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Oral Candida Predicts Streptococcus mutans Emergence in Underserved US Infants

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Abstract

Despite the cariogenic role of Candida suggested from recent studies, oral Candida acquisition in children at high risk for early childhood caries (ECC) and its association with cariogenic bacteria Streptococcus mutans remain unclear. Although ECC disproportionately afflicts socioeconomically disadvantaged and racial-minority children, microbiological studies focusing on the underserved group are scarce. Our prospective cohort study examined the oral colonization of Candida and S. mutans among 101 infants exclusively from a low-income and racial-minority background in the first year of life. The Cox hazard proportional model was fitted to assess factors associated with the time to event of the emergence of oral Candida and S. mutans. Oral Candida colonization started as early as 1 wk among 13% of infants, increased to 40% by 2 mo, escalated to 48% by 6 mo, and remained the same level until 12 mo. S. mutans in saliva was detected among 20% infants by 12 mo. The emergence of S. mutans by year 1 was 3.5 times higher (hazard ratio [HR], 3.5; confidence interval [CI], 1.1–11.3) in infants who had early colonization of oral Candida compared to those who were free of oral Candida (P = 0.04) and 3 times higher (HR, 3.0; CI, 1.3–6.9) among infants whose mother had more than 3 decayed teeth (P = 0.01), even after adjusting demographics, feeding, mother's education, and employment status. Infants' salivary S. mutans abundance was positively correlated with infants' Candida albicans (P < 0.01) and Candida krusei levels (P < 0.05). Infants' oral colonization of C. albicans was positively associated with mother's oral C. albicans carriage and education (P < 0.01) but negatively associated with mother's employment status (P = 0.01). Future studies are warranted to examine whether oral Candida modulates the oral bacterial community as a whole to become cariogenic during the onset and progression of ECC, which could lead to developing novel ECC predictive and preventive strategies from a fungal perspective.

Keywords: yeasts, infant, dental caries, candidiasis, health equity, perinatal care

Introduction

Early childhood caries (ECC), the single most common chronic childhood disease, disproportionately afflicting up to 72.7% of underprivileged preschool children in both developing and industrialized countries (Dye et al. 2015), substantially and adversely affects children, families, and public health systems (Dye et al. 2007). ECC is a disease initiated by changes in the oral environment from a state of homeostasis to dysbiosis, with transmissible pathogens that are capable of forming virulent biofilms (plaques) on tooth surfaces (Caufield et al. 2005; Gomez and Nelson 2017). Traditional microbial risk markers for ECC include Streptococcus mutans and Lactobacillus species (Caufield et al. 1993; Klein et al. 2004; Li et al. 2005; Kanasi et al. 2010; Fontana et al. 2011; Slayton 2011; Zhan et al. 2012; Klinke et al. 2014). Intriguingly, recent advances in pediatric caries research suggested the cariogenic role of Candida albicans (Koo and Bowen 2014; Bowen 2016; Bowen et al. 2018).

C. albicans displays cariogenic traits, in that it is acidogenic, aciduric, and capable of dissolving hydroxyapatite (Samaranayake et al. 1986; Klinke et al. 2009), and it leads to more severe dental caries when infected together with *S. mutans* in the rat model (Falsetta et al. 2014). A unique adhesive interaction between *C. albicans* and *S. mutans* mediated by extracellular polysaccharides (EPSs) and a synergistic interaction between

C. albicans and S. mutans promote biofilm development in vitro and in vivo (Gregoire et al. 2011; Metwalli et al. 2013; Falsetta et al. 2014; Koo and Bowen 2014; Sztajer et al. 2014; Hwang et al. 2015; Ellepola et al. 2019). Furthermore, the presence of oral C. albicans of preschool children was associated with oral bacterial dysbiosis with an abundance of taxa with greater virulence and more conducive for ECC (Xiao, Grier, et al. 2018). In addition, biological mothers of children with severe-ECC (S-ECC) were also highly infected with oral C. albicans (Xiao et al. 2016). Most of the strains in the

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A supplemental appendix to this article is available online.

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mother–child pairs were genetically identical, suggesting maternal sources for *C. albicans* acquisition in the oral cavity of children affected by the disease (Xiao et al. 2016).

Understanding the acquisition of cariogenic microbes is important for the development of improved preventive strategies for ECC. However, the major unanswered questions related to C. albicans and ECC remain: 1) the acquisition of C. albicans in the oral cavity of children at high risk for ECC and 2) the colonization association between C. albicans and S. mutans in the oral cavity. Moreover, although ECC disproportionately afflicts socioeconomically disadvantaged and racial-minority children, microbiological studies focusing on the underserved group are scarce. To close the gap in knowledge, we conducted a prospective birth cohort study to investigate the colonization of Candida and S. mutans among underserved US infants with high risk for ECC and its maternal contribution. We define infants at high risk for ECC as those from families with low income and racial-minority background. The overarching hypothesis was that early oral colonization of Candida in infants at high risk for ECC is associated with the mother's carriage of oral Candida and that the early oral mucosal infection of Candida contributes to the early oral colonization and enrichment of S. mutans in early life.

