Oral Microbiome: *Streptococcus mutans*/Caries Concordant-Discordant Children

Márcia Dinis¹, Melissa Agnello²,³, Lujia Cen⁴, Bhumika Shokeen⁵, Xuesong He⁴, Wenyuan Shi⁴, David T. W. Wong⁶, Renate Lux⁵ and Nini Chaichanasakul Tran¹

¹ Section of Pediatric Dentistry, School of Dentistry, University of California, Los Angeles, Los Angeles, CA, United States, ² Section of Oral Biology, School of Dentistry, University of California, Los Angeles, Los Angeles, CA, United States, ³ M2Biome LLC, San Francisco, CA, United States, ⁴ Microbiology, The Forsyth Institute, Cambridge, MA, United States, ⁵ Section of Periodontics, School of Dentistry, University of California, Los Angeles, Los Angeles, CA, United States, ⁶ Center for Oral/Head and Neck Oncology Research, Laboratory of Salivary Diagnostics, School of Dentistry, University of California, Los Angeles, Los Angeles, CA, United States

Dental caries remains the most common chronic disease in children, and the respective etiology is not fully understood. Though *Streptococcus mutans* is an important factor in the initiation and progression of caries, its presence is not always associated with the disease. The existence of caries discordant populations, in which *S. mutans* counts do not correlate with caries experience, poses a challenging problem. This study explored the possible correlation of *S. mutans* and other microorganism levels on caries-associated ecology of caries-concordant and discordant populations. A total of forty-seven children were analyzed in this study and stratified into four clinical groups based on their *S. mutans* levels in saliva (HS/LS: High/low *S. mutans*) and caries experience. *Streptococcus mutans* levels were determined by culture-based selective plating. The salivary microbiome of caries concordant and discordant populations was investigated by 16S rRNA gene sequencing and downstream bioinformatics analysis. The salivary microbial communities significantly clustered based on *S. mutans* levels and independent of their caries experience. In addition to *S. mutans* levels, significant differences in the abundance of other species were observed between HS and LS groups. Interestingly, disease-associated species such as *Veillonella dispar*, *Streptococcus* spp., and *Prevotella* spp. were significantly increased in HS groups and may contribute, in combination with *S. mutans*, to the caries progression. Furthermore, health-associated species exhibited higher abundance in the LS groups, such as *Veillonella rogosae*, *Haemophilus* sp., and *Alloprevotella* spp. but their possible contribution to the caries process remains to be elucidated. This study provides evidence that *S. mutans* may play a role in shaping the salivary microbial community. Our results highlight that future caries research should consider additional species as health/disease microbial markers in conjunction with *S. mutans* to improve diagnosis and caries management of the caries-discordant population.

Keywords: caries, *Streptococcus mutans*, saliva, microbiome, pediatric dentistry
INTRODUCTION

Dental caries is one of the most prevalent childhood diseases in the United States and worldwide (Dye et al., 2015; Kassebaum et al., 2017). Although considered a preventable disease, the persistence of high caries necessitates the improvement in diagnostics and therapeutic interventions for young and vulnerable populations. A multifactorial etiology of dental caries comprises of a complex interaction of acid-producing bacteria, fermentable carbohydrates, and host factors (Selwitz et al., 2007).

From the microbiological perspective, *Streptococcus mutans* has been considered a key pathogen in the initiation and progression of caries (Tanzer et al., 2001). Hence, *S. mutans* levels have been used as part of caries risk assessment (Guo and Shi, 2013; Edelstein et al., 2016). However, contradicting reports exist in the literature regarding the correlation between *S. mutans* counts and caries experience. Previous studies established a causal relationship between *S. mutans* levels in plaque or saliva and caries experience in children (Alaluusua and Renkonen, 1983; Loesche, 1986; Damle et al., 2016; Edelstein et al., 2016), while others reported distinct populations whose caries status is not correlated with *S. mutans* levels have been observed (Carlsson et al., 1985; Matee et al., 1999; Aas et al., 2008; Mantzourani et al., 2009; Guo and Shi, 2013). Despite a considerable body of evidence, the ecological plaque hypothesis (Marsh, 1994), which echoes the fact that *S. mutans* may not be the sole etiological factor. In this scenario, caries would result from a disruption of the homeostasis of the resident microflora driven by changes in local environmental conditions (Wade, 2013). Emerging evidence associated additional bacterial species within the salivary microbiome with caries experience in children. Besides *S. mutans*, other acid-producing microorganisms such as certain species of the genera *Veillonella*, *Sarcinia*, and *Lactobacillus* have also been identified in caries-affected children populations (Guo and Shi, 2013; Jiang et al., 2016; Xu et al., 2018). Additionally, there are studies linking *Actinomyces* and *Bifidobacterium* genera with cavitated caries lesions (Brailsford et al., 1999; Aas et al., 2008; Mantzourani et al., 2009; Guo and Shi, 2013). Despite a considerable body of evidence, the microbiological ecology of the caries etiology is not fully understood, limiting our ability to explain the existence of the caries discordant population. Moreover, a comprehensive investigation of the oral microbiome in this population is scarce.

