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	between the two groups, including Veillonella HOT 780 and Porphyromonas HOT 284, which were 4.6-fold and 9-fold higher, respectively, in the S-ECC group, and Streptococcus gordonii and Streptococcus sanguinis, which were 5-fold and 2-fold higher, respectively, in the caries-free group. Extremely high levels of Streptococcus mutans were detected in individuals within the S-ECC group. Overall, First Nations children with S-ECC have a significantly different plaque microbiome than caries-free counterparts, with the S-ECC group containing higher levels of known cariogenic organisms.

Microbiome Associated with Severe Caries in Canadian First Nations Children

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Abstract

Young Indigenous children in North America suffer from an extraordinarily higher degree of severe early childhood caries (S-ECC) than the general population, leading some to speculate that it may actually be an altogether different disease, characterized by different microbes. To address this knowledge gap, here we have conducted the first microbiome analysis in an Indigenous population using modern molecular techniques. We investigated the caries-associated microbiome among Canadian First Nations children with S-ECC. Thirty First Nations children < 72 months of age with S-ECC and twenty caries-free children were recruited in Winnipeg, Canada. Parents or caregivers completed a questionnaire on general and dental health, diet, and demographics. The plaque microbiome was investigated by sequencing the 16S rRNA gene. Sequences were clustered into OTUs and taxonomy assigned using the Human Oral Microbiome Database, then analyzed at the community level with alpha- and beta-diversity measures. Results revealed that children with S-ECC came from households with lower income, were more likely to be bottle-fed, and were weaned from the bottle at a later age than those that were caries-free. The microbial communities of the S-ECC and caries-free groups did not differ in terms of species richness or phylogenetic diversity. Beta diversity analysis showed that the samples significantly clustered into groups based on caries status. Twenty-eight species-level OTUs were significantly different between the two groups, including *Veillonella* HOT 780 and *Porphyromonas* HOT 284, which were 4.6-fold and 9-fold higher, respectively, in the S-ECC group, and *Streptococcus gordonii* and *Streptococcus sanguinis*, which were 5-fold and 2-fold higher, respectively, in the caries-free group. Extremely high levels of *Streptococcus mutans* were detected in individuals within the S-ECC group. Overall, First Nations children with S-

ECC have a significantly different plaque microbiome than caries-free counterparts, with the S-ECC group containing higher levels of known cariogenic organisms.

Introduction

Early childhood caries (ECC), defined as decay involving the primary dentition in children < 72 months of age, is the most common chronic disease of childhood (American Academy of Pediatrics 2015). ECC is a critical public health concern due to its high prevalence, high treatment costs, negative effect on quality of life, and potential long-term complications (Martins-Júnior et al. 2013; Schroth et al. 2009; Schroth et al. 2016). Severe-early childhood caries (S-ECC) is an aggressive form of decay that is overrepresented among Indigenous children in North America, including Canadian First Nations, Métis and Inuit, and American Indian and Alaska Natives, and reflects an underlying extreme oral health disparity in these populations (American Academy of Pediatrics 2011; Irvine et al. 2011). In some Canadian First Nations on-reserve communities, the prevalence of decay in the primary dentition can exceed 90% (Schroth et al. 2005). S-ECC is a major cause of hospital visits for young children (Sheller et al. 1997), and frequently requires rehabilitative dental surgery under general anesthesia due to the extent of decay and the young age of the children affected (American Academy of Pediatrics 2015; Schroth and Smith 2007). Alarmingly, children living in communities with a high proportion of Aboriginal residents have pediatric dental surgery rates nearly eight times higher than those living in communities with a low Aboriginal population among children 1-5 years old (Canadian Institute for Health Information 2013; Schroth et al. 2016)

In addition to the well-known microbial and host-related causal factors of caries, the etiology of ECC includes many additional factors, such as socioeconomic status, nutrition, and education (Fisher-Owens et al. 2013; Reisine and Douglass 1998). The early presentation and rapid progression in young Canadian First Nations, Métis, American Indian and Alaska Native children suggests that ECC in these populations may have distinct attributes and etiology (QUEST 2015; Schroth et al. 2009), which warrants further study.

In the current study, we utilized next-generation sequencing to analyze the plaque microbiome from Canadian First Nations and Métis children with and without S-ECC in order to investigate the role of the oral microbiome and identify any unique microbial characteristics that may account for the more aggressive presentation. Defining the etiologic microbiota for S-ECC in these populations will potentially facilitate improvements in care and caries prevention policies, important steps for reducing the extent of S-ECC and improving overall quality of life.

Materials and Methods

Study Population and Design

The study protocol was approved by the University of Manitoba's Health Research Ethics Board and was reviewed by the Assembly of Manitoba Chiefs' Health Information Research Governance Committee. First Nations children < 72 months of age and their parent or primary caregiver were recruited between March 2015 and May 2016. Children with S-ECC had severe tooth decay involving multiple primary teeth, and were recruited from the Misericordia Health Centre (MHC) in Winnipeg, Canada on the day of their scheduled dental rehabilitative surgery under general anesthetic. Caries-free children were recruited from the community and assessed to ensure that there was no evidence of caries (dmft=0). Children < 72 months of age and identified by their parent or legal caregiver as being Canadian First Nations or Métis were included in the study. Children who had taken antibiotics within the last three months were excluded. All parents or caregivers of participating children provided written informed consent.