Methods

Study Population

The reported mother–infant dyads were obtained from an ongoing parent cohort study (186 mother–infant dyads) that examines the association between oral *Candida* and ECC onset in children from birth to 2 y of age. The study protocol was approved by the University of Rochester Research Subject Review Board (#1248). Pregnant women and their infants were recruited from patients visiting the University of Rochester Highland Family Medicine (HFM) or Eastman Institute for Oral Health (EIOH) Perinatal Dental Clinic. Both clinics, HFM and EIOH, serve a large body of low-income patients, with a range of 40% African Americans, 40% White, and 20% others. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

Eligibility

Included mothers were 18 y of age and older, pregnant with a singleton fetus and in their third trimester (beyond 28 gestational weeks), and eligible for New York State—supported medical insurance determined by income level (≤138% federal poverty line). Mothers were excluded if they had received oral and/or systemic antifungal therapy within 90 d of the baseline study visit or had severe systemic medical conditions (e.g., human immunodeficiency virus infection) that made them prone to yeast infections. Infants included were those born to the study mothers, except for those who met the following exclusion criteria: 1) preterm (<37 wk of gestation), 2) with low birth weight (<2,500 g), 3) with Down syndrome, 4) with

orofacial deformity (cleft lip, cleft palate, oral-pharyngeal mass), or 5) received oral and/or systemic antifungal treatment before the baseline study visit.

Oral Examination and Data/Sample Collection

Comprehensive oral examination and data/sample collection occurred at 6 time points: prenatal (during mother's third trimester) for the mothers and 1 wk, 2 mo, 4 mo, 6 mo, and 12 mo after delivery for the infants. A comprehensive oral examination (caries score, plaque index, and oral candidiasis) and oral sample collection (saliva, plaque, and mucosal swab) were performed by 1 of 3 calibrated dentists in a dedicated examination room at the University of Rochester clinics, using standard dental examination equipment, materials, and supplies, under portable lighting, using methods detailed previously (Xiao et al. 2016, 2019) and in the Appendix. Inter- and intra-examiner agreement for the evaluated criteria was calculated by κ statistics and exceeded 83% at the calibration.

The medical background data were collected through self-reporting and confirmation using electronic medical records. The data included 1) physician-diagnosed systemic diseases (e.g., hypertension, diabetes mellitus, asthma, anxiety, depression, kidney disease, liver disease), 2) medications, and 3) smoking status (yes/no) (for mothers). Data on demographic and socioeconomic status, oral hygiene practice, and feeding method were collected via questionnaires.

Primary Outcomes: S. mutans/Candida Detection and Quantification

Oral samples (saliva/plaque/swab) were stored on ice after collection and transferred to the lab located at the University of Rochester Center for Oral Biology within 2 h for microbiological assays. The methods used to identify and quantify *Candida* spp. and *S. mutans* have been detailed previously (Xiao et al. 2016, 2019; Zeng et al. 2020) and in the Appendix.

Statistical Analysis

Infants were grouped based on their oral Candida and S. mutans status. The characteristics of the 2 groups (oral Candida positive vs. oral Candida negative; S. mutans positive vs. S. mutans negative) were compared using the t test for continuous data and χ^2 or Fisher's exact tests for categorical data. One-way analysis of variance (ANOVA) and Tukey's test were used to compare the Candida carriage between swab samples taken from different mucosal sites. Spearman's rank was used to measure the correlation between variables, including infant's Candida spp. and S. mutans carriage, mother's decayed teeth number, mother's C. albicans and S. mutans carriage, mother's education level, and infant night bottle-feeding frequency. To analyze factors associated with oral Candida and S. mutans colonization time to event, we used log-rank tests and fitted Cox proportional hazards regression models, detailed in Appendix 2. All statistical tests were 2-sided with a significance level of

 Table 1. Demographic, Socioeconomic, and Oral Characteristics of Mother–Infant Dyads.