In this study, we investigated the caries concordant-discordant clinical populations for their salivary *S. mutans* levels and the respective microbiome. These unique clinical populations may hold the key to a better understanding of the ecological relationship between *S. mutans* and other microbial species and the impact on dental caries in children.

MATERIALS AND METHODS

Ethics

The study was reviewed and approved by the University of California, Los Angeles Institutional Review Board (13-001075). Informed written consent was obtained from parents or legal guardians of all participants prior to initiation of the study. Additionally, child assent was obtained from participants that were considered to be capable of providing assent, taking into account their age, maturity, and psychological state.

Recruitment and Sampling

Study participants were recruited from the pediatric patient population of the Children’s Dental Center at the University of California, Los Angeles (UCLA). A total of sixty healthy (ASA I) children between 4 and 14 years of age were recruited and enrolled in the study. Each participant completed a brief questionnaire on demographics, oral hygiene habits, and dental treatment history. Participants were enrolled based on the following inclusion criteria: children, who were not taking any medication, and had no antibiotic usage within the past 6 months. Participants were excluded from the study if they had generalized rampant caries, periodontitis, halitosis, open sores or ulcerations, chronic systemic diseases, reduced saliva production. Oral clinical evaluation and radiographic exams were performed by a single pediatric dental resident at the UCLA School of Dentistry. The caries experience was evaluated using the decayed, missing, and filled tooth (dmft/DMFT) index, according to the criteria proposed by World Health Organization (1997).

All participants were asked to refrain from oral hygiene procedures, eating and drinking for at least 2 h before oral sampling. Saliva samples were collected between 8:00 am and 10:00 am. Participants were instructed to rinse their mouths with water prior to collection. Then, 5 mL of unstimulated saliva was collected by drooling/spitting directly into collection tubes or through direct sampling using a soft, sterile plastic pipette (Henson and Wong, 2010). The collected sample was split, one aliquot was immediately used for *S. mutans* levels quantification via plating on selective media, and the remaining portion was stored in 20% glycerol at −80°C until further analysis.

The participants were stratified into four groups based on their *S. mutans* levels and caries experience: high (HS, ≥1×10^5 CFUs/mL) and low (LS, <1.0×10^4 CFUs/mL) *S. mutans* counts; and high (HC, dmft/DMFT ≥ 4) and low (LC, dmft/DMFT < 2) caries scores. Of the samples derived from the initially enrolled sixty children, forty-seven passed additional quality control for microbiome sequencing and were subsequently included in the study: HSHC (high *S. mutans*/high caries, n = 13), HLSLC (high *S. mutans*/low caries, n = 10), LSCLC (low *S. mutans*/high caries, n = 13), and LSLC (low *S. mutans*/low caries, n = 11) (Table 1).

Quantification of Salivary *S. mutans* Levels

Saliva samples were serially diluted, and 100 µl of each dilution was plated on brain heart infusion (BHI, Bacto™ Brain Hearth Infusion, Becton, Franklin Lakes, NJ, United States, Dickinson
and Company, Franklin Lakes, NJ, United States) and selective mitis-salivarius-bacitracin (MSB, Difco™ Mits Salivarius Agar, Becton, Franklin Lakes, NJ, United States, Dickinson and Company, Franklin Lakes, NJ, United States) agar plates. The plates were incubated anaerobically at 37°C for 2 days. Total bacteria were counted on BH media, while *S. mutans* was identified and enumerated according to their specific colony morphology on the MSB selective plates. Colonies were further verified by an *S. mutans*-specific monoclonal antibody as previously described (Gu et al., 2006). Briefly, 10 µL of collected sample was mixed with 10 µL of hybridoma cell line culture supernatant producing an anti-*S. mutans* monoclonal antibody (Mab) (10 µg/µL); incubated at room temperature for 30 min. Then, 1 µL of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin G (IgG) was added and incubated at room temperature for 30 min. The mixture was examined using fluorescent microscopy.

