Association between Oral *Candida* and Bacteriome in Children with Severe ECC

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Abstract

Candida albicans is an opportunistic fungal organism frequently detected in the oral cavity of children with severe early childhood caries (S-ECC). Previous studies suggested the cariogenic potential of *C. albicans*, in vitro and in vivo, and further demonstrated its synergistic interactions with *Streptococcus mutans*. In combination, the 2 organisms are associated with higher caries severity in a rodent model. However, it remains unknown whether *C. albicans* influences the composition and diversity of the entire oral bacterial community to promote S-ECC onset. With 16s rRNA amplicon sequencing, this study analyzed the microbiota of saliva and supragingival plaque from 39 children (21 S-ECC and 18 caries-free [CF]) and 33 mothers (17 S-ECC and 16 CF). The results revealed that the presence of oral *C. albicans* is associated with a highly acidogenic and acid-tolerant bacterial community in S-ECC, with an increased abundance of plaque *Streptococcus* (particularly *S. mutans*) and certain *Lactobacillus/Scardovia* species and salivary/plaque *Veillonella* and *Prevotella*, as well as decreased levels of salivary/plaque *Actinomyces*. Concurrent with this microbial community assembly, the activity of glucosyltransferases (cariogenic virulence factors secreted by *S. mutans*) in plaque was significantly elevated when *C. albicans* was present. Moreover, the oral microbial community composition and diversity differed significantly by disease group (CF vs. S-ECC) and sample source (saliva vs. plaque). Children and mothers within the CF and S-ECC groups shared microbiota composition and diversity, suggesting a strong maternal influence on children's oral microbiota. Altogether, this study underscores the importance of *C. albicans* in association with the oral bacteriome in the context of S-ECC etiopathogenesis. Further longitudinal studies are warranted to examine how fungal-bacterial interactions modulate the onset and severity of S-ECC, potentially leading to novel anticaries treatments that address fungal contributions.

Keywords: *Streptococcus mutans*, early childhood caries, microbiota, glucosyltransferase, maternal influence, *Candida* species

Introduction

Although largely preventable, early childhood caries (ECC) remains the most common chronic childhood disease, with nearly 1.8 billion new cases per year globally (Dye et al. 2012; Vos et al. 2017). Severe ECC (S-ECC) occurs earlier in life than ECC, with more morbidity, often requiring total oral rehabilitation under general anesthesia with multiple tooth extractions, restorations, and crowns (Hajishengallis et al. 2017). The average cost of each total oral rehabilitation is nearly \$7,000 in the United States (Rashewsky et al. 2012), and multiple interventions are common (Graves et al. 2004; Berkowitz et al. 2011), despite complementary pharmacologic treatments, such as topical fluoride/antibacterial applications and dietary counseling (O'Sullivan and Tinanoff 1996; Li and Tanner 2015). Hence, S-ECC constitutes a major challenge to the public health system (Dye et al. 2012) and demands enhanced understanding of disease etiopathogenesis and more effective therapeutic strategies to reduce recurrence and costs.

The microbial etiology of S-ECC has been linked with polybacterial infection of teeth. However, available microbiological data also show that fungal organisms are associated with this pediatric oral disease. In particular, *Candida* species

(especially *Candida albicans*) were often detected at higher levels in the oral cavity of children with S-ECC as compared with caries-free (CF) children, and fungal presence positively correlated with caries severity and *Streptococcus mutans* carriage (as reviewed by Xiao et al. 2018). Moreover, laboratory findings provided plausible biological evidence of the cariogenic

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A supplemental appendix to this article is available online.

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traits of *C. albicans*: 1) it is acidogenic and aciduric (Klinke et al. 2009); 2) it is capable of dissolving hydroxyapatite, a major tooth-enamel component (Nikawa et al. 2003); and 3) it increases *S. mutans* accumulation and extracellular matrix production in mixed-species biofilms (Gregoire et al. 2011; Metwalli et al. 2013; Falsetta et al. 2014; Sztajer et al. 2014; Hwang et al. 2015). Importantly, these in vitro findings were supported by rodent models showing that *C. albicans* can be cariogenic (Klinke et al. 2011) while synergizing with *S. mutans* to cause more severe and rampant caries on smooth surfaces (Falsetta et al. 2014).

