# *Candida albicans* and Early Childhood Caries: A Systematic Review and Meta-analysis

**Jin Xiao**[1], **Xuelian Huang**[1], **Naemah Alkhers**[1], **Hassan Alzamil**[1], **Sari Alzoubi**[1], **Tong Tong Wu**[2], **Daniel A Castillo**[3], **Frank Campbell**[4], **Joseph Davis**[5], **Karli Herzog**[5], **Ronald Billings**[1], **Dorota T Kopycka-Kedzierawski**[1], **Evlambia Hajishengallis**[5], and **Hyun Koo**[6]

[1]Eastman Institute for Oral Health, University of Rochester Medical Center, Rochester, NY, USA

[2]Department of Biostatistics and Computational Biology, University of Rochester, NY, USA

[3]Miner Library, University of Rochester Medical Center, Rochester, NY, USA

[4]Biomed Library, University of Pennsylvania, Philadelphia, PA, USA

[5]Division of Pediatric Dentistry, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA, USA

[6]Department of Orthodontics and Pediatric Dentistry & Community Oral Health Divisions, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA, USA

## 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 (Mental-Haenszel estimate) was used for meta-analysis, and the summary effect measure was calculated by odds ratio (OR) and 95% CI. Fifteen cross-sectional studies were included for the qualitative assessment, and nine studies for meta-analysis. Twelve studies revealed higher oral *C. albicans* prevalence in ECC children than in caries-free children, while two studies indicated an equivalent prevalence. A pooled estimate, OR (6.51) and 95% CI (4.94, 8.57), indicated 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* was 5.26 for salivary, 6.69 for plaque, and 6.3 for oral swab sample. 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).

## Keywords

## Introduction

Early childhood caries (ECC) is the single most common childhood oral disease that disproportionately affects poor and minority children (<6 year of age), in the US 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 [de Carvalho et al., 2006; Hossain et al., 2003; Marchant et al., 2001b; Raja et al., 2010; Rozkiewicz et al., 2006b]. 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) [Pereira et al., 2017; Xu et al., 2014].

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 [de Carvalho et al., 2006; Hossain et al., 2003; Marchant et al., 2001b; Raja et al., 2010; Rozkiewicz et al., 2006b]. Moreover, *in vivo* studies using rodent caries models have demonstrated the cariogenic potential of *C. albicans* [Klinke et al., 2011], particularly when co-infected with *S. mutans*. Co-infection 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 not 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 [Peretz et al., 2011; Moreira et al., 2001]. Additionally, a recent study

indicated a 100% prevalence rate of salivary *C. albicans* in healthy children aged 12 to 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 grey 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.

### Inclusion/Exclusion criteria

This systematic review included experimental and epidemiological study designs such as randomized controlled trials, non-randomized controlled trials, quasi-experimental, 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 was 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 hand out, case report, 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 (K=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 four 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 (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/tools/ cohort). Articles were scaled as "Fair", "Good", or "Poor" following the protocol guidelines. 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 $I^2$ statics. For categorical data, odds ratio (OR), 95% confidence intervals (CI) and p-value were calculated in a forest plot using a fixed effects model (Mental-Haenszel estimate). Sub-group 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 emails 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 Appendix.

### Study characteristics

From the fifteen cross-sectional studies used for qualitative analysis (see Table 1), six studies were assessed as "Good" and nine 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 United States. 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 four studies did not detail the subject recruitment site. Children in all fifteen studies were younger than 72 months of age, with the majority of the studies examining children from 1-5 years of age; one study from Scotland assessed children at an earlier age (<1yr old). Oral samples were collected from at least one 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 one 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, four studies examined samples collected from carious lesions (Table 1). Samples were plated onto Sabouraud dextrose agar (SDA) and/or CHROMagar selective for *C. albicans* isolation. Microbiological and molecular methods were employed for *C. albicans* identification, including colony shape and color, germ tubes test, Auxacolor Test

(Sano® Diagnostics Pasteur, France), β-N-acetylgalactosaminidase assay, API 20C (BioMerieux), API ID 32C and Polymerase Chain Reaction (PCR).