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**TABLE 1 | Demographic and oral hygiene information from the clinical study questionnaire.**

| Characteristic              | HSHC (n = 13) | HSLC (n = 10) | LSHC (n = 13) | LSLC (n = 11) |
|----------------------------|---------------|---------------|---------------|---------------|
| **Gender**                 |               |               |               |               |
| Female                     | 10            | 6             | 3             | 7             |
| Male                       | 3             | 4             | 10            | 4             |
| **Ethnicity**              |               |               |               |               |
| Hispanic                   | 10            | 10            | 12            | 5             |
| Non-Hispanic               | 3             | 0             | 1             | 6             |
| **Age**                    | (4–12 years)  | (4–14 years)  | (4–12 years)  | (4–13 years)  |
|                           | 7.7 ± 2.5     | 10 ± 3.0      | 8.3 ± 2.1     | 9.2 ± 2.9     |
| **dmft/DMFT index**        | (4–10)        | (0–2)         | (4–10)        | (0–2)         |
|                           | 6.5 ± 2.3     | 1.1 ± 0.9     | 4.8 ± 1.5     | 0.2 ± 0.6     |
| **Professional dental cleaning frequency** |           |               |               |               |
| None                       | 0             | 2             | 1             | 0             |
| Once a year                | 11            | 7             | 10            | 9             |
| More than once a year      |               |               |               |               |
| **Tooth brushing frequency** |             |               |               |               |
| Not brushing               | 0             | 0             | 0             | 0             |
| Once a day                 | 3             | 0             | 0             | 1             |
| More than once per day     | 10            | 10            | 13            | 10            |
| **Tooth Flossing frequency** |             |               |               |               |
| No flossing                | 2             | 3             | 5             | 1             |
| Less than once a day       | 0             | 0             | 0             | 0             |
| Once a day                 | 8             | 4             | 1             | 1             |
| More than once per day     | 3             | 3             | 7             | 9             |

High *S. mutans*: $\geq 1.0 \times 10^6$ CFU/mL.
Low *S. mutans*: $<1.0 \times 10^6$ CFU/mL.
High Caries: dmft/DMFT $\geq 4$.
Low Caries: dmft/DMFT $< 2$.
HSHC, High *S. mutans* High Caries; HSLC, High *S. mutans* Low Caries; LSHC, Low *S. mutans* High Caries; LSLC, Low *S. mutans* Low Caries.
The bold values correspond to the average of age and caries experience parameters per group and their respective standard deviations.

Salivary Microbial Community Analysis

The DNA extracted from the saliva samples was sent for microbiome sequencing at the UCLA Microbiome Core. Amplicon libraries were prepared in triplicate by using 16S Metagenomic Sequencing Library Preparation Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System, Illumina® according to the manufacturer’s instructions. Briefly, 10–50 ng of genomic DNA was used as a template in a PCR reaction to amplify the V4 region of the 16S rRNA encoding gene and barcoded using 515f/806r primers. Afterward, the product was purified using AMPure beads (Beckman Coulter) and hundred ng of each library was pooled, gel-purified, and quantified (Bioanalyzer, Agilent), and 12 pM of the mixture, spiked with 20% PhiX, and sequencing was performed on an Illumina MiSeq sequencer system (Illumina, San Diego, CA, United States).

Sequencing reads were de-multiplexed and adaptor sequences removed. Quality filtering removed bad reads and chimera sequences prior to analysis. Sequencing data were analyzed using Quantitative Insights into Microbial Ecology (QIIME) version 1.9.1 (Caporaso et al., 2010). Sequences were clustered into operational taxonomic units (OTUs) using UCLUST, aligned, and the taxonomy was assigned with the Human Oral Microbiome Database (HOMD) as reference. For determination of alpha diversity, OTU tables were rarefied to 93,000 reads, and Shannon indices were calculated. For the analysis of beta diversity, weighted UniFrac distances were calculated, followed by principal coordinates analysis (PCoA).

Statistical Analysis

The statistical tests were performed using GraphPad Prism (GraphPad Prism version 8.0.0, GraphPad Software, San Diego, CA, United States), and statistical significance was defined as follows: *p < 0.05, **p < 0.005, and ***p < 0.0005.