Health-Related Questionnaire

All parents and caregivers completed an interviewed questionnaire proctored by members of the study team. Information was collected on nutritional habits, oral hygiene habits, socioeconomic and demographic characteristics, and history of previous dental visits.

Sample Collection and DNA Isolation

Plaque samples were collected from each subject by swabbing a sterile interdental brush on all available tooth surfaces. For children with S-ECC, the attending pediatric dentist obtained samples while in the operating room prior to surgery. For caries-free children, samples were collected by RJS at the Children's Hospital Research Institute of Manitoba or at community sites in Winnipeg. Samples were immediately frozen and stored at -80°C in 15% glycerol in PBS after collection until undergoing analysis. DNA was extracted from each sample using the Epicentre MasterPure DNA Purification Kit (Madison, WI), following the manufacturer's instructions after mechanical digestion with glass beads and lysozyme treatment at 37 degrees for two hours.

Sequencing and Analysis

Total DNA was sent on ice to the Forsyth Institute (Cambridge, MA) for library prep and sequencing using the MiSeq (Illumina, San Diego, CA) platform. Samples were prepped for sequencing using a previously published protocol (Caporaso et al. 2011). Briefly, 10-50 ng of DNA was used in a PCR reaction with barcoded V3-V4 primers and purified using AMPure beads (Beckman Coulter). 100 ng of each library was then pooled, gel-purified, and quantified (Bioanalzer, Agilent), and 12 pM of the mixture, spiked with 20% PhiX, was run on the MiSeq. Reads were then de-multiplexed and adaptor sequences removed. Quality filtering removed bad reads and chimeric sequences prior to analysis.

Data was analyzed using QIIME (Quantitative Insights into Microbial Ecology) v. 1.9.1(Caporaso et al. 2010). Sequences were clustered into operational taxonomic units (OTUs)

using UCLUST (Edgar 2010), and then aligned and taxonomy assigned with the HOMD database (Chen et al. 2010) as reference. For alpha (within-sample) diversity, OTU tables were rarefied to 30,000 reads and chao1 and Faith's phylogenetic diversity measures were calculated. For beta (between-sample) diversity, weighted and unweighted Unifrac distances (Lozupone and Knight 2005) were calculated, followed by principle coordinates analysis.

Statistical Analysis

Questionnaire and microbiological data were linked in an Excel spreadsheet (Microsoft Office) and analyzed using Number Cruncher Statistical Software (NCSS) version 10 (Kaysville, Utah) and GraphPad Prism v. 7. Bivariate analyses, such as Chi-Square, Fisher's exact test, and t-tests, were performed where appropriate. A P-value ≤ 0.05 was considered statistically significant. For sequencing data, differences in the relative abundances of taxa between the groups were determined with the Kruskal-Wallis test controlling the false discovery rate (FDR) to correct for multiple comparisons (Hochberg and Benjamini 1990). A corrected P-value of ≤ 0.05 was considered significant. Differences in weighted and unweighted Unifrac distances between the groups were analyzed using analysis of similarity (ANOSIM) with $P \leq 0.05$ considered significant.

Results

Demographics and Health-Related Questionnaire Data

A total of 50 children were recruited, 30 with S-ECC and 20 caries-free. The mean age of all children was 40.7 ± 11.6 months. Results from the health-related questionnaire are presented in Table 1. There were no significant differences in mean age ($P = 0.11$) or sex ($P = 0.20$) between the groups. A considerable proportion (56.7%) of children with S-ECC resided in First Nations communities while all of the caries-free children lived in the Winnipeg region. We

found a significant difference in household income (P = 0.032) between the groups, with S-ECC children coming from households with lower incomes compared to caries-free children.

There were significant differences in the proportion of children with S-ECC that were bottle fed in comparison with caries-free children (P = 0.021). Children with S-ECC also were bottle fed for a significantly longer duration (P = 0.028), and the age in which the child was weaned from the breast was significantly lower in S-ECC children (3.3 ± 5.4 months vs. 12.9 ± 11.4; P = 0.015). Children with S-ECC were also less likely to be exclusively breastfed at any point in their infancy (P = 0.0015).

Overall Sequencing Results

Plaque samples were obtained from 20 caries-free subjects and 30 subjects with S-ECC. Sequencing of the 16S rRNA gene variable V3-V4 region generated a total of 3,502,879 sequences after quality filtering, with an average of 66855 (range: 34190-89179) sequences per sample and a median length of 421. Sequences were clustered into OTUs, with a 20-sequence minimum for defining an OTU, and taxonomy was assigned using the HOMD (Human Oral Microbiome Database) as a reference (Chen et al. 2010).