Clinical findings combined with experimental in vivo data point toward active fungal participation in S-ECC and lead to the possibility that the presence of *C. albicans* is associated with changes in the oral bacterial community that promotes S-ECC. In this study of mother-child dyads (S-ECC and CF), we investigated whether the presence of *C. albicans* in S-ECC is correlated with a distinctive oral bacterial composition of saliva and plaque. Furthermore, we explored associations between the oral microbiota of the children and their mothers.

To our knowledge, this study provides the first clinical evidence about the impact of fungi (*C. albicans*) on the oral bacteriome in S-ECC in mother-child dyads. Our results suggest a potential role of *C. albicans* in promoting a cariogenic bacteriome, indicating that future longitudinal and therapeutic studies may need to include the fungal component to assess children at risk for S-ECC and to maintain a healthy oral ecosystem.

Methods

Oral Examination and Sample Collection

Ethical approval of the study was obtained from the University of Rochester Research Subject Review Board. A cohort of mother-child dyads (S-ECC and CF) was enrolled from patients seen at the Eastman Institute for Oral Health Pediatric Dentistry Clinic, University of Rochester (2015 to 2016). Subjects who had severe systemic diseases or antibiotic treatment within the 3 previous months were excluded. Sociodemographic and oral hygiene behavior characteristics were collected. A comprehensive oral examination was performed on the day of the sample collection by 1 of 2 calibrated dentists (intraexaminer kappa = 0.82, interexaminer kappa = 0.82), who used a standard dental mirror and Community Periodontal Index probe with portable headlight. S-ECC was diagnosed per criteria of the American Academy of Pediatric Dentistry. The number of carious teeth and surfaces was charted with index variables of *decayed*, *missing due to decay*, or *filled*, according to the codes proposed by the World Health Organization's *Oral Health Surveys Basic Methods* (1997). Plaque status for mothers and children were assessed separately according to criteria modified from Silness and Le (1964) and Ribeiro Ade et al. (2002). Nonstimulated whole saliva was collected from subjects through a saliva jet connected to a suction pump at least 2 h after any toothbrushing, eating, or drinking. Supragingival dental plaque was collected from the whole dentition with a standard dental scaler.

Sample collection devices were pretreated and tested as DNA free.

C. albicans Identification and Bacterial Community Profiling

Previous established methods were used to isolate and identify *C. albicans* (Xiao et al. 2016) and to perform oral microbiome sequencing and related bioinformatics analysis (Merkley et al. 2015; Grier et al. 2017), as detailed in the Appendix.

Glucosyltransferase Enzymatic Activity in Plaque

Plaque glucosyltransferase (Gtf) enzyme activity was examined with methods detailed in the Appendix.

Statistical Analysis

One-way analysis of variance with the Tukey-Kramer test was used to compare the proportions of the most populated genus/species relative abundance among 3 groups: CF, S-ECC *C. albicans* positive (S-ECC CA+), and S-ECC *C. albicans* negative (S-ECC CA−). DESeq2 negative binomial Wald test was used to compare the microbial abundance at species level among groups at each time: *C. albicans* conditions (positive and negative) among children with S-ECC, child and mother who were CF, and child and mother with S-ECC. Nonparametric *t* test and 2-sample *t* test were used to compare the alpha and beta diversity between groups at a time. Two-sample *t* test was used to compare plaque Gtf enzymatic activity differences between the S-ECC and CF groups and between the groups with the presence and absence of oral *C. albicans*. Spearman's rank test was used to analyze the correlation between plaque Gtf activity and caries severity. $P < 0.05$ was considered statistically significant.

Results

Sample Characteristics

A total of 39 children (21 S-ECC and 18 CF) and 33 biological mothers (17 S-ECC and 16 CF) were enrolled in the study (6 children were not accompanied by their mothers at their study visits). Chi-square analysis ($P > 0.05$) revealed no significant sociodemographic or behavior differences between the S-ECC and CF groups with regard to age, sex, race, ethnicity, and toothbrushing frequency (Table). Notably, for children and mothers, the plaque score was significantly higher in the S-ECC group than the CF group ($P < 0.05$). Mothers with S-ECC had a higher score for decayed teeth than did mothers who were CF ($P < 0.05$).

