Review

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Candida albicans and Early Childhood Caries: A Systematic Review and Meta-Analysis

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Keywords

Candida albicans · Child dentistry · Clinical studies · Early childhood caries · Fungal pathogen · Odds ratio · Risk factor · Yeast infection

Abstract

Oral *Candida albicans* has been detected in children with early childhood caries (ECC) and has demonstrated cariogenic traits in animal models of the disease. Conversely, other studies found no positive correlation between *C. albicans* and caries experience in children, while suggesting it may have protective effects as a commensal organism. Thus, this study aimed to examine whether oral *C. albicans* is associated with ECC. Seven electronic databases were searched. The data from eligible studies were extracted, and the risk of bias was evaluated. A fixed effects model (Mantel-Haenszel estimate) was used for meta-analysis, and the summary effect measure was calculated by odds ratio (OR) and 95% confidence interval (CI). Fifteen cross-sectional studies were included for the qualitative assessment and 9 studies for meta-analysis. Twelve studies revealed higher oral *C. albicans* prevalence in ECC children than in caries-free children, while 2 studies indicated an equivalent prevalence. A pooled estimate, with OR = 6.51 and 95% CI = 4.94–8.57, indicated a significantly higher ECC experience in children with oral *C. albicans* than those without *C. albicans* ($p < 0.01$). The odds of experiencing ECC in children with *C. albicans* versus children without *C. albicans* were 5.26 for salivary, 6.69 for plaque, and 6.3 for oral swab samples. This systematic review indicates that children with oral *C. albicans* have >5 times higher odds of having ECC compared to those without *C. albicans*. Further prospective cohort studies are needed to determine whether *C. albicans* could be a risk factor for ECC, and whether it is dependent on different sample sources (saliva/plaque).

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Early childhood caries (ECC) is the single most common childhood oral disease that disproportionately affects poor and minority children (<6 years of age), in the USA and worldwide [Dye et al., 2012; Kassebaum et al., 2015]. In addition, severe early childhood caries (S-ECC) occurs in children younger than 3 years of age and in children 4–6 years of age with elevated caries scores [Colak et al., 2013]. S-ECC often progresses rapidly leading to rampant and painful destruction of primary teeth. Treatment of S-ECC is most often provided under general anesthesia in the hospital operating room. As such, costs associated with treatment of ECC/S-ECC constitute a major public health expense [Hajishengallis et al., 2017].

ECC is a "family malady" in that the disease is infectious, transmissible, and is often associated with poor (sugar-laden) dietary habits [Douglass and Clark, 2015]. In addition to *Streptococcus mutans* and *Lactobacillus* species, other microorganisms also appear to be involved in the formation of cariogenic biofilms [Hajishengallis et al., 2017]. In this regard, the fungus *Candida albicans* is frequently detected together with *S. mutans* in the plaque/biofilms from children with dental caries [Marchant et al., 2001b; Hossain et al., 2003; de Carvalho et al., 2006; Rozkiewicz et al., 2006b; Raja et al., 2010]. This observation is intriguing, as *C. albicans* usually does not colonize teeth effectively on its own. Rather, *C. albicans* adheres mainly to oral mucosa or acrylic surfaces, while interacting with commensal streptococci to cause mucosal infections (oral candidiasis) [Xu et al., 2014; Pereira et al., 2017].

To date, the role of *C. albicans* in the pathogenesis of ECC remains unclear. A number of studies support a potentially positive association between oral *Candida* carriage and caries experience in children, with detection rates up to 89% in ECC children versus 2–22% in caries-free children [Marchant et al., 2001b; Hossain et al., 2003; de Carvalho et al., 2006; Rozkiewicz et al., 2006b; Raja et al., 2010]. Moreover, in vivo studies using rodent caries models have demonstrated the cariogenic potential of *C. albicans* [Klinke et al., 2011], particularly when coinfected with *S. mutans*. Coinfection has been shown to lead to rampant caries under experimental conditions conducive to ECC (e.g., with exposure to a sugar-rich diet) [Falsetta et al., 2014].

Conversely, some clinical studies have neither shown significant differences in oral *Candida* prevalence between clinically caries-free and caries-active populations [Neves et al., 2015; Thomas et al., 2016] nor a positive association between the presence of *C. albicans* and caries risk in children [Moreira et al., 2001; Peretz et al., 2011]. Additionally, a recent study indicated a 100% prevalence rate of salivary *C. albicans* in healthy children aged 12–71 months, regardless of caries status [Thomas et al., 2016].

Given the conflicting available evidence in the literature, this systematic review and meta-analysis aims to evaluate whether oral detection (saliva, plaque, and oral mucosal swab) of *C. albicans* is associated with ECC.

Methods

Search Strategy

Database and gray literature searches were conducted in October 2016 and updated in March 2017 to identify published information on oral *C. albicans* as a risk factor for ECC. A medical librarian developed individual search strategies and retrieved citations from PubMed, Embase, Scopus, Web of Science, LILACS, Cochrane Library, and ClinicalTrials.gov. A combination of text words and controlled vocabulary terms were used (*Candida*, candidiasis, thrush, child, infant, breast feeding, newborn, dental caries). A detailed search strategy is found in the Appendix (see online suppl. Appendix; see www.karger.com/doi/10.1159/000481833 for all online suppl. material).