Microbial Community Structure

Alpha (within-sample) diversity was calculated at a maximum depth of 30,000 sequences per sample, with the rarefaction curves shown in **Figure 1.** The samples from caries-free and S-ECC subjects do not differ in terms of species richness (**Figure 1a**) nor phylogenetic diversity **(Figure 1b**). Principle coordinate analysis (PCoA) was used on weighted and unweighted Unifrac distances to examine clustering of samples between groups (beta diversity) **(Figure 2)**. Weighted Unifrac distances take into account abundance of each taxon, while unweighted distances are calculated only based on presence/absence data (Lozupone and Knight 2005). The

samples significantly cluster based on caries status (caries-free vs. S-ECC) for both weighted and unweighted distance measures ($P < 0.05$, ANOSIM).

Taxonomic Identification and Relative Abundance

Taxonomy assignment revealed 10 phyla, 4 of which were differentially represented in the caries-free vs. S-ECC groups: Firmicutes (39.4% vs. 47.2%, P=0.01), Actinobacteria (14.4% vs. 6.8%, P=0.002), Fusobacteria (16.8% vs. 11.3%, P=0.008), and TM7 (0.5% vs. 0.24%, P=0.008). A total of 95 genera and 290 species were detected, and those with the highest relative abundance are listed in **Table 2**. 28 species-level OTUs were significantly different ($P < 0.05$) in the S-ECC vs. caries-free groups; 12 were significantly more abundant in the ECC group and 16 in the caries-free group. Most of these species have been associated with either health or caries previously; for example, the caries-free group had 5-fold and 2-fold higher abundances of *Streptococcus gordonii* and *Streptococcus sanguinis,* respectively, than the S-ECC group, while the S-ECC group had 7-fold and 9-fold higher levels of an *Haemophilus* species (HOT 036) and a *Porphyromonas* species (HOT 284), respectively. In addition, a *Veillonella* species (HOT 780) was 4.6-fold higher in the S-ECC group, although the relative abundances were low. Interestingly, subjects that had a high relative abundance (defined as >2%) of *Prevotella melaninogenica* were 8 times more likely to be in the S-ECC group than those that did not have high levels ($P = 0.04$, Fisher's exact test). Conversely, subjects that had high relative abundances of *Rothia aeria* or *Corynebacterium matruchotii* were significantly more likely to be caries-free than those that did not have high levels (60% vs. 0% and 55% vs. 10% respectively, $P < 0.01$ for both).

Streptococcus mutans was detected in all samples, with a 3-fold higher amount detected in the S-ECC group compared to the caries-free group. However, the S-ECC group contained

subjects with extremely high values. Six subjects in the S-ECC group were carrying greater than 5% *S. mutans*, two subjects with over 10%, and one subject with an extraordinarily high level of almost 23% of the total species detected. For comparison, in the caries-free group there was only one subject with over 5% *S. mutans* **(Figure 3).** *Scardovia wiggsiae* has also been recently characterized as a possible important factor in early childhood caries (Tanner et al. 2011b); in the current study, S-ECC children had on average 7-fold higher levels of this organism than the caries-free children, although the relative abundances were low (0.007% vs. 0.001%).

Discussion

To the best of our knowledge, this is the first study to use advanced microbial analyses to investigate the oral microbiome of North American Indigenous children, specifically, Canadian First Nations and Métis children, affected by S-ECC. Despite recent advances in the understanding of the role of the oral microbiome in health and disease, knowledge of its importance in the etiology of ECC is lacking, especially in Indigenous populations. The literature clearly reveals that Indigenous children suffer considerable oral health disparities compared to other children of the same age. Much of this stems from the historical and ongoing effects of colonialism and racism that have resulted in major socioeconomic and healthcare inequities (American Academy of Pediatrics 2011). With rates of S-ECC in these populations drastically higher than rates in the general population, and with the potential for S-ECC to negatively impact systemic health and quality of life, it is of critical importance to investigate the underlying causes and identify any potential unique risk factors that may exist (Schroth et al. 2009; Schroth et al. 2016).

The results of the health questionnaire confirm previously reported behavioral and socioeconomic risk factors, including less frequent brushing, bottle-feeding, later age at weaning, and lower household income (American Academy of Pediatrics 2011; Reisine and Douglass 1998). Although previously known as "nursing caries" or "baby-bottle tooth decay," names that reflected the presumed behavioral and feeding-related etiology, it has become apparent more recently that S-ECC is a complex, multifactorial disease and that there is a major microbiological component (Irvine et al. 2011; QUEST 2015).