	Overall (n = 101)	Infant Streptococcus mutans Status			Infant Candida albicans Status		
Categories		Negative (n = 71), %	Positive (n = 30), %	P Value	Negative (n = 62), %	Positive (n = 39), %	P Value
Male gender	49.5	52.1	46.7	0.67	51.60	46.2	0.68
Race							
Black or African American	61.4	59.2	66.7	0.49	56.5	69.2	0.26
White	18.8	18.3	20.0		19.4	17.9	
Non-Hispanic ethnicity	87.1	85.9	90.0	0.75	83.9	92.3	0.36
Vaginal birth route	79.2	81.7	73.3	0.42	79.0	79.5	1.00
Feeding (I wk)							
Breastfeeding	33.7	36.3	26.7	0.52	37.1	28.2	0.05
Bottle feeding	29.7	26.8	36.7		21.0	43.6	
Both	36.6	36.6	36.7		41.9	28.2	
Feeding (2 wk)							
Breastfeeding	24.8	28.2	16.7	0.36	30.6	15.4	0.01
Bottle feeding	37.6	33.8	46.7		25.8	56.2	
Both	37.6	38.0	36.7		43.5	28.2	
Feeding (4 mo)	57.0	50.0	50.7		15.5	20.2	
Breastfeeding	12.9	14.1	10.0	0.80	17.7	5.1	0.02
0	52.5	50.7	56.7	0.60	49.I	69.2	0.02
Bottle feeding							
Both	34.7	35.2	33.3		40.3	25.6	
Feeding (6 mo)			. =	2.54			
Breastfeeding	9.9	11.3	6.7	0.56	12.9	5.1	0.27
Bottle feeding	55.4	52.1	63.3		50.0	64. I	
Both	34.7	36.6	30		37.1	30.8	
Night bottle feeding (formula) (yes)	77.2	71.8	73.3	0.61	71.0	87.2	0.09
Solid food intake by 6 mo	30.7	29.6	33.3	18.0	29.0	33.3	0.67
Daycare (yes)							
l mo	2.0	1.4	3.3	0.51	1.6	2.6	1.00
2 mo	3.0	4.2	0.0	0.55	4.8	0.0	0.28
4 mo	8.9	9.9	6.7	0.72	11.3	5.1	0.48
6 mo	8.9	8.5	10.0	1	8.1	10.3	0.73
Siblings (yes)	67.3	66.2	70.0	0.80	64.5	71.8	0.25
Infant salivary Candida detection	67.3	57.7	90.0	0.002	NA	NA	NA
Infant salivary S. mutans detection	29.7	NA	NA	NA	9.1	30.7	0.002
Maternal factors (yes)	27.7	14/1	1471	1471	7.1	30.7	0.002
Employment	48.5	30.6	28.8	1.00	56.5	35.9	0.04
Emotional condition	34.7	28.8		0.82	33.9	35.9	0.04
			31.4				
Smoking	17.8	30.1	27.8	1.00	14.5	23.1	0.30
Diabetes	6.9	29.8	28.6	1.00	9.7	2.6	0.24
Hypertension	11.9	30.3	25.0	1.00	12.9	10.3	0.76
Asthma	12.9	31.8	15.4	0.33	14.5	10.3	0.76
Antibiotics or sulfa (within 90 d	3.9	4.2	3.3	1.0	6.4	0	0.16
before the baseline) (yes)							
Marital status (married)	17.8	16.9	20.0	0.78	22.6	10.3	0.18
Number of children							
I	32.7	33.8	30.0	0.80	35.5	28.2	0.25
2	28.7	26.8	33.3		32.3	23.1	
>2	38.6	39.4	36.7		32.3	48.7	
Education							
Middle school	7.9	7.0	10.0	0.67	9.7	5.1	0.004
High school	52.5	50.7	56.7		43.5	66.7	
Associate's degree	16.8	19.7	10.0		12.9	23.1	
≥ College	22.8	22.5	23.3		33.9	5.1	
Salivary Candida (10 ³ CFU/mL),	8.3 ± 36.5	5.9 ± 27.1	24.8 ± 73.1	0.003	1.8 ± 8.7	13.2 ± 47.3	0.005
mean ± SD	J.J ± JU.J	J./ ± 4/.1	Z 1.0 ± 73.1	0.003	1.0 ± 0.7	15.4 ± 17.5	0.003
Salivary C. albicans							
	44.6	50.7	30.0	0.02	51.6	33.3	0.19
No				0.02		33.3	0.17
I-400 CFU/mL	24.8	26.8	20.0		22.6	28.2	
>400 CFU/mL	30.7	22.5	50.0	0.40	25.8	38.5	0.00
Salivary S. mutans (10^5 CFU/mL), mean \pm SD	9.4 ± 13.9	9.2 ± 14.2	10.8 ± 1.8	0.68	8.8 ± 14.3	10.0 ± 13.6	0.90

(continued)

Table I. (continued)

	Overall (n = 101)	Infant Streptococcus mutans Status			Infant Candida albicans Status		
Categories		Negative (n = 71), %	Positive (n = 30), %	P Value	Negative (n = 62), %	Positive (n = 39), %	P Value
Salivary S. mutans							
<10 ⁵ CFU/mL	22.8	22.5	23.3	1.00	29.0	12.8	0.06
≥10 ⁵ CFU/mL	77.2	77.5	76.7		71.0	87.2	
Plaque S. mutans							
<10 ⁶ CFU/mL	59.4	64.8	46.7	0.12	67.7	46.2	0.03
≥106 CFU/mL	40.6	35.29	53.3		32.3	53.8	
Decayed teeth number, mean \pm SD	3.3 ± 4.2	2.9 ± 3.9	6.0 ± 5.5	0.01	2.4 ± 2.8	4.0 ± 5.0	0.01
Decayed teeth							
≤3	66.3	73.2	50.0	0.02	64.2	61.5	0.42
>3	33.7	26.8	50.0		30.6	38.5	

CFU, colony-forming units; NA, not applicable.