Sequencing Data

Illumina sequencing produced an average of >91,000 reads per sample after quality control and operational taxonomic unit

Table. Sociodemographic and Oral Examination Data from the Study Subjects.

Variable	Children			Mothers		
	S-ECC (n = 21)	CF (n = 18)	P Value	S-ECC (n = 17)	CF (n = 16)	P Value
Age, y	4.0 ± 0.9	3.8 ± 1.6	0.52	31.8 ± 6.8	32.6 ± 7.1	0.87
Female	43 (9)	33 (6)	0.8			
Race						
Caucasian	66 (14)	44 (8)	0.28	66 (11)	38 (6)	0.29
African American	19 (4)	39 (7)		17 (3)	38 (6)	
Asian	14 (3)	17 (3)		17 (3)	24 (4)	
Ethnicity: Hispanic	10 (2)	16.7 (3)	0.59	12 (2)	19 (3)	0.66
Toothbrushing, times/day						
2	52 (11)	88 (15)	0.15	70 (12)	94 (15)	0.13
1	33 (7)	12 (2)		24 (4)	6 (1)	
<1	10 (2)	0 (0)		6 (1)	0 (0)	
Plaque score	2.0 ± 0.7	0.8 ± 1.0	0.001	2.0 ± 0.6	1.0 ± 0.9	0.001
dmft/DMFT	10.3 ± 5.1	0	0.000	9.8 ± 6.9	3.8 ± 3.5	0.002
dmfs/DMFS	25.2 ± 13.9	0	0.000	24.6 ± 22.3	9.5 ± 14.1	0.01

Values are presented as mean ± SD or % (n).

CF, caries free; S-ECC, severe early childhood caries.

identification among all sequenced samples. Five samples with markedly fewer reads than the threshold of 38,000 were excluded from all analyses (Appendix Fig. 1). The final taxonomic analysis included 37 salivary samples (19 S-ECC and 18 CF) and 69 plaque samples (children, 21 S-ECC and 15 CF; mothers, 17 S-ECC and 16 CF). Detection of *C. albicans* and other *Candida* species in the S-ECC and CF groups are shown in Appendix Figure 2, and *C. albicans* was the most prevalent isolate as compared with other *Candida* strains in children with S-ECC.

Saliva versus Plaque Microbiota

Overall, the operational taxonomic unit abundance of the microbiota in the S-ECC and CF groups was determined by sample type (saliva or plaque), caries status (S-ECC or CF), and presence of *C. albicans* (Fig. 1). The microbiota composition differed notably between saliva and plaque samples, regardless of caries status. At the genus level, the 2 most abundant genera in children's saliva (Fig. 1A) were *Streptococcus* and *Veillonella*, whereas in plaque (Fig. 1B), the most abundant genera were *Veillonella*, *Streptococcus*, *Actinomyces*, *Selenomonas*, and *Leptotrichia*. At the species level, 3 dominant taxa represented 60% of the relative abundance in the salivary community (Fig. 1C): *Streptococcus ET G 4d04*, *Veillonella atypica dispar parvula*, and *Streptococcus vestibularis salivarius*. In contrast, the 5 most abundant species, representing 30% to 50% of the bacterial plaque community (Fig. 1D), were *V. atypica dispar parvula*, *S. mutans*, *S. ET G 4d04*, *Streptococcus oral taxon B66*, and *Streptococcus gordonii*.

Oral Microbiota in the Presence or Absence of *C. albicans*

The composition of the oral microbiota reflected more acidogenic and aciduric species in children with S-ECC versus those

who were CF, and this difference was enhanced by the presence of *C. albicans*. To further compare the influence of caries status and *C. albicans*, saliva and plaque samples were grouped as follows: CF, S-ECC CA+, and S-ECC CA− (Fig. 1).