We analyzed the plaque microbiome in each group by sequencing a region of the 16S rRNA gene. The results show a statistically significant separation between the microbiomes of the S-ECC and caries-free groups (**Figure 2**), indicating that the composition of the entire microbial community is an important determining factor. Interestingly, the groups did not differ in terms of species or phylogenetic diversity **(Figure 1**). Some studies have shown that increased diversity, including species richness, is associated with health (Belstrøm et al. 2014; Gross et al. 2012; Xiao et al. 2016); however, there is conflicting evidence, with a few studies reporting the opposite (Griffen et al. 2012; Johansson et al. 2016; Xu et al. 2014). Most likely, diversity in general is not as important as a caries determinant as is the presence and quantity of particular organisms and the environment in which they reside.

Sequencing revealed 10 phyla, 4 of which were significantly differentially represented in each group. The S-ECC group had a higher abundance of Firmicutes, while Actinobacteria and Fusobacteria were higher in the caries-free group. This supports a recent longitudinal study in young children, in which Actinobacteria decreased and Firmicutes increased as caries progression proceeded (Gross et al. 2012). Focusing only on the most abundant taxa, seven of the top 25 genera detected were significantly different between the groups. *Alloprevotella* was

significantly increased in the S-ECC group; this genus was also reported to be increased in a study of adult subjects with caries (Xiao et al. 2016). The genera *Leptrotrichia, Rothia, Corynebacterium, Actinomyces, Cardiobacterium,* and TM7 [G-1] were significantly higher in the caries-free group. These have been frequently identified in plaque and associated with health (Johansson et al. 2016; Tanner et al. 2011a; Xiao et al. 2016; Xu et al. 2014). Interestingly, *Cardiobacterium* is an oropharyngeal commensal known for its role in endocardial infections, and although it has been occasionally reported to be health-associated and in decreased abundance in caries in adults and children (Tanner et al. 2011a; Xiao et al. 2016), its role in oral health has not been thoroughly investigated.

Of the top 25 most abundant species detected, *Granulicatella elegans, Prevotella melaninogenica,* and a *Haemophilus* species (HOT 036) were significantly more abundant in the S-ECC group. *G. elegans* and *Prevotella melaninogenica* have been previously reported to be increased in children with S-ECC compared to caries-free (Kanasi et al. 2010; Ling et al. 2010; Tanner et al. 2011a). Conversely, we found that *Rothia aeria, Corynebacterium matruchotii*, *Actinomyces naeslundii*, and *Streptococcus sanguinis* were significantly increased in the caries-free group. Both *C. matruchotii* and *A. naeslundii* have been previously associated with health and caries-free status (Gross et al. 2010; Ma et al. 2015; Marchant et al. 2001; Tanner et al. 2011a). *S. sanguinis* is a known health-related species that has been shown to have an inverse and antagonistic relationship with *S. mutans* (Caufield et al. 2000; Kreth et al. 2008). *Rothia spp*. are commonly detected in plaque (Aas et al. 2008; Bik et al. 2010; Kanasi et al. 2010; Ling et al. 2010), but *Rothia aeria* has not been previously particularly associated with health. Caries-free subjects in our study have almost five times the amount of *R. aeria* on average compared to the S-ECC children. Interestingly, another *Rothia* species, *R. dentocariosa,* has been previously

associated with S-ECC (Jiang et al. 2016), but in our study the caries-free group had >2-fold higher levels. This discrepancy may be one example of the uniqueness of this particular population, and reinforces the need for further study of dental health in Indigenous children.

The relative abundance of *Streptococcus mutans,* the quintessential cariogenic organism, was three times higher in the S-ECC group than in the caries-free group; but the average value masks the extremely high levels in some children in the S-ECC group (**Figure 3)** of up to 23% of the total taxa detected. Interestingly, a recent study comparing the microbiomes of European adolescents with and without caries from two different countries showed that the relative importance of *S. mutans* in determining caries status was different based on where the populations resided; the role of *S. mutans* as an important etiologic factor was more pronounced in the population lacking access to caries prevention and treatment strategies compared to one in which there was adequate dental care (Johansson et al. 2016). This supports the idea that the cariogenic etiology of certain populations may be unique based on socioeconomic or geographic factors, and the high levels of *S. mutans* in some subjects in our study may be a reflection of that. In addition, similarly high levels of *S. mutans* were detected in a separate Indigenous population (Shi, W. *unpublished data).*

Notably, there were 2 subjects (out of 20 total) in the caries-free group that have a high relative abundance (>2%) of *S. mutans*; these 2 subjects also have high levels (>2%) of both *Rothia aeria* and *Corynebacterium matruchotii,* two species significantly associated with caries-free status in this study. In the S-ECC group, there were 10 subjects (out of 30 total) with high abundances of *S. mutans,* with none exhibiting high levels of *R. aeria* and *C. matruchotii.* This suggests that certain health-related species may protect against the risk of carrying a high

abundance of *S. mutans,* and indicates that the balance and structure of the microbial community as a whole may be the most important factor in determining caries risk.

This study is not without limitations. Due to budgetary constraints, we relied on a convenience sample of children with S-ECC on the day of their dental surgery. All controls were from Winnipeg, and those with S-ECC were from different First Nations communities or off-reserve communities, including Winnipeg. Some of the questions posed to parents and caregivers were retrospective in nature, which might have resulted in recall bias, and the potential for response bias on the part of parents and caregivers is noted.