All of the clinical studies used visual-tactile examination techniques; three studies performed intra- and inter-examiner calibration. A K value>0.8 was considered acceptable agreement. Different caries diagnostic criteria were used in the selected studies. Three studies utilized World Health Organization criteria, one study used the British Association for the study of community dentistry criteria, one study used the International Caries Detection and Assessment System (ICDAS), and one 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 severe ECC (S-ECC) was used in several studies (Table 1).

### Oral *C. albicans* prevalence and carriage in ECC children

*C. albicans* prevalence in ECC children ranged from 24%-100% in saliva, 44%-80% in plaque, 14.7%-44% in swab samples, and 60%-100% in carious lesions. *C. albicans* prevalence in caries free children ranged from 10%-100% in saliva, 7%-19% in plaque, and 6%-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 Chi-square, Mann-Whitney *U*, and Pearson $\chi^2$ 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 [Xiao et al., 2016] reported that colony forming unit levels of *C. albicans* in saliva and plaque sample of S-ECC children were 3-log higher than in caries-free children. Thomas [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 [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*. 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 without *C. albicans* was 5.26 for salivary (Fig. 3A), 6.69 for plaque (Fig. 3B), and 6.30 for swab sample (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) [Lozano Moraga et al., 2017; Wu et al., 2015; Xiao et al., 2016]. Wu [Wu et al., 2015] found that *C. albicans* detection rate was positively correlated with ECC severity in terms of *dmft*. Xiao [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 Lozanzo Moraga showed that *C. albicans* was more prevalent in the group with severe caries examined by means of ICDAS (P<0.05) [Lozano Moraga et al., 2017]. Uygun-Can [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 >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 organisms on human mucosal surfaces [Cannon et al., 1995; Samaranayake and Matsubara, 2017; Thein et al., 2009]. 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, socio-economic 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, co-existing 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 [Xiao et al., 2016] showed that none of the factors such as antibiotic usage, birth weight, inhaler use, brushing frequency, and daycare attendance had 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 co-cultured with *S. mutans in vitro* [Gregoire et al., 2011; Kim et al., 2017; Metwalli et al., 2013; Pereira-Cenci et al., 2008; Sztajer et al., 2014] and *in vivo* [Falsetta et al., 2014; Hwang et al., 2017], and 3) capacity of causing advanced 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 [de Carvalho et al., 2006; Neves et al., 2015; Radford et al., 2000; 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, one 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 [Qiu et al., 2015; Wu et al., 2015; Yang et al., 2012]. Finally, in one 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 non-oral 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 multi-factorial 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 two relatively

comparable studies were the ones included in the swab sample sub-analysis, 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) Variability of methodologies *for C. albicans* identification.

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 one 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 SDA 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 SDA, 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 development of ECC/S-ECC.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

## References

Cannon RD, Holmes AR, Mason AB, Monk BC. Oral candida: Clearance, colonization, or candidiasis? Journal of dental research. 1995; 74:1152–1161. [PubMed: 7790592]

Colak H, Dulgergil CT, Dalli M, Hamidi MM. Early childhood caries update: A review of causes, diagnoses, and treatments. Journal of natural science, biology, and medicine. 2013; 4:29–38.

Coronado-Castellote L, Jimenez-Soriano Y. Clinical and microbiological diagnosis of oral candidiasis. J Clin Exp Dent. 2013; 5:e279–286. [PubMed: 24455095]

de Carvalho FG, Silva DS, Hebling J, Spolidorio LC, Spolidorio DM. Presence of mutans streptococci and candida spp. In dental plaque/dentine of carious teeth and early childhood caries. Arch Oral Biol. 2006; 51:1024–1028. [PubMed: 16890907]

Douglass JM, Clark MB. Integrating oral health into overall health care to prevent early childhood caries: Need, evidence, and solutions. Pediatric dentistry. 2015; 37:266–274. [PubMed: 26063555]

Dye, BA., Li, X., Thorton-Evans, G. NCHS Data Brief. 2012. Oral health disparities as determined by selected healthy people 2020 oral health objectives for the united states, 2009-2010; p. 1-8.