The demographic and *S. mutans* quantification data were compared between groups and assessed for the significance of their differences with one-way ANOVA followed by Kruskal–Wallis multiple comparisons test. Spearman correlation analysis was performed to investigate possible correlations between demographic and oral hygiene parameters.

For salivary microbiome sequencing data, differences in the relative abundances of taxa between the groups were determined with the Kruskal–Wallis test, controlling the false discovery rate to correct for multiple comparisons, and a corrected $p \leq 0.05$ was considered significant. Differences in weighted UniFrac distances between the groups were analyzed with analysis of similarity (ANOSIM), and $p \leq 0.05$ was considered statistically significant.
RESULTS

Study Demographics
A total of forty-seven children were enrolled and stratified into four groups based on their S. mutans levels and caries experience: HSHC (n = 13), HSLC (n = 10), LSHC (n = 13), and LSLC (n = 11). The respective age, gender, ethnicity, dmft/DMFT index, and oral hygiene parameters of the participants are summarized in Table 1. In brief, the gender distribution was 26 females (55.3%) and 21 males (44.7%), and their ages ranged from 4 to 14 years with an average of 8.8 (±2.7) years. The study population was comprised of 78.7% Hispanic and 21.3% non-Hispanic children, and the caries experience varied from a dmft/DMFT score of 0 to 10, with an average of 5.6 (±2.1) in the high caries groups (HSHC, LSHC) and 0.6 (±1.0) in the low caries groups (HSLC, LSLC). The frequency of their oral hygiene habits, such as professional cleaning, as well as their tooth brushing, mouth rinse, and flossing routine, did not vary between groups. Overall, there were no significant differences between the groups for all of the above-mentioned parameters, except for the caries status that was a significant difference between high and low caries groups.

Caries Experience and Streptococcus mutans Levels in Saliva
While the caries status for both HC (HSHC, LSHC) and LC (HSLC, LSLC) groups was similar to each other, differences in dmft/DMFT between groups reflecting the contrasting caries experience were statistically significant (HSHC vs. HSLC, HSHC vs. LSLC, HSLC vs. LSHC, and LSHC vs. LSLC) (Figure 1A). For salivary S. mutans quantification, as expected, there were no significant differences between groups with similar S. mutans levels (HSHC vs. HSLC, and LSHC vs. LSLC) despite the difference in caries experiences (Figure 1B). Only those with dissimilar S. mutans levels were statistically different (HSHC vs. LSHC, HSHC vs. LSLC, HSLC vs. LSHC, and HSLC vs. LSLC) (Figure 1B).

Salivary Microbiome Composition of Clinical Populations
Next, the salivary microbiome of caries concordant and discordant clinical populations was investigated. A total of 8,671,057 high-quality sequences from forty-seven samples, with a mean of 108,490 ± 30,338 (range 93,113–230,591), were generated after processing the data obtained from 16S rRNA amplicon sequencing.

Taxonomy analysis revealed 11 phyla, of which the Firmicutes was the only phylum that significantly differed between the samples with high and low S. mutans levels (55.3 vs. 47.3%, p = 0.02). A total of 120 genera comprising 310 species were detected. The salivary microbial community composition based on clinical groups, S. mutans levels, and caries experience is represented at the genus (Figures 2A,B) and species levels (Figures 2C,D). Twelve major genera, which constituted 90% of the total, dominated the communities and: Streptococcus (32.6% ± 2.1), Haemophilus (10.7% ± 1.3), Neisseria (10.0% ± 3.3), Veillonella (8.9% ± 2.2), Prevotella (8.8% ± 1.6), Rothia (6.1% ± 0.6), Gemella (2.9% ± 0.2), Fusobacterium (2.9% ± 1.2), Actinomyces (2.9% ± 0.3), Granulicatella (2.6% ± 0.3), Leptotrichia (2.0% ± 0.2), and Aggregatibacter (1.1% ± 0.3). Of these, only the abundance of Veillonella was significantly different between groups with high and low S. mutans levels (10.7 vs. 7.1%, p = 0.003). The abundance...
of *Streptococcus* appeared elevated in samples with high *S. mutans* levels (34.0 vs. 31.2%), while levels of the genera *Neisseria* (7.7 vs. 12.5%), *Rothia* (5.7 vs. 6.6%), and *Fusobacteria* (2.3 vs. 3.5%) were lower, albeit those differences were not statistically significant. Of note, the genera *Megasphaera*, *Atopobium*, and *Bifidobacterium* were significantly elevated in samples with high *S. mutans* levels (Table 2). Moreover, within the high *S. mutans* groups all these genera were higher in the HSLC compared to the HSHC group.