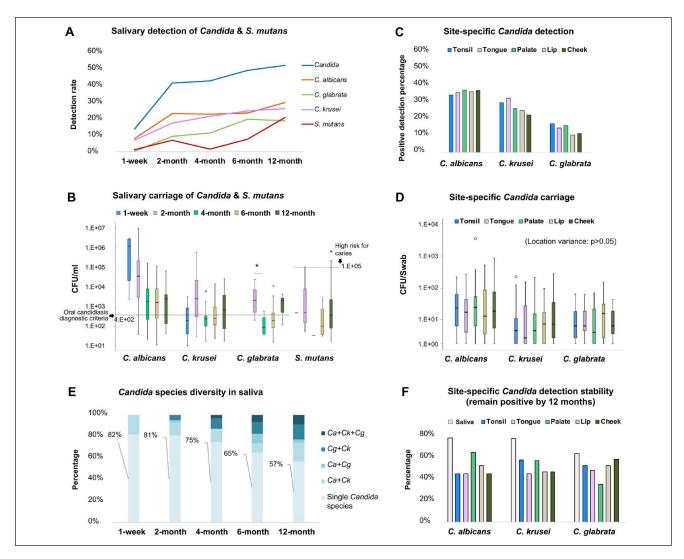


Figure 1. Oral Candida species and Streptococcus mutans emergence in early infancy. (A) Saliva detection of Candida species and Streptococcus mutans detection from birth to 12 mo. (B) Detection of Candida species at different sites of the infant's oral cavity. (C) Salivary carriage of Candida species and S. mutans in infants. A horizontal line marked above Candida species indicating 400 colony-forming units (CFU)/mL is drawn in the plot, which is an oral candidiasis criterion based on the salivary Candida CFU established by Epstein et al. (1980). A horizontal line marked above S. mutans indicating 1.0×10^5 CFU/mL is drawn in the plot, which indicates a threshold of high risk for dental caries. (D) Carriage of Candida species at different sites of the infant's oral cavity from oral swab samples. (E) Candida species diversity in salivary samples. (F) Site-specific detection stability of oral Candida. The detection stability is defined as that infants who had positive Candida remain positive until 12 mo. Ca, Candida albicans; Ck, Candida krusei; Cg, Candida glabrata.

5%. SAS (SAS Institute) was used for all statistical analyses. Sample size justification is detailed in Appendix 2.

Results

A total of 101 mother–infant dyads were included in the study, with 61% Black or African American, 18.8% White, and 19.9% other or mixed races. Overall, 83 and 75 infants were retained by 6 and 12 mo, separately. The demographic, socioeconomic, and feeding characteristics and oral conditions of infants and their mothers are shown in Table 1. No statistical differences were detected between the C. albicans and S. mutans detection status groups in terms of infant's gender, race, ethnicity, and birth route (P > 0.05). Infants with bottle feeding in the first 4 mo had a higher detection rate of C. albicans compared to those breastfed or fed with both methods (P < 0.05). Infants whose mothers had more decayed teeth, higher plaque index, and more C. albicans carriage had a higher detection of S. mutans by 12 mo (P < 0.05).

Candida and S. Mutans Colonization in Underserved Infants

The oral colonization of *Candida* spp. and *S. mutans* from birth to 12 mo among the study infants is demonstrated in Figure 1A (saliva) and Appendix Figure 2 (plaque at 12 mo). Oral *Candida* colonization started as early as 1 wk among 13% of infants. The detection of salivary *Candida* continued to increase, reached 40% by 2 mo, remained stable until 4 mo, and further escalated to 48% by 6 mo and remained the same level until 12 mo. Among the *Candida* spp., *C. albicans* and *Candida krusei* were the most commonly detected strains, followed by *Candida glabrata*. *S. mutans* in saliva was detected among 7% of the infants by 6 mo, among whom the majority of 6-mo-old infants did not have erupted teeth. By 12 mo, *S. mutans* was detected in saliva among 20% of the infants and in plaque among 25% of the infants (Appendix Fig. 2).

No differences in abundance of *Candida* spp. and *S. mutans* were detected from birth to 12 mo, except the carriage of *C. glabrata* at 2 mo and 4 mo (P < 0.05) (Fig. 1B). Significantly, although only a limited number of infants were diagnosed with oral candidiasis upon clinical examination, more than 80% of the infants could be diagnosed as having oral candidiasis based on the laboratory standard established by Epstein et al. (1980) using the salivary *Candida* colony-forming unit (CFU).

Among the infants who had positive *Candida* detection, we further assessed the detection and carriage levels across the mucosal sites, with results shown in Figure 1C. No detection difference was found for *C. albicans* across the oral cavity sites (32%-35%), including tonsil, tongue, palate, inner lip, and cheek mucosa. Tongue (31%) was the most prevalent site for *C. krusei* detection, followed by tonsil (28%), palate (24%), inner lips (23%), and check mucosa (21%). No quantitative differences were found between mucosal sites in the oral cavity in terms of *Candida* abundance in swab samples (P > 0.05) (Fig. 1D). The abundance of *Candida* at each site remained stable over time (Appendix Fig. 3).

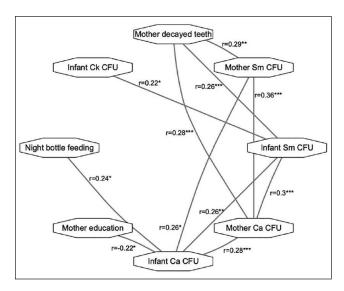


Figure 2. Correlation between mother's oral health and infant's Candida and Streptococcus mutans carriage. Spearman's rank correlation analysis among 101 infants (0–12 mo) indicates that infant's S. mutans carriage was positively associated with infant's Candida albicans and Candida krusei levels, as well as mother's C. albicans level. Infant C. albicans level was positively associated with more frequent night bottle feeding and higher maternal C. albicans level. Ca, Candida albicans; CFU, colony-forming unit; Ck, Candida krusei; Sm, Streptococcus mutans. *Correlation is significant at the 0.05 level (2-tailed). ***Correlation is significant at the 0.01 level (2-tailed).

We further assessed the diversity and detection stability of oral *Candida* in infants. Interestingly, most infants in early life carried only 1 type of *Candida* spp. at an early age (82% at 1 wk to 75% at 4 mo); more older infants (12 mo) tended to carry more than 1 type of *Candida* (43%) (Fig. 1E). We define detection stability as that infants who had positive *Candida* remain positive until 12 mo (Fig. 1F). Seventy-four percent of the infants who had salivary *C. albicans* and *C. krusei* remained positive until 12 mo. The detection stability of *C. albicans* from palate sites was 62%, whereas the detection stability of other sites ranged from 43% to 50%.