Inclusion/Exclusion Criteria

This systematic review included experimental and epidemiological study designs such as randomized controlled trials, nonrandomized controlled trials, quasiexperimental, before and after studies, prospective and retrospective cohort studies, case-control and analytical cross-sectional studies that examined the oral presence of *C. albicans* in children (age <72 months), with or without ECC. Statistical data from selected studies were reported as odds ratio (OR), relative risk (RR), prevalence ratio (PR), confidence intervals (95% CI), *p* values, or frequency of an absolute number of events/total number of individuals per group. In vitro and animal studies were excluded, as were papers with abstract only, literature reviews, letters to the editor, editorials, patient handouts, case reports, case series, or studies that included children with severe systematic diseases such as HIV and leukemia.

The independent reviewers were calibrated in accordance with inclusion/exclusion criteria using a sample of 20% of the retrieved studies. Agreement between reviewers was good ($\kappa = 0.79$). The inclusion and exclusion criteria were applied independently to the remainder of the studies, and any disagreement was resolved by consensus within the 4 reviewers.

Data Extraction

Descriptive data, including clinical and methodological factors such as country, study design, subject recruitment site, dental examination and calibration, age of the subjects, sample sources, *C. albicans* isolation and identification methods, prevalence of *C. albicans*, as well as results from statistical analysis were obtained using an extraction form (see online suppl. Appendix).

Qualitative Assessment and Quantitative Analysis

Selected articles were assessed using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies (National Heart, Lung and Blood Institute: http://www.nhlbi.nih.gov/health-pro/guidelines/in-develop/cardiovascular-risk-reduction/

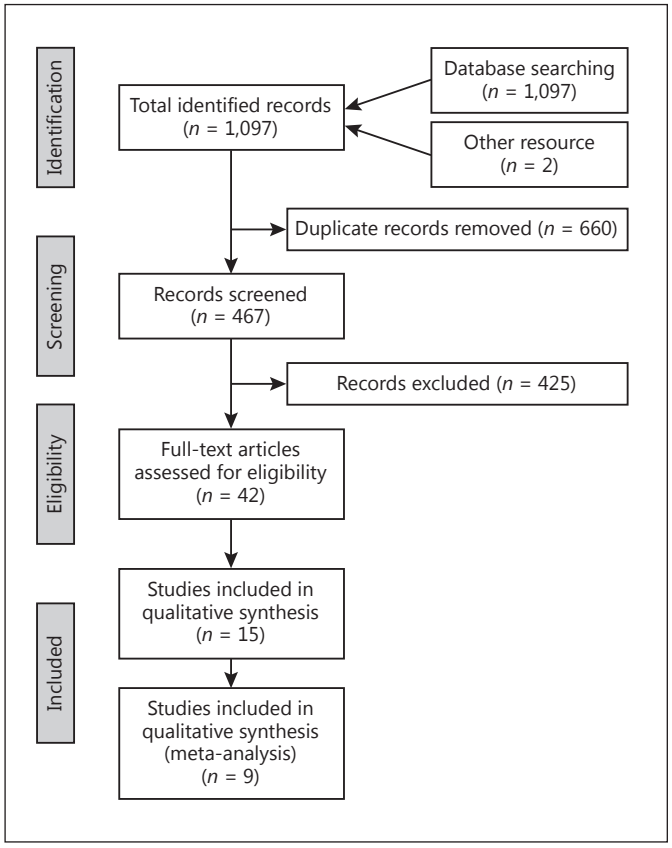

Fig. 1. Screening and assessing studies for inclusion eligibility. The 4-phase Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram was used to determine the number of studies identified, screened, eligible, and included in the systematic review and meta-analysis (http://www.prisma-statement.org).

tools/cohort). Articles were scaled as "fair," "good," or "poor" following the protocol guidelines. The OpenMeta(Analyst) (http://www.cebm.brown.edu/openmeta/) software program was used for meta-analysis. Studies with similar designs (cross-sectional design) were included in the forest plot. Heterogeneity among the studies was evaluated using I^2 statistics. For categorical data, OR, 95% CI and p value were calculated in a forest plot using a fixed effects model (Mantel-Haenszel estimate). Subgroup analysis was performed based on the sample sources (saliva, plaque, and swab).

Results

The literature analyses identified a total of 1,097 papers, including 1,095 articles from database searches and 2 articles from manual searching (Fig. 1). A total of 660 duplicate references were removed. The remaining 467 studies were imported into an Endnote Library for further review. From those, 425 studies were excluded after title/abstract screening. The remaining 42 articles were selected for a full-text review. Authors were contacted by e-mails when articles were not available. After the full-text analysis, 27 studies were eliminated based on the exclusion criteria and 15 articles were chosen for qualitative assessment. Nine articles were further assessed quantitatively using meta-analysis (1 article that used lesion site instead of tooth number to record caries was excluded, and 5 articles were excluded due to unspecified caries diagnostic criteria). The full list of excluded papers and meta-analysis results without exclusion of papers with unspecified caries diagnostic criteria are shown in the online supplementary Appendix.