At the genus level, *Streptococcus* was the most abundant in all saliva samples; moreover, higher abundance of *Streptococcus* was detected in samples from the CF group (47%) as compared with the S-ECC CA− (40%) and S-ECC CA+ (37%) groups. There was an opposite trend with salivary *Veillonella*, which was most abundant in the S-ECC CA+ group (36%), decreased in S-ECC CA− (27%), and down to 22% in CF. Interestingly, when CF samples were compared with the S-ECC CA+ samples in regard to other species, we observed for S-ECC CA+ a significantly higher ($P < 0.05$) abundance of *Prevotella*, *Cardiobacterium*, *Capnocytophaga*, *Kingella*, *Abiotrophia*, *Lactobacillus*, *Dialister*, *Fusobacterium*, *Atopobium*, *Alloprevotella*, *Bifidobacterium*, *Scardovia*, *Dolosigranulum*, and *Moraxella* and a significantly lower abundance ($P < 0.05$) in *Lautropia*, *Propionibacterium*, and *Porphyromonas*. In parallel, when the plaque microbiota was compared between S-ECC CA+ and CF samples, a higher abundance ($P < 0.05$) of *Veillonella* was detected in S-ECC CA+, while a lower abundance ($P < 0.05$) was observed for *Selenomonas*, *Leptotrichia*, *Capnocytophaga*, *Alloprevotella*, *Slackia*, *Parvimonas*, *Neisseria*, *Propionibacterium*, *Cardiobacterium*, *Tannerella*, *Dialister*, *Lactobacillus*, and *Atopobium*.

At the species level, salivary microbiota in S-ECC samples versus CF samples had a significantly higher abundance of *V. atypica dispar parvula* and *S. vestibularis salivarius* and a lower abundance of *S. ET G 4d04*, regardless of the presence of *C. albicans*. In plaque samples of children with S-ECC versus those who were CF, a drastically higher abundance of *V. atypica dispar parvula* and *S. mutans* was noted, and this difference was even greater when *C. albicans* was present. The abundance of a well-known cariogenic species, *S. mutans*, was markedly different across groups: 1% in CF, 2% in S-ECC CA−, and 12% in S-ECC CA+ ($P < 0.05$: CF vs. S-ECC CA+,

Figure 1. Relative abundance of bacterial genera and species in the microbiome of children by caries status and the presence of *Candida albicans* in saliva and plaque samples. One-way analysis of variance with Tukey-Kramer test was used to compare the proportions of the most populated genus/species relative abundance among 3 groups: CF and S-ECC *C. albicans* positive and negative. The upper graph reveals that the presence of *C. albicans* is associated with a distinctive oral microbiota in children with S-ECC, with highly acidogenic and acid-tolerant bacterial genera in addition to an increased abundance of *Veillonella* and *Prevotella* in (A) saliva and (B) plaque. (C) The lower graph shows low levels of *S. mutans* in saliva as compared with other streptococcal species. However, in (D) plaque, *S. mutans* abundance was dramatically increased in samples from the S-ECC group versus the CF group when *C. albicans* was present. * $P < 0.05$ between CF and S-ECC–*C. albicans* (–). $^{\dagger}P < 0.05$ between CF and S-ECC–*C. albicans* (+). $^{\S}P < 0.05$ between S-ECC–*C. albicans* (–) and S-ECC–*C. albicans* (+). CF, caries free; S-ECC, severe early childhood caries.

Figure 2. Relative fold change in abundance of species in (A) saliva and (B) plaque from children with S-ECC in the presence or absence of *Candida albicans* (Ca). Bars show the fold change for each species, calculated as abundance in the presence of *C. albicans* divided by the abundance in its absence. The DESeq2-negative binomial Wald test was used to compare the relative abundance of the listed bacterial species; all strains listed have a raw *P* value < 0.05. Strains marked with an asterisk (*) have a significant difference in abundance with an adjusted *P* value < 0.05.