Falsetta ML, Klein MI, Colonne PM, Scott-Anne K, Gregoire S, Pai CH, Gonzalez-Begne M, Watson G, Krysan DJ, Bowen WH, Koo H. Symbiotic relationship between streptococcus mutans and candida albicans synergizes virulence of plaque biofilms in vivo. Infection and immunity. 2014; 82:1968–1981. [PubMed: 24566629]

Ghasempour M, Sefidgar SA, Eyzadian H, Gharakhani S. Prevalence of candida albicans in dental plaque and caries lesion of early childhood caries (ecc) according to sampling site. Caspian J Intern Med. 2011; 2:304–308. [PubMed: 24551436]

Gregoire S, Xiao J, Silva BB, Gonzalez I, Agidi PS, Klein MI, Ambatipudi KS, Rosalen PL, Bauserman R, Waugh RE, Koo H. Role of glucosyltransferase b in interactions of candida albicans with streptococcus mutans and with an experimental pellicle on hydroxyapatite surfaces. Applied and environmental microbiology. 2011; 77:6357–6367. [PubMed: 21803906]

Gunther LS, Martins HP, Gimenes F, Abreu AL, Consolaro ME, Svidzinski TI. Prevalence of candida albicans and non-albicans isolates from vaginal secretions: Comparative evaluation of colonization, vaginal candidiasis and recurrent vaginal candidiasis in diabetic and non-diabetic women. Sao Paulo Med J. 2014; 132:116–120. [PubMed: 24714993]

Hajishengallis E, Parsaei Y, Klein MI, Koo H. Advances in the microbial etiology and pathogenesis of early childhood caries. Molecular oral microbiology. 2017; 32:24–34. [PubMed: 26714612]

Hossain H, Ansari F, Schulz-Weidner N, Wetzel WE, Chakraborty T, Domann E. Clonal identity of candida albicans in the oral cavity and the gastrointestinal tract of pre-school children. Oral Microbiol Immunol. 2003; 18:302–308. [PubMed: 12930522]

Hwang G, Liu Y, Kim D, Li Y, Krysan DJ, Koo H. Candida albicans mannans mediate streptococcus mutans exoenzyme gtfb binding to modulate cross-kingdom biofilm development in vivo. PLoS pathogens. 2017; 13:e1006407. [PubMed: 28617874]

Janus MM, Willems HM, Krom BP. Candida albicans in multispecies oral communities; a keystone commensal? Adv Exp Med Biol. 2016; 931:13–20. [PubMed: 27271681]

Kadir T, Uygun B, Akyüz S. Prevalence of candida species in turkish children: Relationship between dietary ıntake and carriage. Archives of Oral Biology. 2005; 50:33–37. [PubMed: 15598415]

Kassebaum NJ, Bernabe E, Dahiya M, Bhandari B, Murray CJ, Marcenes W. Global burden of untreated caries: A systematic review and metaregression. Journal of dental research. 2015; 94:650–658. [PubMed: 25740856]

Kim D, Sengupta A, Niepa TH, Lee BH, Weljie A, Freitas-Blanco VS, Murata RM, Stebe KJ, Lee D, Koo H. Candida albicans stimulates streptococcus mutans microcolony development via cross-kingdom biofilm-derived metabolites. Sci Rep. 2017; 7:41332. [PubMed: 28134351]

Klinke T, Guggenheim B, Klimm W, Thurnheer T. Dental caries in rats associated with candida albicans. Caries research. 2011; 45:100–106. [PubMed: 21412001]

Klinke T, Kneist S, de Soet JJ, Kuhlisch E, Mauersberger S, Forster A, Klimm W. Acid production by oral strains of candida albicans and lactobacilli. Caries research. 2009; 43:83–91. [PubMed: 19246906]