Study Characteristics

From the 15 cross-sectional studies used for qualitative analysis (Table 1), 6 studies were assessed as "good" and 9 as "fair" using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies. These studies were all cross-sectional and were conducted in 11 different countries including Scotland, England, Germany, Brazil, Turkey, Iran, China, India, Poland, and the USA. Seven studies specified that the subjects were enrolled in a university dental clinic or nursing school setting. Four studies enrolled study subjects from local kindergartens, while another 4 studies did not detail the subject recruitment site. Children in all 15 studies were younger than 72 months of age, with the majority of the studies examining children from 1 to 5 years of age; 1 study from Scotland assessed children at an earlier age (<1 year old). Oral samples were collected from at least 1 of the following sources: saliva, plaque, oral mucosal swab, and carious lesions.

Salivary samples were collected in 6 studies. Among them, a tongue-loop method instead of harvesting whole saliva was used in 1 study [Radford et al., 2000]. Plaque samples were collected in 10 studies, while oral swab samples were collected in 2 studies. In addition to sound tooth surfaces, 4 studies examined samples collected from carious lesions (Table 1). Samples were plated onto Sabouraud dextrose agar and/or CHROMagar selective for *C. albicans* isolation. Microbiological and molecular methods were employed for *C. albicans* identification, including colony shape and color, germ tube test, Auxacolor Test (Sano® Diagnostics Pasteur, France), β-N-acetylgalactosaminidase assay, API 20C (BioMerieux), API ID 32C, and polymerase chain reaction.

All of the clinical studies used visual-tactile examination techniques; 3 studies performed intra- and interex-

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Table 1. Characteristics of studies included in systematic review

Authors	Country, study design	Subject recruitment site	Dental examination, calibration	Caries diagnosis	Age	Total subjects	Sample sources	*C. albicans* isolation method	*C. albicans* identification methods
Radford et al., 2000	Scotland, CS	Didn't specify	VTE, 1 calibrated examiner	WHO 1979, dmft	1–12 months	1,393	Saliva (tongue loop method)	Sabouraud dextrose agar	Colony shape and odor
Marchant, et al., 2001a	England, CS	University dental clinic	VTE, didn't specify	British Association for the Study of Community Dentistry criteria	3–5 years	29	Carious dentin; Caries-free plaque	CHROMagar Candida	Colony color; β-N-acetylgalactosaminidase assay; API 20C (BioMerieux)
Hossain, et al., 2003	Germany, CS	University dental clinic/ nursery schools	VTE, didn't specify	International standardization for caries, dmft	53.3–61.4 months	108 (M: 58/F: 50)	Saliva, plaque, carious lesion	CHROMagar Candida, Sabouraud agar	Germ tubes/Auxacolor test (Sano® Diagnostics Pasteur, France) Randomly amplified polymorphic DNA analysis
Ugun-Can, et al., 2007	Turkey, CS	University dental clinic/ nursery schools	VTE, didn't specify	WHO 1997, dmft	4–6 years	115	Swab	Sabouraud dextrose agar	Germ tube test and API 20C (BioMerieux), chlamydospore formation on cornmeal agar
De Carvalho et al., 2006	Brazil, CS	Didn't specify	VTE, didn't specify	Method reported in Drury et al. [1999]	1–5 years	56	Supragingival plaque, carious dentin	CHROMagar Candida	Colony color, germ tube test
Rozkiewicz et al., 2006a	Poland	Didn't specify	VTE, didn't specify	Didn't specify	4–5 years	52	Supragingival plaque, carious lesion	Sabouraud dextrose agar	API 20C AUX (BioMerieux)
Ghasempour, et al., 2011	Iran, CS	Kindergarten	VTE, didn't specify	Carious lesion on the cervical side of incisor, dmft	2–5 years	60	Whole supragingival plaque	Sabouraud dextrose agar with chloromycetin and CHROMagar Candida	Germ tube test
Yang et al., 2012	China, CS	University dental clinic/ nursery schools	VTE, didn't specify	Method reported in Drury et al. [1999], dmft/s	3–6 years ECC: 4.5 + 0.6 years CF: 4.1 + 0.8 years	41	Whole supragingival plaque	CHROMagar Candida	Colony color; PCR, primer described in Miyakawa et al. [1993]
Wu et al., 2015	China, CS	Didn't specify	VTE, didn't specify	Didn't specify, dmft	3–5 years	399	Whole supragingival plaque	CHROMagar Candida	Colony characteristics. PCR, primer ITS1/ITS2
Qiu et al., 2015	China, CS	Kindergarten	VTE, didn't specify	Method reported in Drury et al. [1999]	3–5 years	363 (M: 200/F: 163)	Whole supragingival plaque	CHROMagar Candida	PCR, primer CA-INT-L, CA-INT-R
Neves et al., 2015	Brazil, CS	University dental clinic	VTE, intra κ = 0.836; inter κ = 0.838	Didn't specify, dmft	2–4 years	14	Saliva	CHROMagar Candida	Colony characteristics
Lozano Moraga et al., 2017	Chile, CS	Kindergarten	VTE	ICDAS	2–5 years	61 (M: 27/F: 34)	Saliva	Sabouraud agar with tetracycline, CHROMagar Candida	API ID32C, and PCR (primer ITS1/ITS4 for *Candida* spp, HWP1 gene for *C. albicans* and *C. dubliniensis*)
Xiao et al., 2016	USA, CS	University dental clinic	VTE, intra κ = 0.82; inter κ = 0.82	WHO 1997, *dmft/s*	12–71 months	35	Saliva, whole supragingival plaque, swab	CHROMagar Candida	Germ tube test
Zhang et al., 2016	China, CS	Kindergarten	VTE, intra κ = 0.83; inter κ = 0.81	Didn't specify, dmft	3–5 years	397 (M: 202/F: 195)	Supragingival plaque	CHROMagar Candida	Colony color, germ tube test, PCR (primer ITS1/ITS2)
Thomas et al., 2016	India, CS	University dental clinic	VTE, didn't specify	Didn't specify,	12–71 months	40	Saliva	CHROMagar Candida	Colony color, germ tube test and API 20C (BioMerieux)