S-ECC CA- vs. S-ECC CA+). In contrast, the abundance of several strains was significantly lower ($P < 0.5$) in S-ECC CA+ as compared with CF samples, including *S. oral taxon B66*, *Actinomyces viscosus naeslundii oris*, and *Leptotrichia BU064*. Conversely, we detected unique species in CF samples, such as *Leptotrichia oral taxon 225* and *Leptotrichia shahii*.

Relative Change in Taxa Abundance Associated with *C. albicans*

We next determined the relative change in microbial abundance associated with *C. albicans* in children with S-ECC, as shown in Figure 2. The presence of *C. albicans* was associated with a significant fold change of taxa in saliva and plaque ($P < 0.05$). In saliva, *C. albicans* was associated with a higher abundance of *Leptotrichia* and a lower abundance of *Capnocytophaga gingivitis*, *Aggregatibacter AY349380*, *Cardiobacterium hominis*, *Capnocytophaga granulosa*, and *Prevotella oral taxon 781*. In plaque, *C. albicans* was associated with a 4-fold higher abundance of *S. mutans*, as well as a higher abundance of *Lactobacillus gasseri* and *Lactobacillus vaginalis* and a lower abundance of *Lactobacillus plantarum*.

Community Diversity and Cluster Analysis

Further analysis of the microbial community revealed that bacterial composition in the plaque was more diverse than in saliva, with a higher mean alpha diversity (Shannon index) value in the CF and S-ECC groups ($P < 0.05$; Fig. 3A). However, the presence of *C. albicans* did not alter the microbial alpha diversity in S-ECC salivary or plaque samples when

compared within the same sample sources ($P > 0.05$; Fig. 3B). Appendix Figure 3 shows an additional rarefaction curve of the Shannon index of groups with different caries and *C. albicans* statuses. In addition, principal coordinate analysis of microbial community dissimilarity identified distinct profiles between plaque and saliva in the CF and S-ECC groups ($P < 0.05$; Fig. 3C). In the S-ECC group, however, the presence of *C. albicans* was not associated with community dissimilarity within saliva or plaque samples (Fig. 3D).

Maternal Influence on Children's Oral Microbiota

To investigate maternal influence on the oral microbiome of children with S-ECC, we examined the species-level relative abundance and diversity between plaque samples obtained from mother-child pairs. Overall, the microbial composition between mother and child plaque samples was similar (Fig. 4A). Data analysis also revealed no significant differences in diversity or dissimilarity between mothers and children, within either CF or S-ECC dyads ($P > 0.05$; Fig. 4B, C). Thus, maternal and child microbiota were similar within dyads.

Functional Correlation with Plaque Gtf Activity

Since the detection of *C. albicans* is associated with an increased abundance of *S. mutans* and acidogenic-aciduric species in plaque, we examined whether the activity of a cariogenic virulence factor (Gtfs) can be altered by the fungal presence. Insoluble glucans produced by streptococcal Gtfs were shown to be key virulence traits in ECC (Mattos-Graner

et al. 2000; Vacca Smith et al. 2007). Thus, we determined the plaque Gtf enzymatic activity by measuring insoluble glucan synthesis via scintillation spectroscopy (Appendix Fig. 4). Interestingly, the Gtf activity was significantly higher in S-ECC than in the CF group ($P < 0.05$), and it was further elevated with the presence of *C. albicans* within S-ECC ($P < 0.05$). Moreover, Spearman's rank test demonstrated a strong correlation between plaque Gtf activity and caries severity among children with S-ECC ($r = 0.67$, $P < 0.05$).

Discussion

Microbiome studies have largely focused on the bacterial components and their role in causing dysbiotic microbiota and ecologic shifts that result in oral diseases (Marsh 2018; Tanner et al. 2018). Here, our clinical findings demonstrate an association of *C. albicans* with alterations of the oral bacterial composition and virulence potentially more conducive for S-ECC. The presence of *C. albicans* was associated with an enrichment of a highly acidogenic-aciduric bacterial community, with an increased abundance of plaque *Streptococcus* (particularly *S. mutans*) and certain species of *Lactobacillus/Scardovia* and salivary/plaque *Veillonella* and *Prevotella*, as well as decreased levels of salivary/plaque *Actinomyces*. In parallel, our data indicate that *C. albicans* could play an important role in enhancing plaque Gtf activity, which is associated with caries pathogenesis, as demonstrated in rodent models and clinical studies (Bowen and Koo 2011). Therefore, fungal counterparts may play an active role in the caries microbiome and should be included in future longitudinal studies investigating the microbial etiopathogenesis of this costly and intractable disease.