Overall, this study yielded important information on the microbiome in First Nations and Métis children with S-ECC and those free from caries. The only previous study to investigate the microbiology of Canadian First Nations children with S-ECC was a longitudinal observation in 1985 (Milnes and Bowden 1985). Therefore, this is the first study to investigate the microbiome of this population using modern molecular techniques. We confirmed previous reports that implicate behavioral as well as microbiological factors in the development of S-ECC, with *S. mutans* as the major cariogenic factor, along with many other species. Furthermore, we found that the S-ECC and caries-free groups represent disparate plaque microbial communities, supporting the notion that there is potentially a distinct caries-causing community that can eventually be identified and used for diagnosis and prognosis. It is clear that socioeconomics, cultural factors, and microbiology all play a role in the high rates of S-ECC experienced by Canadian Indigenous populations, but the finer details are still very much unknown. Therefore, it is imperative to continue to study the underlying causes (including the microbiome) of the extreme oral health disparities that these populations face in order to provide prevention and treatment services that accurately reflect the underlying etiology.

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Conflict of Interest Statement

Melissa Agnello has no conflict of interest; Jesse Marques has no conflict of interest; Lujia Cen has no conflict of interest; Betty-Anne Mittermuller has no conflict of interest; Alice Huang has no conflict of interest; Nini Chaichanasakul Tran has no conflict of interest; Wenyuan Shi is the founding scientist for C3 Jian, Inc., which has licensed an anti-*S. mutans* technology from UC Regents that could be indirectly related to this research project; Xuesong He has no conflict of interest; Robert J Schroth has no conflict of interest

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Figure Legends

Figure 1. Rarefaction curves of diversity indices. A) chao1 (species richness) and B) Faith's phylogenetic diversity index. S-ECC plaque samples are in red, caries-free in blue.

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Table 1. Demographics and Health Characteristics of Study Population

Variable	Caries Status		
	Caries-free	S-ECC	P Value
Mean Age in Months*	37.4 ± 10.3	42.8 ± 12.2	0.11
Sex[t]			
Male	9 (32.1%)	19 (67.9%)	0.20
Female	11 (50.0%)	11 (50.0%)	
Average Weight at Birth*	3529.9 ± 699.0	3421.3 ± 573.2	0.57
Premature[t]			
Yes	2 (16.7%)	10 (83.3%)	0.073[a]
No	18 (48.7%)	19 (51.4%)	
Feeding Habits[t]			
Child was breastfed			
Yes	13 (52.0%)	12 (48.0%)	0.083
No	7 (28.0%)	18 (72.0%)	
Child was *exclusively* breastfed			
Yes	12 (70.6%)	5 (29.4%)	**0.0015**
No	8 (24.2%)	25 (75.8%)	
Child was bottle fed			
Yes	16 (34.8%)	30 (65.2%)	**0.021[a]**
No	4 (100.0%)	0 (0.0%)	
Age the child was weaned from the breast*	12.9 ± 11.4	3.3 ± 5.4	**0.014**
Age the child was weaned from the bottle*	17.9 ± 8.9	25.8 ± 12.0	**0.028**
Number of times per day child snacks*	3.7 ± 1.7	3.9 ± 1.4	0.71
Oral Hygiene Habits[t]			
Child brushes ≥ daily	17 (51.2%)	16 (48.5%)	**0.032[a]**
Child brushes < daily	3 (17.7%)	14 (82.4%)	
Yearly Household income[t]			
> 28,000$	7 (70.0%)	3 (30.0%)	**0.032[a]**
< 28,000$	12 (32.4%)	25 (67.6%)	
Family Size[t]			
Other children	2 (50.0%)	2 (50.0%)	1.00[a]
Only child	18 (39.1%)	28 (60.9%)	
Receives Social Assistance[t]			
Yes	13 (37.1%)	22 (62.9%)	0.41
No	7 (50.0%)	7 (50.0%)	
Lives in a First Nation Community[t]			
Yes	0 (0.0%)	17 (100.0%)	**0.000010[a]**
No	20 (60.6%)	13 (39.4%)	
Age of child at first dental visit	20.8 ± 16.0	27.8 ± 14.6	0.11

(months):