Candida Maternal Relatedness

We compared the detection of *Candida* spp. between mothers and infants (Appendix Fig. 1). The subset that included both positive mothers and infants continued to increase from 9% at 1 wk to 38% at 6 mo and remained stable at 41% from 6 mo to 12 mo. Strikingly, 74% to 76% of the mothers and infants who were positive for *Candida* detection shared the same type of *Candida* species at 6 and 12 mo.

Candida and S. mutans Abundance Correlation in Infants

We examined the correlation between infants' *Candida* and *S. mutans* carriage and their correlation to the mothers' *Candida* and *S. mutans* carriage, mother's oral condition, mothers' educational level, and infant night bottle-feeding frequency (see Fig. 2). Infant's *C. albicans* carriage level was positively

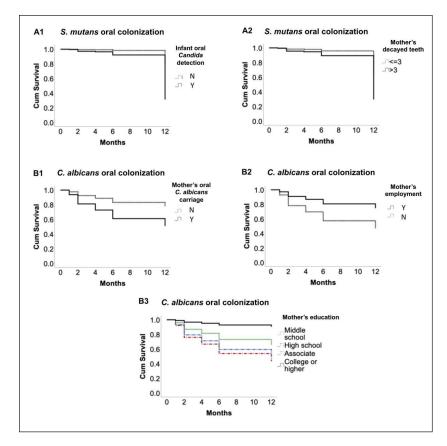


Figure 3. Oral Streptococcus mutans and Candida colonization event time probability estimation. (A1) Infants' S. mutans colonization survival estimation between infants with and without oral Candida detection prior to S. mutans colonization. (A2) Infants' S. mutans colonization survival estimation between infants mother who has ≤ 3 decayed teeth or > 3 decayed teeth. All variants are significantly associated with S. mutans colonization (P < 0.05). (B) Factors that are associated with infants' oral Candida colonization: mother's oral C. albicans carriage (B1), mother's employment status (B2), and mother's education status (B3). All variants are significantly associated with infant's oral C. albicans colonization (P < 0.05).

associated mother's C. albicans carriage (P < 0.001), infant's S. mutans carriage (P < 0.01), mother's S. mutans carriage (P < 0.05), and more frequent night bottle feeding (P < 0.01). Infant C. albicans carriage was negatively associated with mother's higher education (P < 0.01). Furthermore, infant S. mutans carriage was positively associated with mother's C. albicans carriage (P < 0.001), infant C. krusei carriage (P < 0.01), and mother's decayed teeth number (P < 0.01), in addition to infant's C. albicans level.

Factors Associated with S. mutans Colonization Time to Event

From the log-rank tests, we identified several variables that were significantly associated with increased risk for infant's *S. mutans* colonization by 12 mo. We plotted the survival curve to visualize their impact (Fig. 3A). Early colonization of oral *Candida* was associated with *S. mutans* colonization and carriage by age 1 (Fig. 3A1). The emergence of *S. mutans* by year 1 was 3.5 times higher (hazard ratio [HR], 3.5; confidence

interval [CI], 1.1–11.3) in infants who had early colonization of oral *Candida* compared to those who were free of oral *Candida* (P = 0.04) and 3 times higher (HR, 3.0; CI, 1.3–6.9) among infants whose mother had more than 3 decayed teeth (P = 0.01), even after adjusting demographics, feeding, mother's education, and employment status (Table 2).

Factors Associated with Candida Colonization Time to Event

The plots of factors that affect C. albicans colonization from birth to 12 mo are indicated in Figure 3B. The Cox regression results (Table 2) revealed that infants whose mothers had oral C. albicans were 2.2 (1.1, 4.6) times more susceptible to C. albicans oral colonization (P = 0.03). Infants' C. albicans oral colonization time to event was also positively associated with lower education of mothers (high school vs. \geq college: HR, 8.6; CI, 2.0–37.5, P < 0.01; associate's degree vs. \geq college: HR, 7.9; CI, 1.7–37.8, P < 0.01) and negatively associated with mothers' employment status (yes/no; HR, 0.4; CI, 0.2-0.8, P = 0.01). We did not identify factors that were statistically significantly associated with oral C. krusei colonization in infants (Table 2).

Discussion

Despite that previous cross-sectional and case-control studies have revealed that children with oral *C. albicans* had higher odds

of experiencing ECC than children without *C. albicans* (Xiao, Huang, et al. 2018), prospective cohort studies were lacking to strengthen the evidence supporting the association between oral *C. albicans* and ECC. Our study revealed a novel finding that early colonization of oral *Candida* is associated with *S. mutans* colonization among underserved US infants in the first year of life. We demonstrated the sequential order of how the oral colonization of *Candida* affects *S. mutans* early emergence in infants' oral cavity; the emergence of *S. mutans* was 3.5 times higher in infants who had early colonization of oral *Candida*. Furthermore, we also revealed a quantitative correlation between infants' *S. mutans* carriage and their oral *C. albicans* and *C. krusei* carriage.

The concept of investigating the colonization of microorganisms from 2 different kingdoms (yeast and bacteria) in the context of ECC is clinically novel. Several pathways are speculated to link the presence of *C. albicans* and the emergence of *S. mutans* in underserved children who are at high risk for S-ECC. First, *C. albicans* can interact with *S. mutans* via EPSs

Table 2. Cox Regression Models for Salivary Streptococcus mutans, Candida albicans, and Candida krusei Emergence in the First Year of Life.