The saliva microbiome analysis at the species level revealed that *S. mutans* abundance was similar within the high (HSHC vs. HSLC, 0.29 vs. 0.52%) and low (LSHC vs. LSLC, 0.007 vs. 0.005%) groups, but significantly different between groups with divergent *S. mutans* levels (HSHC vs. LSHC, HSHC vs. LSLC, HSLC vs. LSHC, and HSLC vs. LSLC) (Figure 3A). Importantly, linear regression analysis demonstrated that the *S. mutans* levels detected by microbiome sequencing were correlated with those quantified using selective culture (Figure 3B).

The correlation between *S. mutans* levels and the abundance of other bacterial species was further analyzed. Fourteen species-level operational taxonomic units were significantly
TABLE 2 | Relative abundance of the genus and species-level OTUs detected in the saliva, that were significantly different between high and low S. mutans samples.

| % Relative abundance | S. mutans levels |
|----------------------|------------------|
| Genus – level        | High     | Low     | p-value |
| Atopobium            | 0.360%   | 0.168%  | 0.035   |
| Bifidobacterium      | 0.067%   | 0.001%  | 0.009   |
| Megasphaera          | 0.485%   | 0.342%  | 0.035   |
| Veillonella          | 10.732%  | 7.098%  | 0.031   |

| Species – level      | High     | Low     | p-value |
|----------------------|----------|---------|---------|
| Actinomyces sp. [HOT 448] | 0.019%   | 0.007%  | 0.016   |
| Alloprevotella sp. [HOT 473] | 0.050%   | 0.021%  | 0.023   |
| Alloprevotella sp. [HOT 914] | 0.048%   | 0.013%  | 0.044   |
| Haemophilus sp. [HOT 035] | 0.321%   | 0.066%  | 0.046   |
| Kingella oralis      | 0.013%   | 0.004%  | 0.016   |
| Megasphaera micronuciformis | 0.474%   | 0.342%  | 0.033   |
| Prevotella histicola | 0.006%   | 0.002%  | 0.012   |
| Prevotella sp. [HOT 292] | 0.015%   | 0.005%  | 0.016   |
| Prevotella sp. [HOT 313] | 2.667%   | 1.897%  | 0.023   |
| Streptococcus intermedius | 0.286%   | 0.154%  | 0.016   |
| Streptococcus mutans | 0.393%   | 0.006%  | 0.000   |
| Streptococcus parasanguinis_II | 8.755%   | 6.062%  | 0.054   |
| Veillonella dispar    | 10.183%  | 6.432%  | 0.016   |
| Veillonella rogosae   | 0.035%   | 0.056%  | 0.045   |

High S. mutans: ≥1.0 × 10^5 CFU’s/mL.
Low S. mutans: <1.0 × 10^5 CFU’s/mL.
The significant P-value of the group is indicated in bold.

Different in the samples with high S. mutans levels compared to those with lower levels (Table 2). Samples of the high S. mutans group also contained significantly elevated levels of Veillonella dispar, Streptococcus parasanguinis_II, Prevotella sp. [HOT 313], Megasphaera micronuciformis, Streptococcus intermedius, Actinomyces sp. [HOT 448], Prevotella sp. [HOT 292], Kingella oralis, and Prevotella histicola. In the samples with low S. mutans levels, significantly higher abundance was observed for Haemophilus sp. [HOT 035], Alloprevotella sp. ([HOT 473] and [HOT 914]), and Veillonella rogosae (Table 2).

Furthermore, the analysis of the salivary microbial community diversity revealed that there were no significant differences neither between the analyzed clinical groups (Figure 4A), S. mutans levels (Figure 4B), nor the individual caries status (Figure 4C). The beta diversity of the salivary microbial communities was evaluated using weighted UniFrac, and plotted using principal coordinates analysis (Figures 4D–F). The samples were analyzed according to a combination of both S. mutans levels and caries experience (HSHC, HSLC, LSHC, and LSLC) as well as the individual parameters. The samples significantly clustered according to their S. mutans levels (Figure 4E). Moreover, the samples displayed an apparent pattern associated with their caries status, but this was not statistically significant (Figure 4F).