[r]chi square analysis, * = t-test, [a] = Fisher's exact

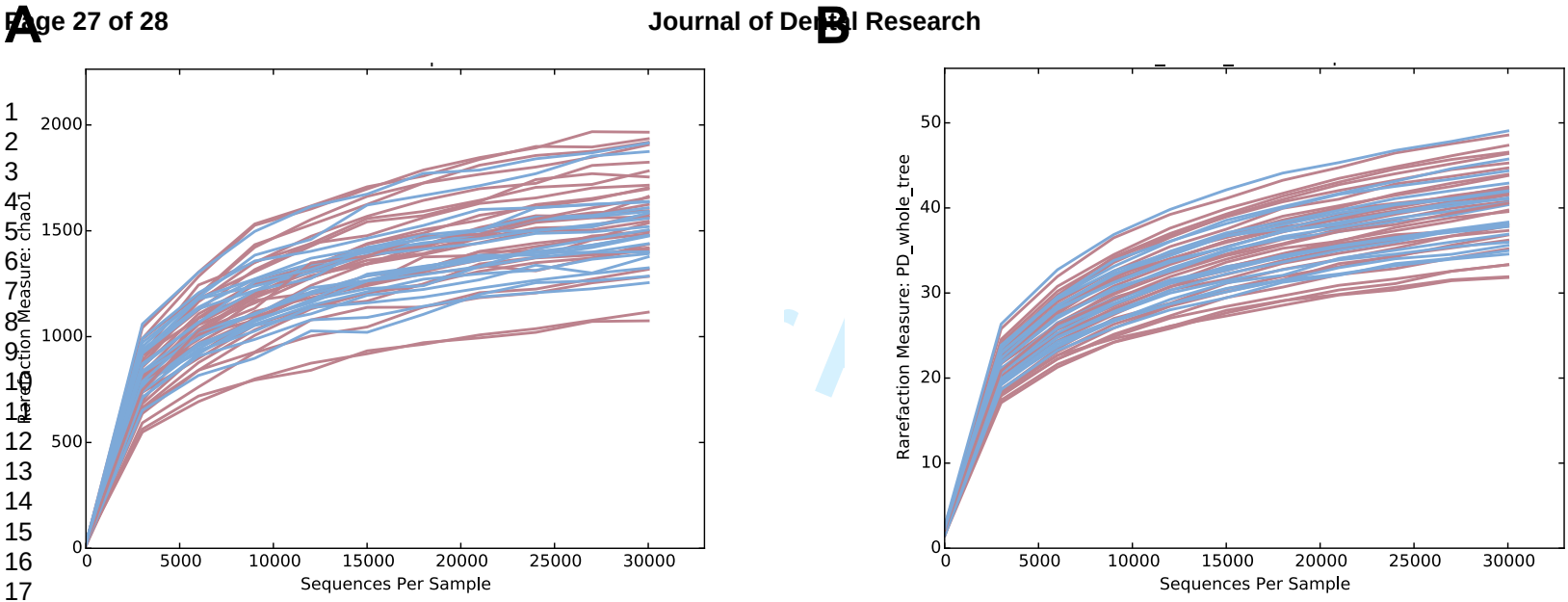
Table 2. Relative Abundance of the Top 25 Species-level and Genera-level Operational Taxonomic Units (OTUs) Detected in Plaque of Caries-Free (CF) Children and Children with S-ECC

Species-level OTU	Median Relative Abundance, % *(Range)*	
	Caries-Free *(n=20)*	**S-ECC** (*n=30*)
Streptococcus HOT 058	23.7 (*12.3 – 42.0)*	26.7 (*11.1 – 42.3*)
Leptotrichia shahii	3.4 (*0 – 16.7*)	2.1 (*0.2 – 11.7*)
Lautropia mirabilis	3.2 *(0.2 – 11.6)*	2.2 *(0.05 – 10.0)*
Haemophilus parainfluenzae	2.0 *(0.01 – 7.2)*	3.1 *(0.12 – 12.6)*
Veillonella dispar	2.2 *(0.15 – 9.4)*	3.0 *(0.33 – 19.0)*
*Rothia aeria**	2.4 (*0.37- 7.6*)	0.52 *(0.004 – 1.7)*
*Corynebacterium matruchotii**	2.0 *(0.66 – 5.4)*	0.85 (*0.13 – 5.4)*
*Actinomyces naeslundii**	1.8 (*0.68 – 6.2)*	0.67 *(0.15 – 5.7)*
Rothia dentocariosa	1.7 *(0.12 – 24.5)*	0.70 *(0.16 – 5.5)*
Abiotrophia defectiva	1.1 *(0.07 – 6.3)*	1.3 *(0.003 – 5.7)*
Gemella haemolysans	0.87 *(0.09 – 3.8)*	1.2 *(0.15 – 5.3)*
Granulicatella adiacens	0.82 *(0.14 – 2.3)*	1.1 *(0.02 – 2.4)*
Porphyromonas HOT 279	0.63 *(0.03 – 3.5)*	1.1 (*0.02 – 8.4)*
*Granulicatella elegans**	0.32 *(0.03 – 1.2)*	1.0 (*0.003 – 4.0)*
Leptotrichia HOT 225	1.0 *(0.03 – 4.5)*	0.45 (*0.008 – 5.9)*
*Fusobacterium nucleatum ss. vincentii_*	0.54 *(0.04 – 3.8)*	0.88 *(0.09 – 4.1)*
Corynebacterium durum	0.80 *(0.14 – 9.4)*	0.41 *(0 – 4.3)*
Streptococcus mutans	0.15 *(0.006 – 10.4)*	0.73 (*0.02 – 22.9)*
*Prevotella melaninogenica**	0.10 *(0.002 – 3.6)*	0.71 *(0.03 – 11.8)*
*Alloprevotella HOT 473**	0.04 *(0 – 1.7)*	0.69 *(0.001 – 9.3)*
Gemella morbillorum	0.58 *(0.11 – 3.3)*	0.69 *(0.07 – 2.0)*
*Haemophilus HOT 036**	0.07 *(0.003 – 0.3)*	0.56 *(0.001 – 3.5)*
*Streptococcus sanguinis**	0.56 *(0.19 – 0.8)*	0.28 *(0.13 – 0.7)*
Neisseria mucosa	0.44 *(0.09 – 1.2)*	0.34 *(0.02 – 1.2)*
Aggregatibacter HOT 458	0.25 *(0.003 – 2.3)*	0.43 *(0.05 – 2.8)*
Genera-level OTU		
Streptococcus	28.3 *(16.8 – 49.6)*	31.3 *(12.8 – 50.0)*
*Leptotrichia**	10.5 *(4.2 – 23.7)*	5.7 *(0.61 – 30.4)*
Neisseria	7.5 *(0.70 – 27.9)*	9.0 *(0.22 – 26.4)*
*Rothia**	4.8 *(0.72 – 29.9)*	1.7 *(0.04 – 10.3)*
Fusobacterium	4.8 *(0.65 – 12.3)*	3.7 *(1.1 – 9.5)*
Haemophilus	2.1 *(0.01 – 7.5)*	4.6 *(0.13 – 12.8)*
Veillonella	2.4 *(0.18 – 10.1)*	4.1 *(0.39 – 19.8)*
*Corynebacterium**	3.3 *(1.4 – 14.8)*	1.6 *(0.01 – 8.1)*
*Actinomyces**	3.2 *(1.4 – 9.4)*	1.8 *(0.25 – 7.4)*