Lozano Moraga CP, Rodriguez Martinez GA, Lefimil Puente CA, Morales Bozo IC, Urzua Orellana BR. Prevalence of candida albicans and carriage of candida non-albicans in the saliva of preschool children, according to their caries status. Acta Odontol Scand. 2017; 75:30–35. [PubMed: 27796162]

Marchant S, Brailsford S, Twomey A, Roberts G, Beighton D. The predominant microflora of nursing caries lesions. Caries research. 2001a; 35:397–406. [PubMed: 11799279]

Marchant S, Brailsford SR, Twomey AC, Roberts GJ, Beighton D. The predominant microflora of nursing caries lesions. Caries research. 2001b; 35:397–406. [PubMed: 11799279]

Metwalli KH, Khan SA, Krom BP, Jabra-Rizk MA. Streptococcus mutans, candida albicans, and the human mouth: A sticky situation. PLoS pathogens. 2013; 9:e1003616. [PubMed: 24146611]

Moreira D, Spolidório DMP, Rodrigues JAdO, Boriollo MFG, Pereira CV, Rosa EAR, HÖfling JF. Candida sp. Biotypes in the oral cavity of school children from different socioeconomic categories in piracicaba-sp, brazil. Pesquisa Odontológica Brasileira. 2001; 15:187–195. [PubMed: 11705265]

Neves A, Lobo L, Pinto K, Pires E, Requejo M, Maia L, Antonio A. Comparison between clinical aspects and salivary microbial profile of children with and without early childhood caries: A preliminary study. Journal of Clinical Pediatric Dentistry. 2015; 39:209–214. [PubMed: 26208063]

Nikawa H, Yamashiro H, Makihira S, Nishimura M, Egusa H, Furukawa M, Setijanto D, Hamada T. In vitro cariogenic potential of candida albicans. Mycoses. 2003; 46:471–478. [PubMed: 14641620]

Pereira D, Seneviratne CJ, Koga-Ito CY, Samaranayake LP. Is the oral fungal pathogen candida albicans a cariogen? Oral diseases. 2017

Pereira-Cenci T, Deng DM, Kraneveld EA, Manders EM, Del Bel Cury AA, Ten Cate JM, Crielaard W. The effect of streptococcus mutans and candida glabrata on candida albicans biofilms formed on different surfaces. Archives of oral biology. 2008; 53:755–764. [PubMed: 18395698]

Peretz B, Mazor Y, Dagon N, Bar-Ness Greenstein R. Candida, mutans streptococci, oral hygiene and caries in children. Journal of Clinical Pediatric Dentistry. 2011; 36:185–188. [PubMed: 22524082]

Qiu R, Li W, Lin Y, Yu D, Zhao W. Genotypic diversity and cariogenicity of candida albicans from children with early childhood caries and caries-free children. BMC oral health. 2015; 15:144. [PubMed: 26576955]

Radford J, Ballantyne H, Nugent Z, Beighton D, Robertson M, Longbottom C, Pitts N. Caries-associated microorganisms in infants from different socio-economic backgrounds in scotland. Journal of dentistry. 2000; 28:307–312. [PubMed: 10785295]

Raja M, Hannan A, Ali K. Association of oral candidal carriage with dental caries in children. Caries research. 2010; 44:272–276. [PubMed: 20516688]

Rozkiewicz D, Daniluk T, Zaremba M, Cylwik-Rokicka D, Stokowska W, Pawi ska M, Dabrowska E, Marczuk-Kolada G, Waszkiel D. Oral candida albicans carriage in healthy preschool and school children. Advances in medical sciences. 2006a; 51(1):187–190. [PubMed: 17458089]

Rozkiewicz D, Daniluk T, Zaremba ML, Cylwik-Rokicka D, Stokowska W, Pawinska M, Dabrowska E, Marczuk-Kolada G, Waszkiel D. Oral candida albicans carriage in healthy preschool and school children. Advances in medical sciences. 2006b; 51(1):187–190. [PubMed: 17458089]