DISCUSSION

Dental caries is a multifactorial oral disease affecting children worldwide, for which the complete pathogenesis is not yet fully understood. The existence of caries discordant populations, in which S. mutans levels do not correlate with the observed caries experience, poses a compelling challenge for caries management. In this study, we use saliva as a source of S. mutans and oral microbiome isolation because it reflects

![FIGURE 3](https://example.com/figure3.png)

**FIGURE 3** | Salivary S. mutans levels detected with culture based method versus microbiome sequencing. The relative abundance of S. mutans detected on saliva upon 16S rRNA sequencing (A) per group (HSHC, HSLC, LSHC, and LSLC), (B) Linear regression analysis between the S. mutans levels detect by culture based (CFU's/mL) and 16S RNA sequencing (% Relative abundance). The graphic bars represent means, and error bars represent the standard deviation (SD). Differences in significance between groups were analyzed using one-way ANOVA followed by Kruskal–Wallis multiple comparisons tests; ***p < 0.0005, ****p < 0.00005.
FIGURE 4 | Alpha- and Beta- diversity analyses of salivary microbial community. Alpha diversity was analyzed using Shannon index (upper panel, A–C). The beta diversity was by principal coordinates analysis (PCoA) of weighted UniFrac distances (lower panel, D–F). The analyses were performed based on the following parameters: (A,D) per group (HSHC, HSLC, LSHC, and LSLC); (B,E) S. mutans levels (High, Low); and (C,F) caries experience (High, Low). The graphic bars represent means, and error bars represent the standard deviation (SD). The differences in significance of the groups was performed by one-way ANOVA followed by Kruskal–Wallis multiple comparisons tests (alpha diversity) or ANOSIM (beta diversity).

the composition of the oral microbiome, mirrors the complex microbial ecosystems of the oral cavity (Belstrøm, 2020), and is an excellent non-invasive diagnostic biofluid. During the initial phase of random participant recruitment for this study, we observed that only 60% of the clinical population could be categorized into caries concordant groups (HSHC and LSLC). In contrast, a substantial portion of the population (40%) displayed caries discordant phenotypes (HSLC and LSHC), implying that the presence and quantity of S. mutans may not be the sole determinant of disease risk. In this study, the possible role of S. mutans levels and/or other microorganisms on caries-associated ecology was explored in concordant and discordant populations.

Our results indicate that S. mutans levels may play a role in shaping the community as significant differences in the groups with high and low counts of this cariogenic pathogen were observed. In the HS groups, apart from S. mutans several other genera and species were significantly higher than in the LS groups (Table 1). The genera with significant differences included the acid producers, Bifidobacterium (Henne et al., 2015) and Atopobium (Kolenbrander et al., 2009), the lactic acid consumer Veillonella (Mashima and Nakazawa, 2014), and Megasphaera, which is closely related to Veillonella (Nallabelli et al., 2016). Previously, these genera have been identified in close association with high S. mutans levels (Aas et al., 2008) that they are thought to either assist in the production or metabolism of lactic acid. Of note Bifidobacterium dentium was found to be present in 30.8% of caries active individual but was not present at all in caries-free individual (Henne et al., 2015), suggesting that this species should be considered alongside S. mutans as another aciduric taxa contributing to dental caries. Although several studies have recognized the contribution of the genus Veillonella in dental caries (Becker et al., 2002; Aas et al., 2008; Gross et al., 2012; Jiang et al., 2016; Agnello et al., 2017; Xu et al., 2018; Hurley et al., 2019), differential roles may exist at species level (Do et al., 2015). In this study, the relative abundance of two different Veillonella species were significantly different between groups. While high levels of V. dispar were detected in the HS groups, V. rogosae was significantly higher in LS groups. Similar to our findings, previous studies have
reported an increased abundance of V. dispar in caries-active children (Arif et al., 2008; Hurley et al., 2019) and V. rogosae in caries-free children (Arif et al., 2008). There is little doubt that the ability of Veillonella to consume lactate can contribute to caries development. Interestingly, M. micronuciformis, a low abundance species which was significantly increased in the HS groups were previously reported in association with caries (Kalpana et al., 2020).