Several studies characterized the oral microbiota in caries-active children (Tanner et al. 2011; Gross et al. 2012; Yang et al. 2012; Ma et al. 2015; Johansson et al. 2016; Richards et al. 2017) and identified *S. mutans* in addition to several caries-associated species, including *Streptococcus salivarius*, *Streptococcus sobrinus*, *Streptococcus parasanguinis*, *Scardovia wiggsiae*, *Spodoptera exigua*, *Lactobacillus salivarius*, *Parascardovia denticolens*, *Porphyromonas*, *Actinomyces*, and *Veillonella*. Our study confirms the detection of higher levels of plaque *S. mutans*, *Lactobacillus*, and *Scardovia* species associated with the S-ECC condition and further underscores the potential *C. albicans* influence on the oral bacteriome, as the fungal presence appears to increase the abundance of these caries-associated bacteria.

Figure 3. Taxa (or operational taxonomic unit) diversity in plaque and saliva samples from CF versus S-ECC and *Candida albicans* (Ca) positive (+) versus negative (-). (A, B) Mean alpha diversity (measured by Shannon diversity indices with 95% CIs) is shown in the upper graphs. The range of alpha diversity is shown as a dashed line. Means are shown by red lines. (A) Comparisons between sample types (saliva and plaque) were significantly different between S-ECC and CF samples ($P < 0.05$). Species diversity was higher in plaque samples than in saliva samples. (B) The presence of *C. albicans* did not alter the microbial alpha diversity in S-ECC salivary or plaque samples when compared within the same sample sources ($P > 0.05$). (C) This graph depicts 2 main findings. Plaque and saliva samples had dissimilar beta diversity ($P < 0.05$), which was also different between CF and S-ECC saliva samples ($P = 0.005$) and plaque samples ($P = 0.001$). (D) This graph compares saliva and plaque samples in children with S-ECC only, in the presence or absence of *C. albicans*. Although the cluster pattern looks different in both *C. albicans* conditions (positive and negative), the differences are not statistically significant. CF, caries free; S-ECC, severe early childhood caries.

Available in vitro and in vivo evidence indicates that the presence of *C. albicans* can promote the development of a cariogenic biofilm environment, which may involve complex physical, chemical, and metabolic bacterial-fungal interactions (O'Donnell et al. 2015; Bowen et al. 2018). For example, *C. albicans* promotes *S. mutans* carriage by inducing glucosyltransferase B and bacterial accumulation via chemical-metabolic interactions (Sztajer et al. 2014; Kim et al. 2017). Once together, cross-feeding interactions favor *C. albicans* and *S. mutans* growth while creating a Gtf activation loop, consistent with the increased abundance of *S. mutans* and plaque Gtf activity observed in this study. Enhanced carbohydrate metabolism via bacterial-fungal interactions can promote the creation of a highly acidified and selective biofilm microenvironment that favors the growth of acidogenic-aciduric and acid-consuming species, while nonaciduric and aerobic species may perish (Bowen et al. 2018). This pathogenic process could, at least in part, explain the increased abundance of *L. gasseri*, *L.*

Figure 4. Species relative abundance and taxa or operational taxonomic unit diversity (Shannon diversity) and dissimilarity (beta diversity) from plaque samples among mother-child dyads. **(A)** For species relative abundance among mother-child samples, a similar microbial composition was seen between mother and child plaque samples. **(B)** Beta diversity results indicate the dissimilarity of plaque microbiome in S-ECC samples between children and their mothers ($P = 0.703$, nonparametric P value) and in CF samples between children and their mothers ($P > .99$, nonparametric P value). **(C)** Alpha diversity is shown (mean Shannon diversity indices with 95% CIs). The range of plaque microbiome diversity is shown as a vertical dashed line, and means are shown as red lines. No alpha diversity difference was seen between the children and their mothers in the S-ECC or CF group ($P > 0.05$). CF, caries free; S-ECC, severe early childhood caries.