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Table 1 (continued)

Authors	Prevalence of *C. albicans* in ECC	Prevalence of *C. albicans* in caries-free samples	Statistical analysis	Correlation between *C. albicans* and ECC	Conclusions	Quality assessment
Radford et al., 2000	24%	10%	Mann-Whitney *U*	Didn't examine	In infants as young as 1 year of age, salivary *S. mutans*, lactobacilli and yeasts but not *S. sobrinus* were isolated significantly more frequently from those with caries compared to those who were caries-free	Good
Marchant et al., 2001a	89% (among 52 carious lesion sites)	7%	χ^2, Mann-Whitney *U*	Didn't examine	The proportion of *C. albicans* was significantly greater in the carious dentin of caries children than in the plaque samples of caries-free children	Good
Hossain, et al., 2003	Saliva: 55.4%; plaque: 66.1%; carious lesion: 85.7%	Saliva: 9.6%; plaque: 7.7%	χ^2 test	Didn't examine	Significantly higher levels of *C. albicans* were found in saliva, dental plaque, carious specimens, and stools of 56 patients with severe caries as compared to 52 healthy control subjects. Results demonstrate a strong correlation between oral and gastrointestinal *C. albicans* colonization	Good
Ugun-Can et al., 2007	Caries score low, moderate, high (14.7, 48.3, 38.4%)	7.7%	Mann-Whitney *U*	Didn't examine	In the 4- to 6-year age group, high frequency of oral *Candida* in children with moderate and high dft indexes was significantly higher than in caries-free children	Good
de Carvalho et al., 2006	Plaque: 50%; carious dentin: 70.8%;	14%	Fisher or χ^2	$p < 0.05$	The frequency of *C. albicans* in ECC was higher when compared to caries-free groups. There is a significant association between the presence of *C. albicans* and early childhood caries	Good
Rozkiewicz et al., 2006a	61%	33%	χ^2	Didn't examine	Carriage of *C. albicans* in caries children was significantly higher than in caries-free children ($p = 0.0479$). However, there was no significant difference of detection frequency of *C. albicans* in caries-free girls (5/11) and caries-active girls (7/13)	Fair
Ghasempour, et al., 2011	Plaque: 80%; carious lesion: 100%	Plaque: 15%	χ^2	Didn't examine	The largest distribution of *C. albicans* in dental plaque and caries lesions was found in the cervical decay group	Fair
Yang,et al., 2012	Carious site: 57.1%; sound site: 14.3%	0%	χ^2	Didn't examine	This study found a high prevalence of *C. albicans* in the dental biofilm of children with S-ECC. The presence of *C. albicans* was significantly higher in carious lesions than on sound tooth surfaces of children with S-ECC, and *C. albicans* genotype A was the dominant component in both carious and sound sites	Fair
Wu et al., 2015	49%	7%	Pearson χ^2	$p = 0.000$	The detection rate of *C. albicans* was closely correlated with the caries filling index classification ($p = 0.000$). There was a *C. albicans* genotype distribution difference between Uygur and Han children	Fair
Qiu et al., 2015	44%	19%	χ^2	Didn't examine	The genotypic distribution of *C. albicans* is associated with the caries experience of children, and the genotype may be related to its acidogenicity at pH 4.0	Fair
Neves et al., 2015	43%	43%	Student *t* test	Didn't examine	The number of *Candida* spp. colonies did not differ between the groups ($p = 0.479$)	Fair
Lozano Moraga et al., 2017	63% (moderate caries: 50%, severe caries: 72.2%)	35.5%	χ^2, nonparametric Kruskal-Wallis	Didn't examine	*C. albicans* was found more prevalent in the group with severe caries ($p < 0.05$)	Fair
Xiao, et al., 2016	Saliva: 77%; plaque: 83%; swab: 44%	Saliva: 12%; plaque: 6%; swab: 6%	χ^2, Spearman rank test	$p < 0.05$	Results indicated a high *C. albicans* carriage rate in the oral cavity (saliva and plaque) of both S-ECC children and their mothers (>80%). Spearman correlation coefficients also indicated a significant correlation between salivary and plaque *C. albicans* and *S. mutans* carriage ($p < 0.01$) and caries severity ($p < 0.05$)	Good
Zhang et al., 2016	48%	24%	Pearson χ^2	$p < 0.05$	There were significant correlations between the presence of *C. albicans* and ECC severity in terms of dmft (Uygur children $p = 0.001$, Han children $p = 0.000$)	Fair
Thomas et al., 2016	100%	100%	χ^2, Mann-Whitney *U*	Didn't examine	*C. albicans* was found in both the S-ECC and caries-free groups. The median *C. albicans* of the S-ECC group was numerically greater than that of the caries-free group, and this difference was highly statistically significant ($p = 0.012$)	Fair

CS, cross-sectional; VTE, visual-tactile examination; CF, caries free.