Cox Regression Models	Hazard Ratio	(95% CI)	
S. mutans emergence time to event $(n = 101)$			
Infant sex (female/male)	0.9 (0.4–2	2.1)	
Infant race			
White/other	1.4 (0.4–4.0)		
Black/other	1.6 (0.4–7.3)		
Feeding method (bottle and breast feeding) by 6 mo			
Breastfeeding/both	0.7 (0.1–4.7)		
Bottle feeding/both	1.3 (0.5–3.4)		
Night bottle feeding with formula by 6 mo (yes/no)	0.7 (0.2–1.9)		
Daycare attendance by 6 mo (yes/no)	2.4 (0.6–	9.3)	
Solid food intake by 6 mo (yes/no)	1.0 (0.4–2.5)		
Teeth eruption by last visit or S. mutans detection (yes/no)	1.0 (0.7–1.4)		
Candida status by last visit or S. mutans detection (yes/no) ^a	3.5 (1.1–11.3)		
Mother employment (yes/no)	1.0 (0.4–2.3)		
Mother education			
Middle school/college or higher	4.0 (1.0–23.4)		
High school/college and higher	1.2 (0.4–3.3)		
Associate's degree/college or higher	0.6 (0.1–2.5)		
Mother decayed teeth (>3/≤3) ^b	3.0 (1.3–6.9)		
C. albicans and C. krusei emergence time to event $(n = 101)$	·		
	C. albicans	C. krusei	

	C. albicans	C. krusei
Infant sex (female/male)	0.7 (0.3–1.4)	0.6 (0.3–1.3)
Infant race		
White/other	2.3 (0.8–6.3)	0.8 (0.3-1.8)
Black/other	1.7 (0.5–5.8)	0.7 (0.2–2.0)
Feeding method (bottle and breast feeding) by 6 mo		
Breastfeeding/both	1.0 (0.1–6.7)	0.2 (0.0-1.5)
Bottle feeding/both	0.9 (0.4–1.9)	0.9 (0.4–1.9)
Night bottle feeding with formula by 6 mo (yes/no)	2.5 (0.7–8.8)	1.4 (0.5–4.0)
Mother employment (yes/no) ^b	0.4 (0.2-0.8)	0.6 (0.3-1.2)
Mother education		
Middle school/college or higher	3.4 (0.5–26.1)	0.5 (0.1–2.8)
High school/college and higher ^b	8.6 (2.0-37.5)	1.1 (0.5–2.6)
Associate's degree/college or higher ^b	7.9 (1.7–37.8)	1.0 (0.4–2.8)
Mother's oral C. albicans carriage (yes/no) ^a	2.2 (1.1–4.6)	1.5 (0.8–3.1)
Delivery method (cesarean-section/vaginal)	0.9 (0.4–1.9)	0.9 (0.4–2.0)
Infant siblings (yes/no)	1.6 (0.8–3.3)	1.0 (0.5–2.0)

^aSignificant at the 0.05 level.

produced by glucosyltransferase exoenzymes when dietary sucrose is available. This EPS-mediated interaction promoted both C. albicans and S. mutans accumulation to saliva-coated hydroxyapatite discs (Gregoire et al. 2011; Falsetta et al. 2014). Second, C. albicans is an early oral cavity colonizer, and its oral colonization could start a few days after birth (Xiao et al. 2020). Although S. mutans is traditionally considered to only establish their colonization on solid surfaces, such as the tooth surfaces (Duchin and van Houte 1978), our recent clinical studies demonstrated a strong correlation between these 2 microorganisms in saliva, independent of tooth surfaces (Xiao et al. 2016; Xiao, Grier, et al. 2018). Thus, we speculate that early oral mucosal infection of C. albicans could promote the oral colonization of S. mutans before tooth eruption through adhesive interaction between these 2 organisms in conditions conducive of ECC (e.g., high sugar intake) and subsequently

increase the salivary load at an early age of the host. To further test the hypothesis that *C. albicans* and *S. mutans* bind to each other through enzyme-synthesized EPSs in vivo, future studies are needed to examine the *C. albicans—S. mutans* binding in saliva using advanced imaging techniques.

Moreover, our results enhanced our understanding of oral *Candida* colonization and its maternal influence among underserved US children in early infancy. *C. albicans* is by far the most commonly detected fungal organism on human mucosal surfaces (Sharma et al. 2018). To date, few prospective cohort studies had investigated the oral colonization of *Candida* spp. after birth during the predentate stage. The oral *Candida* detection among the socioeconomically deprived infants in our study is slightly higher than other reports in which a 44% detection was found among 36 healthy children (6–18 mo) living in Iowa City (Kleinegger et al. 1996), and an 11% to 15%

^bSignificant at the 0.01 level.

detection was revealed among 100 healthy Swedish children during the first year of life (Stecksen-Blicks et al. 2015).

Oral carriage of Candida can be affected by multiple factors, such as age, diet, population demographic and socioeconomic characteristics, gender, and host immunosuppression and medication use (Kleinegger et al. 1996). Interestingly, the Cox model fitted in our study did not reveal significant contributions from the factors mentioned above to infant Candida colonization. Instead, mothers' influence, including mothers' oral C. albicans carriage, education, and employment status, were the sole contributing factors, highlighting the importance of maternal oral health care and socioeconomic status in maintaining infants free of oral yeast. Maternal vulvovaginal candidiasis is a major risk factor for Candida colonization and infection of the infant (Zisova et al. 2016). Twenty-two percent of the infants born to mothers who were clinically suspicious for vaginal candidiasis had positive detection of Candida in the oral mucosa and feces; isolates of the newborns were 100% identical to those of the mothers' vaginal secretion.