Samaranayake L, Matsubara VH. Normal oral flora and the oral ecosystem. Dental clinics of North America. 2017; 61:199–215. [PubMed: 28317562]

Sherrington SL, Sorsby E, Mahtey N, Kumwenda P, Lenardon MD, Brown I, Ballou ER, MacCallum DM, Hall RA. Adaptation of candida albicans to environmental ph induces cell wall remodelling and enhances innate immune recognition. PLoS pathogens. 2017; 13:e1006403. [PubMed: 28542528]

Sztajer H, Szafranski SP, Tomasch J, Reck M, Nimtz M, Rohde M, Wagner-Dobler I. Cross-feeding and interkingdom communication in dual-species biofilms of streptococcus mutans and candida albicans. The ISME journal. 2014; 8:2256–2271. [PubMed: 24824668]

Thein ZM, Seneviratne CJ, Samaranayake YH, Samaranayake LP. Community lifestyle of candida in mixed biofilms: A mini review. Mycoses. 2009; 52:467–475. [PubMed: 19486299]

Thomas A, Mhambrey S, Chokshi K, Chokshi A, Jana S, Thakur S, Jose D, Bajpai G. Association of oral candida albicans with severe early childhood caries - a pilot study. J Clin Diagn Res. 2016; 10:ZC109–112. [PubMed: 27656551]

Ugun-Can B, Kadir T, Akyuz S. Oral candidal carriage in children with and without dental caries. Quintessence Int. 2007; 38:45–49. [PubMed: 17216907]

Willems HM, Kos K, Jabra-Rizk MA, Krom BP. Candida albicans in oral biofilms could prevent caries. Pathogens and disease. 2016:74.

Wu N, Lin J, Wu L, Zhao J. Distribution of candida albicans in the oral cavity of children aged 3-5 years of uygur and han nationality and their genotype in caries-active groups. Genetics and Molecular Research. 2015; 14:748–757. [PubMed: 25730012]

Xiao J, Moon Y, Li L, Rustchenko E, Wakabayashi H, Zhao X, Feng C, Gill SR, McLaren S, Malmstrom H, Ren Y, Quivey R, Koo H, Kopycka-Kedzierawski DT. Candida albicans carriage in children with severe early childhood caries (s-ecc) and maternal relatedness. PLOS ONE. 2016; 11:e0164242. [PubMed: 27741258]

Xu H, Jenkinson HF, Dongari-Bagtzoglou A. Innocent until proven guilty: Mechanisms and roles of streptococcus-candida interactions in oral health and disease. Molecular oral microbiology. 2014; 29:99–116. [PubMed: 24877244]

Yang XQ, Zhang Q, Lu LY, Yang R, Liu Y, Zou J. Genotypic distribution of candida albicans in dental biofilm of chinese children associated with severe early childhood caries. Archives of Oral Biology. 2012; 57:1048–1053. [PubMed: 22717324]