Lautropia	3.2 *(0.19 – 11.6)*	2.2 *(0.05 – 10.2)*
Prevotella	0.93 *(0.17 – 9.1)*	2.5 *(0.20 – 26.5)*
Granulicatella	1.3 *(0.22 – 2.6)*	2.3 *(0.06 – 5.7)*
Gemella	1.6 *(0.20 – 4.9)*	2.0 *(0.29 – 7.0)*
Porphyromonas	1.3 *(0.08 – 5.1)*	1.8 *(0.023 – 9.2)*
Capnocytophaga	1.5 *(0.48 – 5.1)*	0.94 *(0.19 – 2.7)*
Abiotrophia	1.1 *(0.07 – 6.3)*	1.3 *(0.003 – 5.7)*
Kingella	1.2 *(0.61 – 2.5)*	0.80 *(0.065 – 2.1)*
*Alloprevotella**	0.13 *(0.003 – 1.7)*	1.0 *(0.006 – 9.5)*
Aggregatibacter	0.88 *(0.01 – 3.3)*	0.99 *(0.14 – 4.1)*
Campylobacter	0.59 *(0.14 – 2.5)*	0.46 *(0.08 – 3.9)*
Selenomonas	0.29 *(0.02 – 3.7)*	0.54 *(0.02 – 4.2)*
*Cardiobacterium**	0.45 (*0.04 – 2.2)*	0.17 *(0.002 – 0.7)*
Lachnoanaerobaculum	0.43 *(0.16 – 2.6)*	0.32 *(0.03 – 1.6)*
*TM7 [G-1]**	0.33 *(0.004 – 1.4)*	0.11 *(0.005 – 1.8)*
Bergeyella	0.33 *(0.03 – 0.8)*	0.27 *(0.05 – 1.2)*