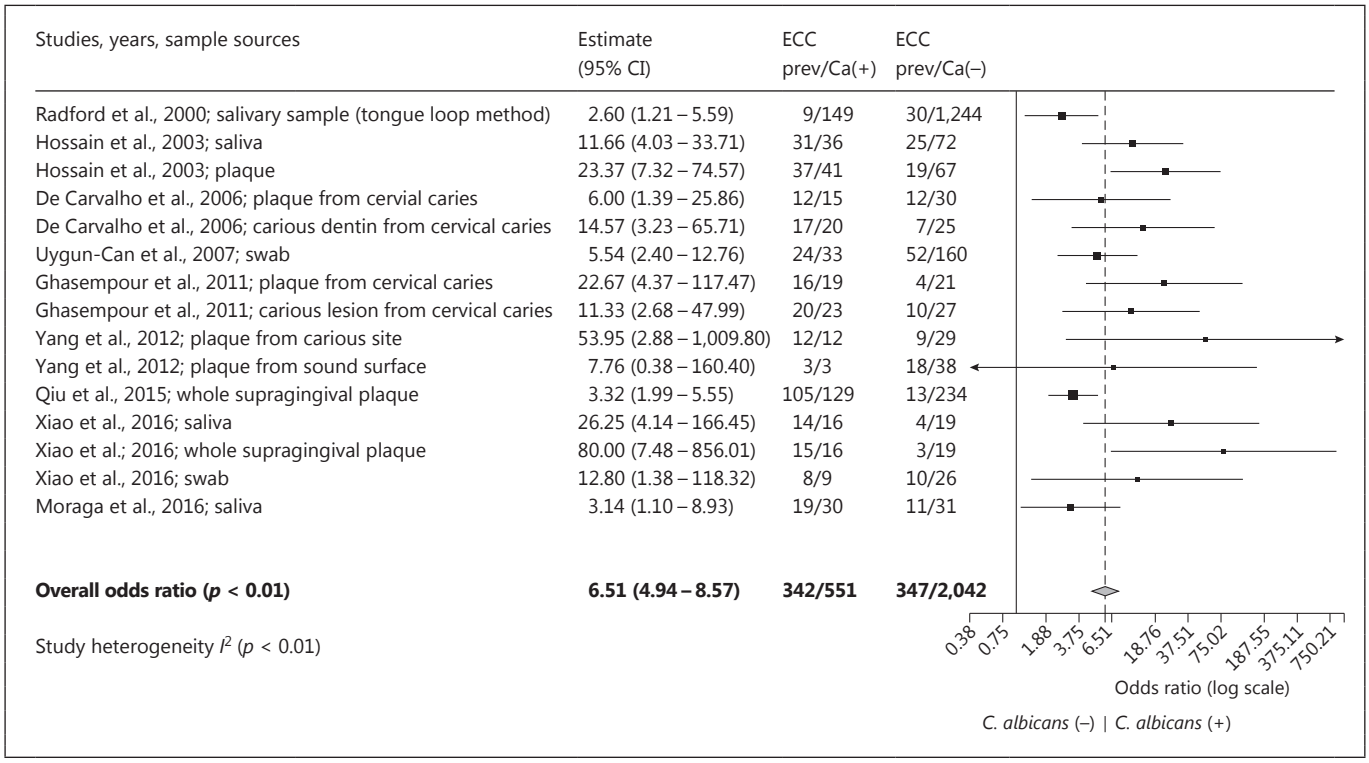

Fig. 2. Odds ratio of ECC prevalence in children with and without oral *C. albicans*. Meta-analysis from all oral sample sources (saliva, plaque, oral mucosal swab, and carious lesions). Evaluations of the presence of *C. albicans* and dental caries (outcome: presence of ECC vs. absence of ECC). Pooled effect measures of odds ratio (OR) and 95% confidence interval (CI) indicated that regarding ECC experience, there is a statistically significant difference between children with the presence of oral *C. albicans* and absence of oral *C. albicans*; the OR is 6.51 (favors the presence of oral *C. albicans*) and $p < 0.01$. Study heterogeneity (I^2) and the related p value were also calculated ($p < 0.01$). The solid line indicates when OR = 1. The dotted line indicates the overall OR value.

aminer calibration. A κ value >0.8 was considered acceptable agreement. Different caries diagnostic criteria were used in the selected studies. Three studies utilized World Health Organization criteria, 1 study used the British Association for the Study of Community Dentistry criteria, 1 study used the International Caries Detection and Assessment System (ICDAS), and 1 study used the International Standardization for Caries. The remaining studies did not clearly specify the caries diagnostic method. The decayed (d), missing (m), filled (f), tooth (t) or surface(s) (dmft/s) index was used in most of the studies for caries severity. The American Academy of Pediatric Dentistry definition and classification of S-ECC was used in several studies (Table 1).