Zhang WT, Lian BJ, Zhao J. the prevalence of candida albicans and its relationship with early childhood caries among children of uygur and han nationalities in kashi city. Zhonghua kou qiang yi xue za zhi = Zhonghua kouqiang yixue zazhi = Chinese journal of stomatology. 2016; 51:269–274. [PubMed: 27220384]
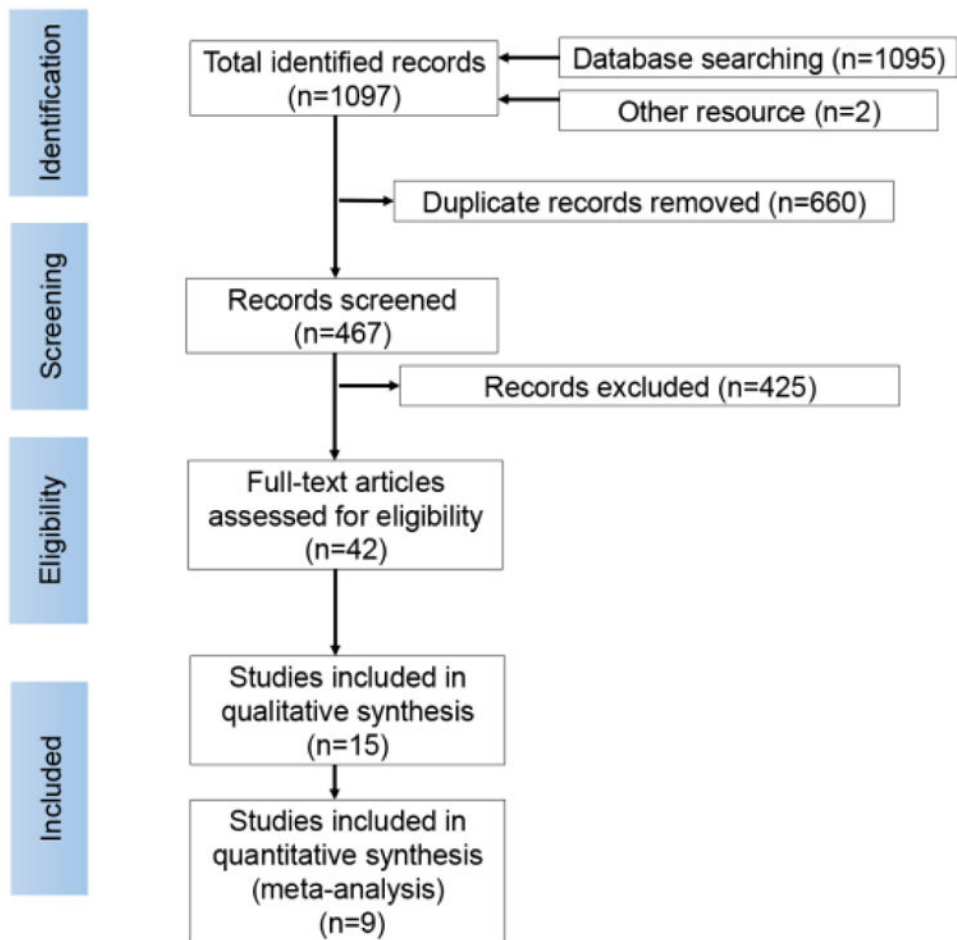
**Fig.1. Screening and assessing studies for inclusion eligibility**
The four-phase Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram was used to determine the number of studies identified, screened, eligible, and included in the systematic review and meta-analysis (http://www.prisma-statement.org).
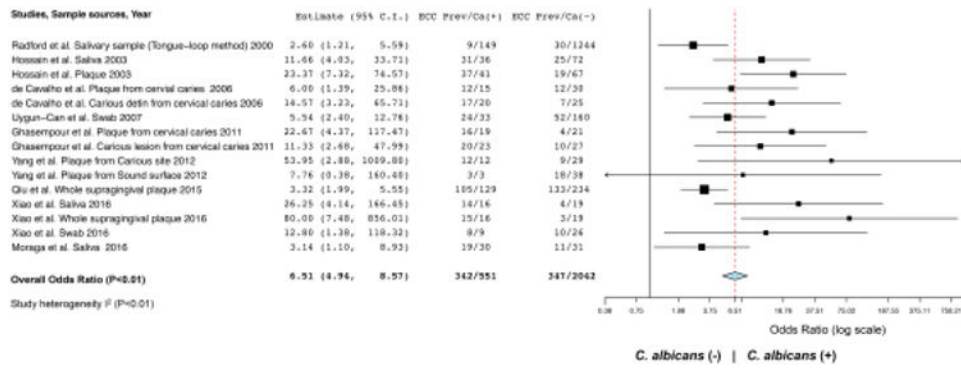
**Fig.2. Odds Ratio of ECC prevalence in children with and without oral *C. albicans***
Meta-analysis from all oral sample sources (saliva, plaque, oral mucosal swab and carious lesions). Evaluations of the presence of *C. albicans* and dental caries (outcome: presence of ECC vs. absence of ECC). Pooled effect measures of odds ratio (OR) and 95% confidence interval (CI0) 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*; OR is 6.51 (favors the presence of oral *C. albicans*) and P<0.01. Study heterogeneity ($I^2$) and the related P value was also calculated (P<0.01). The solid line indicates when OR=1. The red dotted line indicates the overall OR value.