vaginalis, and *S. mutans* in *C. albicans*-positive S-ECC plaque samples. However, additional studies are needed to further determine whether *Candida* has an active role in causation or its presence is simply a result of a disrupted microbiota. In this context, *C. albicans* could enhance EPS production and acidification of cariogenic biofilms, or alternatively, the acidic environment in S-ECC biofilms could cause fungal proliferation. In addition, interaction of *Candida* with oral microbiota under healthy conditions needs further exploration (Janus et al. 2017), with the actual sequence of events elucidated by mechanistic animal and longitudinal clinical studies.

Interestingly, when we compared the oral microbiota of mother-child dyads, we observed that the microbial community structure and diversity within dyads were similar (although species abundance differed across pairs; see Fig. 4). Such strong maternal influence on the oral microbiota of children is supported by the recent observations of Chu et al. (2017), who found that infant and maternal oral microbiota had a similar community structure and taxonomic membership (classified at the genus level). Although *C. albicans* is a commensal strain, the detection rate in the first year of life is about 11% to 15% (Stecksén-Blicks et al. 2015). Vertical and horizontal transmissions were implicated for fungal colonization in neonates. In particular, the vertical transmission rate ranges from 14%,

typed by electrophoretic karyotyping and restriction endonuclease analysis of genomic DNA with pulsed-field gel electrophoresis, to 41%, typed by DNA fingerprinting with a *C. albicans* strain-specific DNA probe (Waggoner-Fountain et al. 1996; Bliss et al. 2008). Given the existing evidence indicating the maternal contribution of *C. albicans* and *S. mutans* to infants (Caufield et al. 2005; Xiao et al. 2016), if the causative role of *C. albicans* is confirmed by further longitudinal cohort study, controlling oral *C. albicans* carriage in mothers who are at high caries risk could be an effective adjunctive approach to reduce or delay the *C. albicans* oral colonization in infants, in addition to addressing other behavioral and dietary factors to prevent S-ECC in children.

The strength of the study, although a case-control report, is the availability of a defined mother-child cohort to analyze the influence of *C. albicans* presence in the bacterial community in plaque and saliva samples and on the activity of a known cariogenic factor (Gtfs). Conversely, we recognize the limitations of this study: 1) it utilized a case-control design instead of a cohort design, so inferences about causation are limited in scope; 2) it was based at a single hospital site; 3) a limited sample size may have compromised the power of our multiple regression analyses, given that S-ECC is a multifactorial disease; and 4) plaque samples were not site specific, so data on

the microbiota could not be localized to the site of carious lesions. Nevertheless, these limitations shall be addressed in a follow-up longitudinal study.

Conclusions

In conclusion, the presence of *C. albicans* appeared to be associated with significant differences in the oral bacteriome profiles between children who had S-ECC and those who were CF. The bacterial composition changes were characterized by an increased abundance of highly acidogenic and aciduric microbiota, such as *S. mutans* and specific *Lactobacillus* and *Scardovia* species, in addition to other bacteria commonly associated with S-ECC. The data also revealed that the presence of *C. albicans* enhanced the plaque Gtf activity, a virulence factor associated with caries. In addition, maternal and infant microbiota were highly related to each other, with a similar microbial community structure and diversity. Altogether, the data highlight the importance of including fungal components (e.g., mycobiome analysis) of the oral microbiome in future longitudinal studies assessing the potential risk factors and therapeutic approaches for S-ECC.

Author Contributions

J. Xiao, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; A. Greier, Y. Liu, S.R. Gill, contributed to data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; R.C. Faustoferr, R.G. Quivey, D.T. Kopycka-Kedzierawski, contributed to data interpretation, critically revised the manuscript; S. Alzoubi, A.L. Gill, contributed to data acquisition and analysis, critically revised the manuscript; C. Feng, contributed to data analysis and interpretation, drafted and critically revised the manuscript; H. Koo, contributed to data interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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