Oral C. albicans *Prevalence and Carriage in ECC Children*

The *C. albicans* prevalence in ECC children ranged from 24 to 100% in saliva, 44 to 80% in plaque, 14.7 to 44% in swab samples, and from 60 to 100% in carious lesions. The *C. albicans* prevalence in caries-free children ranged from 10 to 100% in saliva, 7 to 19% in plaque, and 6 to 7% in swab samples. Statistical differences of *C. albicans* prevalence between ECC and caries-free children were examined in 11 studies using methods such as χ^2, Mann-Whitney *U*, and Pearson χ^2 with $p < 0.05$ (detailed in Table 1). Most studies (13 out of 15) found a higher prevalence of oral *C. albicans* in ECC children than in caries-free children.

In addition to the prevalence rate, *C. albicans* carriage was quantified in a few studies. Xiao et al. [2016] reported that colony-forming unit levels of *C. albicans* in saliva and plaque samples of S-ECC children were 3-log higher than in caries-free children. Thomas et al. [2016] found the median *C. albicans* count to be statistically greater in S-ECC groups than in the caries-free groups (except in the subgroup of children aged 1–3 years). Similarly, Rozkiewicz et al. [2006a] reported that carriage of *C. albicans* in caries-active children was significantly higher than in caries-free children.

Oral C. albicans *Detection and Sample Collection Sites*

Several studies compared *C. albicans* detection in samples collected from different sites. Plaque samples were found to yield a higher *C. albicans* detection than the salivary and swab samples [Xiao et al., 2016]. Plaque samples collected adjacent to carious lesion sites, especially cervical lesions, appeared to have a higher detection rate than those collected from sound tooth surfaces [Yang et al., 2012]. Furthermore, *C. albicans* was detected more frequently in infected dentin than in plaque close to carious lesions [Ghasempour et al., 2011]. Interestingly, plaque and infected dentin collected from proximal caries had a lower detection frequency of *C. albicans* than samples from cervical carious lesions [Ghasempour et al., 2011].

Association between Oral C. albicans *and ECC Experience*

Meta-analysis results from 9 studies evaluated the odds of ECC experience (outcome) associated with the presence of oral *C. albicans*. The pooled estimate of OR (6.51) and 95% CI (4.94–8.57) indicated significantly higher ECC experience in children with *C. albicans* than those without *C. albicans* ($p < 0.01$) (Fig. 2). The odds of experiencing ECC in children with *C. albicans* versus those without *C. albicans* was 5.26 for salivary (Fig. 3a), 6.69 for plaque (Fig. 3b), and 6.30 for swab samples (Fig. 3c); all supported the association between *C. albicans* presence and greater ECC experience.

Several studies further indicated a positive correlation between *C. albicans* prevalence and/or carriage and ECC severity ($p < 0.05$) [Wu et al., 2015; Xiao et al., 2016; Lozano Moraga et al., 2017]. Wu et al. [2015] found that the *C. albicans* detection rate was positively correlated with ECC severity in terms of dmft. Xiao et al. [2016] reported a significant positive correlation between salivary/plaque *C. albicans* carriage and ECC severity (dmft/s) ($p < 0.05$). Results from Lozano Moraga et al. [2017] showed that *C. albicans* was more prevalent in the group with severe caries examined by means of ICDAS ($p < 0.05$). Ugun-Can et al. [2007] found the detection frequency of oral *Candida* to be statistically higher in children with moderate and high dft than that in caries-free children; however, there was no significant difference between low dft and caries-free children.

Discussion

In this systematic review and meta-analysis, we noted a statistically significant difference between *C. albicans* prevalence in the oral cavity of children with ECC compared to those without ECC. Moreover, we found that individuals with oral *Candida* presence were associated with a >5 times odds of experiencing ECC. Despite the heterogeneity of the included studies with regard to the sample sources and *C. albicans* isolation/identification methods, as well as the relatively low evidence strength (e.g., cross-sectional study design, risk bias, small sample size of some studies), the nearly unequivocal conclusions of reported findings, and the magnitude of pooled OR estimates strongly support the association of *C. albicans* with caries experience.

C. albicans is by far the most commonly detected fungal organism on human mucosal surfaces [Cannon et al., 1995; Thein et al., 2009; Samaranayake and Matsubara, 2017]. It is considered an opportunistic pathogen that lives as a benign commensal organism in the mouths of healthy individuals, especially younger children [Thomas et al., 2016]. Its oral carriage can be affected by several factors, such as host age, diet, geographic location, socioeconomic status, gender, immunosuppression, and medication use [Cannon et al., 1995; Kadir et al., 2005; Samaranayake and Matsubara, 2017]. One attribute of *C. albicans* that makes it a successful opportunistic pathogen is its ability to adapt and proliferate in a broad range of host environments [Sherrington et al., 2017] such as acidic conditions [Cannon et al., 1995; Gunther et al., 2014; Sherrington et al., 2017]. In this context, the presence of *C. albicans* may just be serendipitous, coexisting with other oral microorganisms in biofilms or in carious lesions as a natural consequence of the acidified microenvironment. The majority of studies included in this review did not examine the effect of predisposing factors that might potentially be associated with *C. albicans* carriage in ECC children. The regression analysis from Xiao et al. [2016] showed that none of the factors such as antibiotic usage, birth weight, inhaler use, brushing frequency, and daycare attendance had a significant effect on the carriage of salivary and plaque *C. albicans* in S-ECC children.