## (A) Saliva



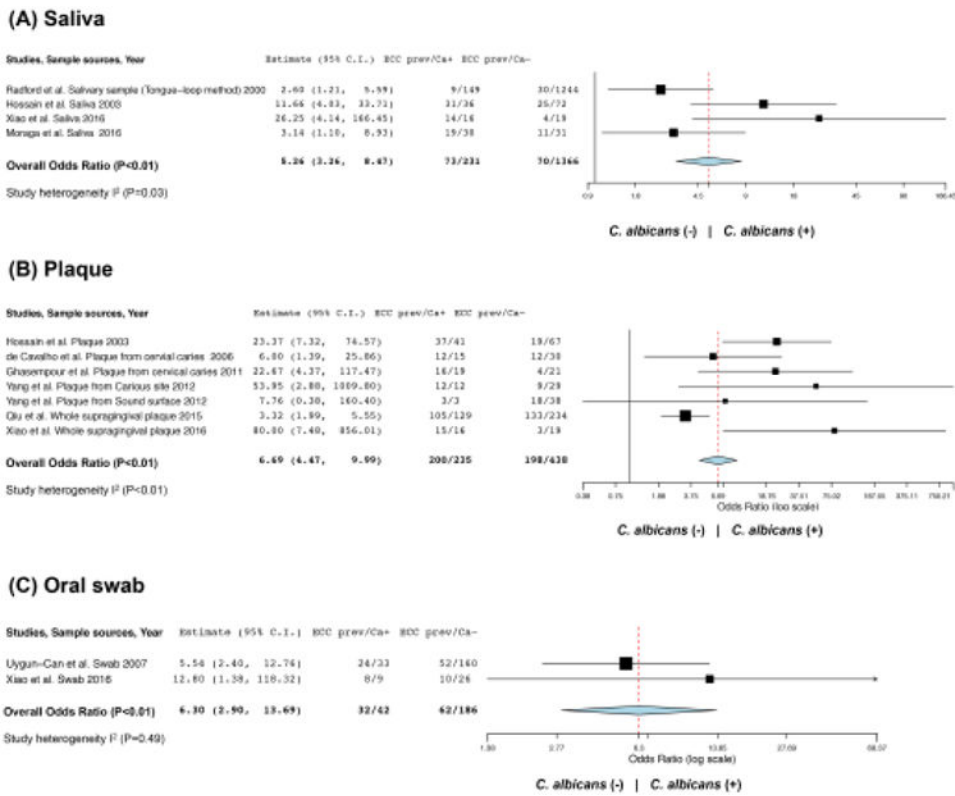## (B) Plaque



## (C) Oral swab



**Fig.3. Odds Ratio of ECC prevalence in children with and without oral *C. albicans* (sub-group 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 (CI0) 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*; OR is 5.26 (favors the presence of salivary *C. albicans*) and P<0.01. Study heterogeneity ($I^2$) and the related P value was 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*; 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*; 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 red dotted line indicates the overall OR value.