Other pertinent bacterial species were observed in high abundance in the HS groups. For Streptococcus species, besides S. mutans, S. parasanguinis, and S. intermedius were significantly higher in the HS groups. However, their association with health or disease is controversial as elevated levels of these species were reported in both caries-active and caries-free children (Becker et al., 2002; Corby et al., 2005; Aas et al., 2008). In addition, significantly higher levels of caries-associated species such as Actinomyces sp. and Prevotella spp. were also observed in the HS groups, both of which have been reported in both the saliva and plaque of children with severe caries lesions (Becker et al., 2002; Corby et al., 2005; Jiang et al., 2013, 2016; Ma et al., 2015; Zhang et al., 2015; Zhu et al., 2018; Kalpana et al., 2020). Particularly, a higher abundance of Prevotella spp. was correlated with the group characterized by elevated S. mutans and caries experience levels. A previous study investigating the microbiome of dentin carious lesions reported similar results (Hurley et al., 2019).

Focusing on the LS groups, no significant trends were observed at the genus level. Besides the health-related V. rogosae mentioned above, at the species level, Alloprevotella spp. and Haemophilus sp. were also significantly increased in the LSLC group. While our findings are consistent with previous reports (Hurley et al., 2019), other studies suggested an association of these species with caries-affected children (Xu et al., 2018; Zhu et al., 2018), yet another group published no distinct association with either one (Jiang et al., 2016).

The microbial community diversity in both the caries concordant and discordant populations were compared based on clinical groups (Figures 4A,D), S. mutans levels (Figures 4B,E), and caries experience (Figures 4C,F). Overall, when comparing between clinical groups or caries experience, there were no significant differences in species abundance, richness, diversity, and no particular clustering pattern was observed. Previous studies that applied the same alpha-diversity index only found a correlation with caries experience when the disease severity progressed into dentin (Bong-Soo et al., 2018; Hurley et al., 2019). Findings from recent reports were consistent with our results that revealed no clustering of microbial communities based on caries status (Jiang et al., 2016; Zhu et al., 2018; Hurley et al., 2019). Further community diversity analysis based on S. mutans levels showed no significant differences in alpha-diversity (Figure 4C). However, the most striking result to emerge from our data was the microbial communities significantly cluster based on S. mutans (HS vs. LS) (Figure 4E). The saliva from children with high S. mutans counts exhibited similar microbial community structure independent of their carious status, while in individuals with low S. mutans levels, the communities were more diverse. This result further supports the influence of S. mutans in shaping the composition of the microbial communities.

Intriguingly, S. mutans accounts for less than 1% of the community composition yet plays a crucial role in shaping the entire community by influencing the abundance of other species and shifting the oral microbiome toward disease. However, our findings highlight other disease-associated species such as V. dispar, S. parasanguinis, S. intermedius, and Prevotella spp., which were significantly increased in HS groups and may contribute, in combination with S. mutans, to the caries progression. In contrast, other health-associated species exhibited higher abundance in the LS groups, such as V. rogosae, Haemophilus sp., and Alloprevotella spp., but their contribution to the balance of health and disease is unknown.

This study is without limitations. The sample number per group could be increased to improve statistical power of the study. Although 16S rRNA gene sequencing can provide adequate phylogenetic information to identify the bacteria at the species level, for some taxa such as different streptococcal species, the identification is limited by the discriminatory power of the hypervariable regions and the lack of a consensus protocol. Furthermore, diet, especially the frequent intake of high carbohydrate beverages and food, is known to contribute to a sharp decrease in pH that drives a proliferation of cariogenic bacteria and microbiome dysbiosis leading to dental caries. Additional mechanistic studies should be undertaken to fully grasp the interactions between these cariogenic species and their respective contribution to caries development. The presence of different S. mutans strains may also account for the discordant population’s phenotypes. Previous studies demonstrated that different strains of the same species might have different contributions to caries in children (Al-Hebshi et al., 2019). Similarly, clinical isolates of S. mutans showed distinct phenotypic traits and thus differences in caries severity (Valdez et al., 2017). Therefore, future work should examine other potential factors contributing to the existence of the caries discordant population such as dentition stage, specific S. mutans strains, diet, and host susceptibility.

**CONCLUSION**

This research has raised an important consideration of the additional species as health/disease microbial markers in conjunction with S. mutans. Our present unique study substantiates the understanding of the caries discordant populations microbiome and provides the first insight into how different microbes may contribute to the balance between health and disease in these populations. We believe that our research will serve as a base for future studies to unravel additional microbial targets that may help improve diagnosis and caries management of the caries discordant population.

**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the
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