F. Acquisition and maturation of oral microbiome throughout childhood: an update. *Dent Res J*. 2014;11(3):291–301.
50. Kaci G, Goudercourt D, Dennin V, et al. Anti-inflammatory properties of *Streptococcus salivarius*, a commensal bacterium of the oral cavity and digestive tract. *Appl Environ Microbiol*. 2014;80(3):928–934.
51. Baker JL, Bor B, Agnello M, Shi W, He X. Ecology of the oral microbiome: beyond bacteria. *Trends Microbiol*. 2017;25(5):362–374.
52. Thakur R, Singh MG, Chaudhary S, Manuja N. Effect of mode of delivery and feeding practices on acquisition of

oral *Streptococcus mutans* in infants. *Int J Paediatr Dent*. 2012;22(3):197–202.
53. Han YW. *Fusobacterium nucleatum*: a commensal-turned pathogen. *Curr Opin Microbiol*. 2015;23:141–147.
54. Murayama R, Abe S, Hasegawa Y, et al. Study of the distribution of *Megasphaera micronuciformis* in oral cavities of the Japanese by species-specific polymerase chain reaction (PCR) assay. *African J Microbiol Res*. 2013;7:5546–5549.
55. Mok SF, Chinna K, Cheah YK, et al. The oral microbiome community variations associated with normal, potentially malignant disorders and malignant lesions of the oral cavity. *Malays J Pathol*. 2017;39(1):1–15.
56. Belstrøm D, Holmstrup P, Fiehn NE, et al. Bacterial composition in whole saliva from patients with severe hyposalivation—a case-control study. *Oral Dis*. 2016;22(4):330–337.
57. Feng X, Zhu L, Xu L, et al. Distribution of 8 periodontal microorganisms in family members of Chinese patients with aggressive periodontitis. *Arch Oral Biol*. 2015;60(3):400–407.
58. Adhikari K, Saimbi CS, Gupta BP. Estimation of transmission of *Porphyromonas gingivalis* from mother to child through saliva. *J Nepal Med Assoc*. 2018;56(212):781–786.
59. Brecher EA, Lewis CW. Infant oral health. *Pediatr Clin*. 2018;65(5):909–921.
60. Jevtić M, Pantelinac J, Jovanović Ilić T, Petrović V, Grgić O, Blažić L. The role of nutrition in caries prevention and maintenance of oral health during pregnancy. *Med Pregl*. 2015;68(11/12):387–393.
61. Eidelman AI, Schanler RJ. Breastfeeding and the use of human milk. *Pediatrics*. 2012;129(3):e827–e841.
62. Centers for Disease Control and Prevention. Infant Feeding Practices Study II. http://www.cdc.gov/ifps/pdfs/data/ifps2_tables_ch3.pdf. Published 2015. Accessed February 1, 2020.
63. Virtanen JI, Vehkalahti KI, Vehkalahti MM. Oral health behaviors and bacterial transmission from mother to child: an explorative study. *BMC Oral Health*. 2015;15(1):75. https://link.springer.com/article/10.1186/s12903-015-0051-5. Accessed February 1, 2020.

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