Conversely, there is evidence from in vitro and in vivo mechanistic studies that strongly support the cariogenic properties of *C. albicans* such as: (1) an acidogenic and aciduric potential (even at pH 4.0) [Klinke et al., 2009] that is capable of dissolving hydroxyapatite [Nikawa et al., 2003] and causing caries in vivo (Klinke et al., 2011); (2) enhanced sucrose-dependent biofilm formation when cocultured with *S. mutans* in vitro [Pereira-Cenci et al., 2008; Gregoire et al., 2011; Metwalli et al., 2013; Sztajer et al., 2014; Kim et al., 2017] and in vivo [Falsetta et al., 2014; Hwang et al., 2017], and (3) capacity of causing advanced

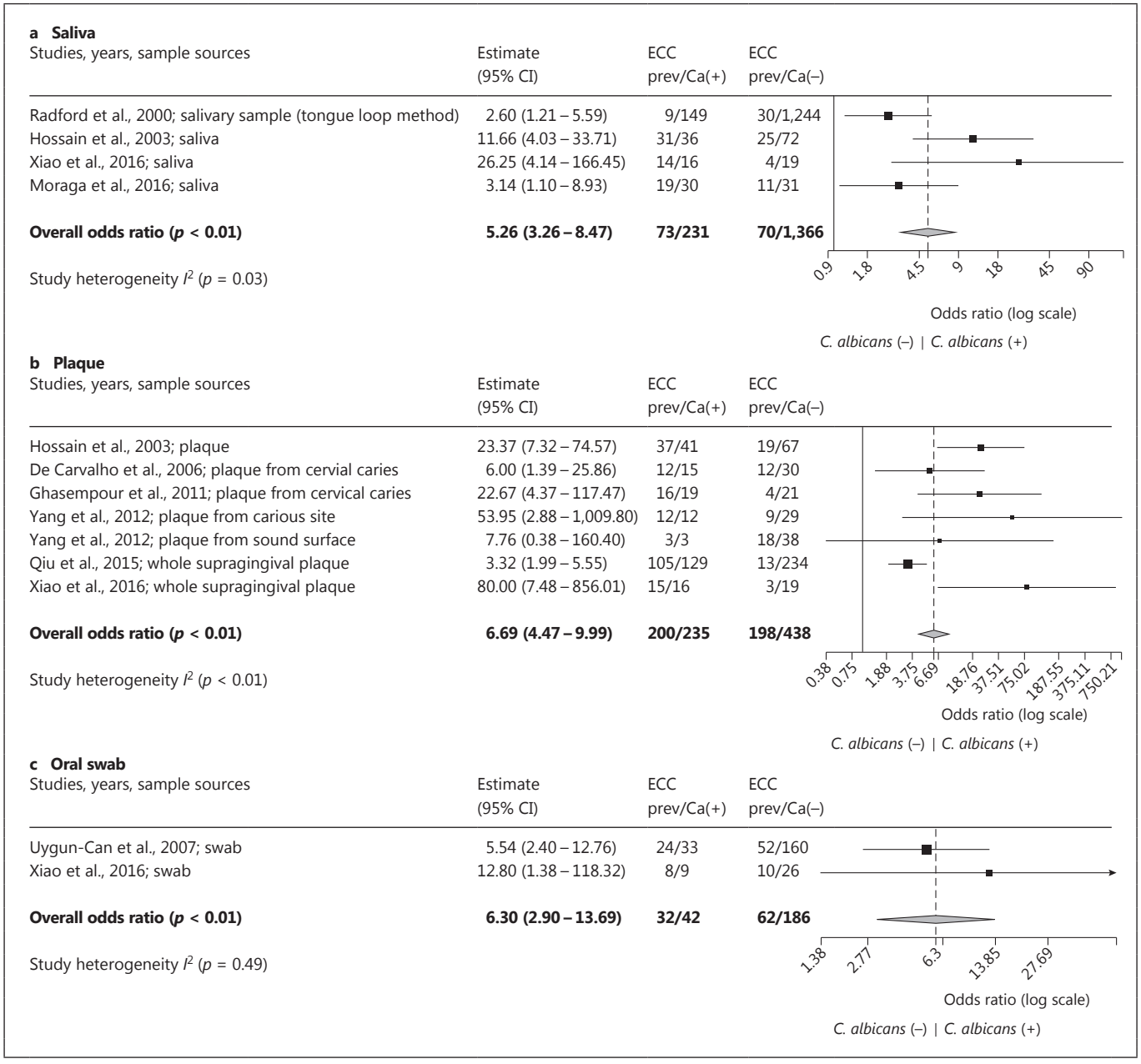

Fig. 3. Odds ratio of ECC prevalence in children with and without oral *C. albicans* (subgroup analysis). Evaluation of the presence of saliva/plaque/oral mucosal *C. albicans* and dental caries (outcome: presence of ECC vs. absence of ECC). **a** Pooled effect measures of odds ratio (OR) and 95% confidence interval (CI) indicated that regarding ECC experience, there is a statistically significant difference between children with the presence of salivary *C. albicans* and absence of salivary *C. albicans*; the OR is 5.26 (favors the presence of salivary *C. albicans*) and $p < 0.01$. Study heterogeneity (I^2) and the related p value were also calculated ($p = 0.03$). **b** Regarding ECC experience, there is a statistically significant difference between children with the presence of plaque *C. albicans* and absence of plaque *C. albicans*; the OR is 6.69 (favors the presence of plaque *C. albicans*) and $p < 0.01$. Study heterogeneity I^2, $p < 0.01$. **c** Regarding ECC experience, there is a statistically significant difference between children with the presence of swab *C. albicans* and absence of swab *C. albicans*; the OR is 6.3 (favors the presence of swab *C. albicans*) and $p < 0.01$. Study heterogeneity I^2, $p = 0.49$. The solid line indicates when OR = 1. The dotted line indicates the overall OR value.