HOT = Human Oral Taxon

**P ≤ 0.05, Kruskal-Wallis test, corrected for multiple comparisons by the FDR method*



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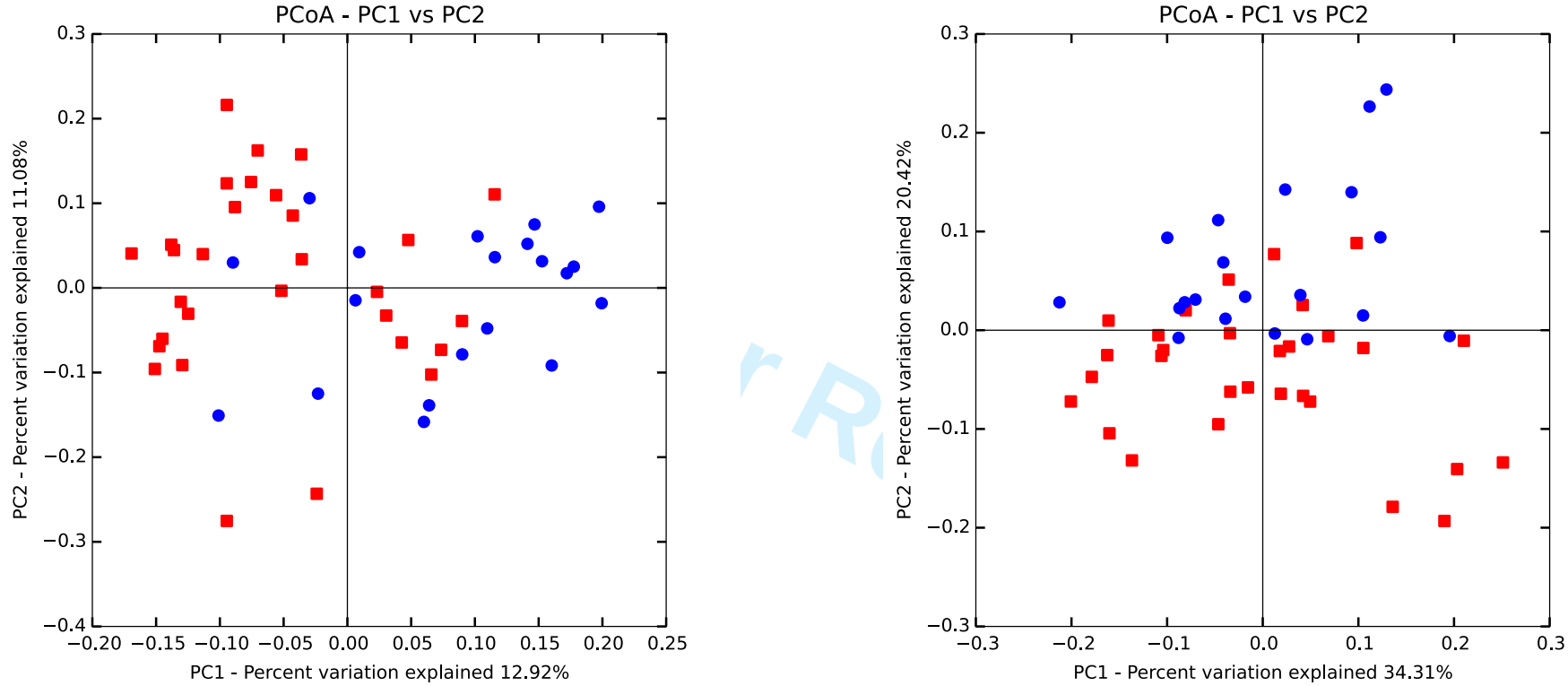

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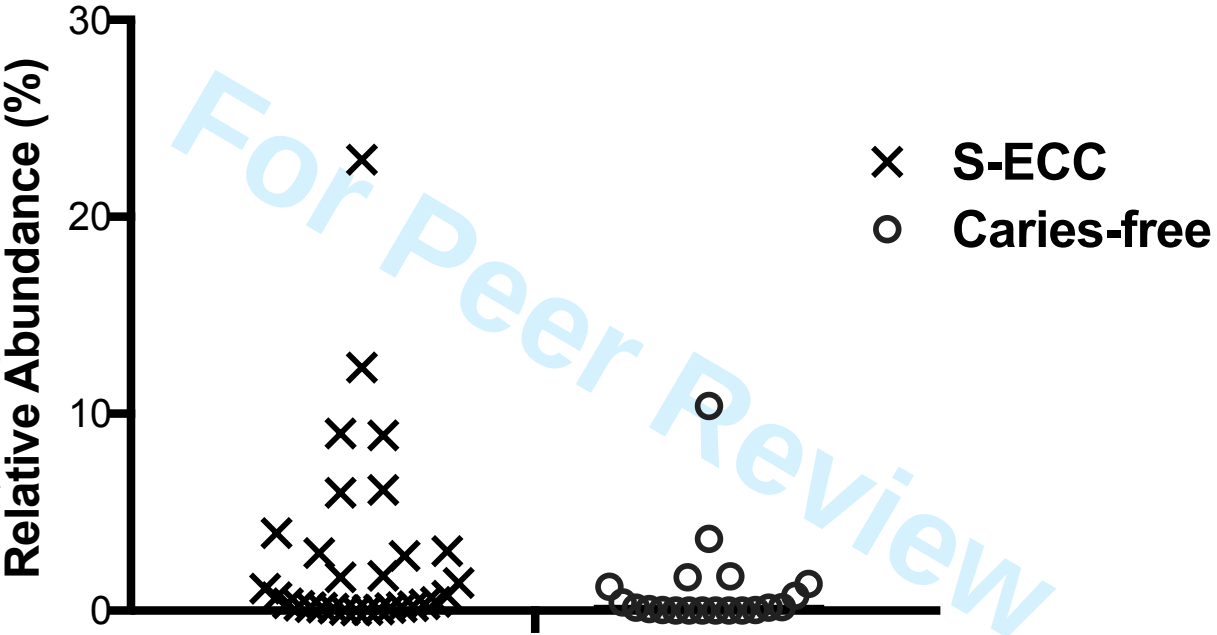

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