The strength of the study is being the first study, to our knowledge, to evaluate the oral Candida and cariogenic S. mutans colonization among racially and socioeconomically disadvantaged infants. Conversely, the following limitations should be considered, which include 1) due to the demographicsocioeconomic characteristics of the study infants, the study results cannot be generalized to other populations and those residing outside of upstate New York. 2) We focused on the colonization association between Candida and S. mutans; however, whether C. albicans modulates the oral bacterial community as a whole to become cariogenic during the onset/progression of ECC remains to be elucidated. Our parent study is monitoring a larger infant cohort for a longer period of time, from birth to 2 y of age, which will elucidate the association between oral Candida and ECC onset and identify other orally colonized bacteria and yeast populations other than S. mutans and C. albicans. The microbiome (bacterial and fungal) analyses are conducted in parallel to examine whether the presence of C. albicans modulates the establishment of oral bacteriome in early infancy.

Conclusions

Our study revealed a novel finding that early colonization of oral *Candida* predicted *S. mutans* emergence in early infancy among underserved US infants, even after adjusting common confounders, including demographics, tooth eruption and infant feeding, and so on. Maternal factors (*Candida* carriage, education, employment, and decayed teeth) significantly affected the colonization and carriage of *Candida* and *S. mutans* in infants, highlighting the importance of maternal oral health condition and socioeconomic status in maintaining infants free of oral pathogens.

Despite the emerging evidence of *C. albicans*' association with ECC, it remains unclear whether *Candida* detection can serve as a reliable microbial risk factor or an intervention target for ECC development. Future prospective studies that monitor the children from birth to ECC onset and assess the effect of antifungal intervention among children with high caries risk

are urgently needed. These studies could lead to developing novel caries-predictive and caries-preventive strategies from a fungal perspective.

Author Contributions

N. Alkhars, Y. Zeng, contributed to data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; N. Alomeir, S. Aboelmagd, M. Youssef, H. Jang, C. Fogarty, Contributed to data acquisition, critically revised the manuscript; N. Al Jallad, contributed to data acquisition and analysis, critically revised the manuscript; T.T. Wu, contributed to data analysis and interpretation, drafted and critically revised the manuscript; J. Xiao, contributed to conception, design, data acquisition, analysis and 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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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

Bowen WH. 2016. Dental caries—not just holes in teeth! A perspective. Mol Oral Microbiol. 31(3):228–233.

Bowen WH, Burne RA, Wu H, Koo H. 2018. Oral biofilms: pathogens, matrix, and polymicrobial interactions in microenvironments. Trends Microbiol. 26(3):229–242.

Caufield PW, Cutter GR, Dasanayake AP. 1993. Initial acquisition of mutans streptococci by infants: evidence for a discrete window of infectivity. J Dent Res. 72(1):37–45.

Caufield PW, Li Y, Dasanayake A. 2005. Dental caries: an infectious and transmissible disease. Compend Contin Educ Dent. 26(5 Suppl 1):10–16.

Duchin S, van Houte J. 1978. Colonization of teeth in humans by *Streptococcus mutans* as related to its concentration in saliva and host age. Infect Immun. 20(1):120–125.

- Dye BA, Hsu KL, Afful J. 2015. Prevalence and measurement of dental caries in young children. Pediatr Dent. 37(3):200–216.
- Dye BA, Tan S, Smith V, Lewis BG, Barker LK, Thornton-Evans G, Eke PI, Beltran-Aguilar ED, Horowitz AM, Li CH. 2007. Trends in oral health status: United States, 1988–1994 and 1999–2004. Vital Health Stat 11. (248):1–92.
- Ellepola K, Truong T, Liu Y, Lin Q, Lim TK, Lee YM, Cao T, Koo H, Seneviratne CJ. 2019. Multi-omics analyses reveal synergistic carbohydrate metabolism in *Streptococcus mutans—Candida albicans* mixedspecies biofilms. Infect Immun. 87(10):e00339–19.
- Epstein JB, Pearsall NN, Truelove EL. 1980. Quantitative relationships between *Candida albicans* in saliva and the clinical status of human subjects. J Clin Microbiol. 12(3):475–476.
- Falsetta ML, Klein MI, Colonne PM, Scott-Anne K, Gregoire S, Pai CH, Gonzalez-Begne M, Watson G, Krysan DJ, Bowen WH, et al. 2014. Symbiotic relationship between *Streptococcus mutans* and *Candida albicans* synergizes virulence of plaque biofilms in vivo. Infect Immun. 82(5):1968–1981.
- Fontana M, Jackson R, Eckert G, Swigonski N, Chin J, Zandona AF, Ando M, Stookey GK, Downs S, Zero DT. 2011. Identification of caries risk factors in toddlers. J Dent Res. 90(2):209–214.
- Gomez A, Nelson KE. 2017. The oral microbiome of children: development, disease, and implications beyond oral health. Microb Ecol. 73(2):492– 503
- Gregoire S, Xiao J, Silva BB, Gonzalez I, Agidi PS, Klein MI, Ambatipudi KS, Rosalen PL, Bauserman R, Waugh RE, et al. 2011. Role of glucosyltransferase B in interactions of *Candida albicans* with *Streptococcus mutans* and with an experimental pellicle on hydroxyapatite surfaces. Appl Environ Microbiol. 77(18):6357–6367.
- Hwang G, Marsh G, Gao L, Waugh R, Koo H. 2015. Binding force dynamics of *Streptococcus mutans*-glucosyltransferase B to *Candida albicans*. J Dent Res. 94(9):1310–1317.
- Kanasi E, Johansson I, Lu SC, Kressin NR, Nunn ME, Kent R Jr, Tanner AC. 2010. Microbial risk markers for childhood caries in pediatricians' offices. J Dent Res. 89(4):378–383.
- Klein MI, Florio FM, Pereira AC, Hofling JF, Goncalves RB. 2004. Longitudinal study of transmission, diversity, and stability of *Streptococcus mutans* and *Streptococcus sobrinus* genotypes in Brazilian nursery children. J Clin Microbiol. 42(10):4620–4626.
- Kleinegger CL, Lockhart SR, Vargas K, Soll DR. 1996. Frequency, intensity, species, and strains of oral *Candida* vary as a function of host age. J Clin Microbiol. 34(9):2246–2254.
- Klinke T, Kneist S, de Soet JJ, Kuhlisch E, Mauersberger S, Forster A, Klimm W. 2009. Acid production by oral strains of *Candida albicans* and lactobacilli. Caries Res. 43(2):83–91.
- Klinke T, Urban M, Luck C, Hannig C, Kuhn M, Kramer N. 2014. Changes in Candida spp., mutans streptococci and lactobacilli following treatment of early childhood caries: a 1-year follow-up. Caries Res. 48(1):24–31.
- Koo H, Bowen WH. 2014. *Candida albicans* and *Streptococcus mutans*: a potential synergistic alliance to cause virulent tooth decay in children. Future Microbiol. 9(12):1295–1297.