caries lesions in a rat model of ECC through synergistic interactions with *S. mutans* [Falsetta et al., 2014]. The cariogenic potential of *C. albicans* has also been supported by clinical studies showing *S. mutans* and *C. albicans* co-detection in plaque, which was found to be strongly associated with ECC [Radford et al., 2000; de Carvalho et al., 2006; Neves et al., 2015; Xiao et al., 2016]. Further mechanistic and longitudinal studies are needed, however, to validate these observations, as some studies have shown no correlation with caries, while other studies consider *C. albicans* as a keystone commensal [Janus et al., 2016] with a possible protective role against dental caries development [Willems et al., 2016].

In addition to examining the association between oral *Candida* and ECC, a few other interesting findings emerged. For example, 1 study examined the maternal relatedness of *C. albicans* isolated from S-ECC children and found that the mothers of S-ECC children were also highly infected with oral *C. albicans* (>80% detection in both saliva and plaque samples) and more than 60% of the S-ECC children were carrying the same *C. albicans* strains as their mothers. This suggests that the mother might be a source for *C. albicans* acquisition in the oral cavity of children affected by the disease [Xiao et al., 2016] which, if validated, may have important implications for ECC prediction and prevention. Additionally, the genotypic distribution of *C. albicans* appeared to be associated with the caries experience of children, with the *C. albicans* genotypic subgroup A being the dominant strain in the plaque biofilm of children with S-ECC [Yang et al., 2012; Qiu et al., 2015; Wu et al., 2015]. Finally, in 1 study there was a strong correlation between oral and gastrointestinal *C. albicans* colonization [Hossain et al., 2003] suggesting that carious teeth may constitute an ecologic niche for *C. albicans* that can contribute to recurrent oral and nonoral candidiasis.

The findings presented here should be interpreted within the following limitations. (1) All the selected studies had a cross-sectional design instead of case-control or cohort design which was a weakness of the available evidence for the question our review attempted to answer. Without prospective cohort studies, it remains unclear whether *C. albicans* is a causative factor for ECC initiation or progression, or whether *C. albicans* presence is merely a consequence of an acidified oral environment following the development of ECC. (2) Small sample size was another limitation of most of the included studies. ECC is a multifactorial disease, with many predisposing factors other than fungal carriage. Limited sample size compromised the power of performing multiple regression analysis used in some studies. (3) The articles included in the meta-analysis were highly heterogeneous; the only 2 relatively comparable studies were the ones included in the swab sample subanalysis, with the $I^2 = 0\%$, $p = 0.49$. (4) Less than half of the included studies were assessed as good quality, with the rest being of fair quality. (5) Methodologies *for C. albicans* identification were variable.

As the detection of *C. albicans* was the outcome measure in this systematic review, it is worth noting that clinical sample collection and processing methods can significantly affect *C. albicans* isolation outcome, especially in the case of dental plaque samples. None of the studies specified the amount of plaque collected or whether the viable counts were normalized. Additionally, cariogenic plaque is relatively sticky due to the rich content of extracellular polysaccharides, requiring adequate sonication to improve cell dispersion and cultivation. Among the selected literature, only 1 study described the sonication steps during plaque sample processing [Xiao et al., 2016]. Furthermore, there were multiple *C. albicans* isolation and identification methods that would provide different levels of sensitivity and specificity across the included studies. For instance, both Sabouraud dextrose agar and CHROMagar Candida were used in the studies, with the latter used more frequently. The yield (i.e., the number of colonies) and detection of yeast strains on CHROMagar Candida were shown to be greater than on Sabouraud dextrose agar, with a high *C. albicans* detection sensitivity (98.6%) and specificity (98.8%) [Coronado-Castellote and Jimenez-Soriano, 2013]. Molecular tools such as DNA-based identification were also used for enhanced precision of *Candida* detection at species level. These observations clearly emphasize the need for standardized methods for both identification and quantification to ensure comparable results while enhancing reproducibility and reliability of the data.

Conclusion

The evidence presented in this systematic review indicates that the prevalence of *C. albicans* in children with ECC is significantly higher than in caries-free children. In addition, children with oral *C. albicans* have higher odds of experiencing ECC compared to children without *C. albicans*. Further prospective observational cohort studies are needed to strengthen the evidence supporting the association between oral *C. albicans* and ECC, and to determine whether or not *Candida* detection can serve as a reliable risk factor or risk indicator for the development of ECC/S-ECC.

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Disclosure Statement

The authors declare no conflict of interests.

Author Contributions

J.X. and H.K. contributed to the study design, J.X., X.H., N.A., H.A., S.A., D.A.C., F.C., J.D., T.T.W. and K.H. performed the data acquisition and analysis. J.X., X.H., F.C., T.T.W., D.T.K.-K., R.B., E.H., and H.K. contributed to the data interpretation, manuscript writing and critical revision of the manuscript.

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