- Li Y, Caufield PW, Dasanayake AP, Wiener HW, Vermund SH. 2005. Mode of delivery and other maternal factors influence the acquisition of Streptococcus mutans in infants. J Dent Res. 84(9):806–811.
- Metwalli KH, Khan SA, Krom BP, Jabra-Rizk MA. 2013. Streptococcus mutans, Candida albicans, and the human mouth: a sticky situation. PLoS Pathog. 9(10):e1003616.
- Samaranayake LP, Hughes A, Weetman DA, MacFarlane TW. 1986. Growth and acid production of *Candida* species in human saliva supplemented with glucose. J Oral Pathol. 15(5):251–254.
- Sharma N, Bhatia S, Sodhi AS, Batra N. 2018. Oral microbiome and health. AIMS Microbiol. 4(1):42–66.
- Slayton RL. 2011. Reducing mutans streptococci levels in caregivers may reduce transmission to their children and lead to reduced caries prevalence. J Evid Based Dent Pract. 11(1):27–28.
- Stecksen-Blicks C, Granstrom E, Silfverdal SA, West CE. 2015. Prevalence of oral Candida in the first year of life. Mycoses. 58(9):550–556.
- Sztajer H, Szafranski SP, Tomasch J, Reck M, Nimtz M, Rohde M, Wagner-Dobler I. 2014. Cross-feeding and interkingdom communication in dual-species biofilms of *Streptococcus mutans* and *Candida albicans*. ISME J. 8(11):2256–2271.
- Xiao J, Fiscella KA, Gill SR. 2020. Oral microbiome: possible harbinger for children's health. Int J Oral Sci. 12(1):12.
- Xiao J, Fogarty C, Wu TT, Alkhers N, Zeng Y, Thomas M, Youssef M, Wang L, Cowen L, Abdelsalam H, et al. 2019. Oral health and *Candida* carriage in socioeconomically disadvantaged US pregnant women. BMC Pregnancy Childbirth. 19(1):480.
- Xiao J, Grier A, Faustoferri RC, Alzoubi S, Gill AL, Feng C, Liu Y, Quivey RG, Kopycka-Kedzierawski DT, Koo H, et al. 2018. Association between oral *Candida* and *Bacteriome* in children with severe ECC. J Dent Res. 97(13):1468–1476.
- Xiao J, Huang X, Alkhers N, Alzamil H, Alzoubi S, Wu TT, Castillo DA, Campbell F, Davis J, Herzog K, et al. 2018. Candida albicans and early childhood caries: a systematic review and meta-analysis. Caries Res. 52(1– 2):102–112.
- Xiao J, Moon Y, Li L, Rustchenko E, Wakabayashi H, Zhao X, Feng C, Gill SR, McLaren S, Malmstrom H, et al. 2016. Candida albicans carriage in children with severe early childhood caries (S-ECC) and maternal relatedness. PLoS One. 11(10):e0164242.
- Zeng Y, Youssef M, Wang L, Alkhars N, Thomas M, Cacciato R, Qing S, Ly-Mapes O, Xiao J. 2020. Identification of non-Streptococcus mutans bacteria from predente infant saliva grown on mitis-salivarius-bacitracin agar. J Clin Pediatr Dent. 44(1):28–34.
- Zhan L, Tan S, Den Besten P, Featherstone JD, Hoover CI. 2012. Factors related to maternal transmission of mutans streptococci in high-risk children—pilot study. Pediatr Dent. 34(4):e86–e91.
- Zisova LG, Chokoeva AA, Amaliev GI, Petleshkova PV, Miteva-Katrandzhieva TM, Krasteva MB, Uchikova EH, Kouzmanov AH, Ivanova ZV. 2016. Vulvovaginal candidiasis in pregnant women and its importance for Candida colonization of newborns. Folia Med (Plovdiv). 58(2):108–114.