**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.a isolation method | C.a identification methods |
|---|---|---|---|---|---|---|---|---|---|
| Radford et al. [Radford et al., 2000], 2000 | Scotland, CS | Didn't specify | VTE, One calibrated examiner | WHO 1979, *dmft* | 1-12 months | 1393 | Saliva (tongue-loop method) | Sabouraud dextrose agar | Colony shape and odor |
| Marchant, et al. [Marchant et al., 2001a], 2001 | 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. [Hossain et al., 2003], 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 (RAPD) analysis |
| Uygun-Can, et al. [Uygun-Can et al., 2007] 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 Cavalho, et al. [de Carvalho et al., 2006], 2006 | Brazil, CS | Didn't specify | VTE, didn't specify | Method reported in *Drury, et al.* | 1-5 years | 56 | Supragingival plaque, carious dentin | CHROMagar Candida | Colony color, germ-tube test |
| Rozkiewicz D et al. [Rozkiewic z et al., 2006a], 2006 | 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. [Ghasemp our et al., 2011], 2011 | Iran, CS | Kindergarten | VTE, didn't specify | Carious lesion on the cervical of incisor, *dmft* | 2-5 years | 60 | Whole Supragingival plaque | Sabouraud dextrose agar with chloromycetin and CHROMagar Candida | Germ tube test |
| Yang, et al. [Yang et al., 2012], 2012 | China, CS | University dental clinic/Nursery schools | VTE, didn't specify | Method reported in *Drury, et al., 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. |
| Wu et al. [Wu et al., 2015], 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 |

| Authors | Country, study design | Subject recruitment site | Dental examination, Calibration | Caries diagnosis | Age | Total subjects | Sample sources | C.a isolation method | C.a identification methods |
|---|---|---|---|---|---|---|---|---|---|
| Qiu et al. [Qiu et al., 2015], 2015 | China, CS | Kindergarten | VTE, didn't specify | Method reported in *Drury, et al.* | 3-5 years | 363 (M:200/F:163) | Whole Supragingival plaque | CHROMagar Candida | PCR, primer CA-INT-L, CA-INT-R |
| Neves et al. [Neves et al., 2015], 2015 | Brazil, CS | University dental clinic | VTE, Intra Kappa=0.83 6; Inter Kappa=0.83 8; | Didn't specify, *dmft dmft* | 2-4 years | 14 | Saliva | CHROMagar Candida | Colony characteristics |
| Moraga, et al. [Lozano Moraga et al., 2017], 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/TS4 for Candida spp, HWP1 gene for *C.a* and *C. dubliniensis* |
| Xiao, et al. [Xiao et al., 2016], 2016 | US, CS | University dental clinic | VTE, Intra Kappa=0.82; Inter Kappa=0.82; | WHO 1997, *dmft/s* | 12-71 months | 35 | Saliva, Whole Supragingival plaque, | CHROMagar Candida | Germ tube test |
| Zhang et al. [Zhang et al., 2016], 2016 | China, CS | Kindergarten | VTE, Intra Kappa=0.83; Inter Kappa=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. [Thomas et al., 2016], 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) |
| Authors | Prevalence of *C.a* in ECC | Prevalence of *C.a* in Caries free | Statistical analysis | Correlation between *C.a* and ECC | Conclusions | | | | Quality Assessment |
| Radford et al. [Radford et al., 2000], 2000 | 24% | 10% | Mann-Whitney *U* | Didn't exam | 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. [Marchant et al., 2001a], 2001 | 89% (Among 52 carious lesion site), | 7% | Chi-square, Mann-Whitney *U* | Didn't exam | The Proportion of *C.a* was significantly greater in the carious dentine of caries children than in the plaque samples of Caries free children. | | | | Good |
| Hossain, et al. [Hossain et al., 2003], 2003 | Saliva: 55.4%; Plaque: 66.1%; Carious lesion: 85.7% | Saliva: 9.6%; Plaque: 7.7% | Chi-squared test | Didn't exam | Significantly higher levels of *C.a* 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.a* colonization. | | | | Good |
| Uygun-Can, et al. [Ugun-Can et al., 2007] 2007 | Caries score low, moderate, high (14.7%, 48.3%, 38.4 %) | 7.7% | Mann-Whitney *U* | Didn't exam | In the 4- to 6-year age group, high frequency of oral *Candida* in children with moderate and high df-t indexes was significantly higher than in caries-free children. | | | | Good |

| Authors | Country, study design | Subject recruitment site | Dental examination, Calibration | Caries diagnosis | Age | Total subjects | Sample sources | C.a isolation method | C.a identification methods |
|---|---|---|---|---|---|---|---|---|---|
| de Cavalho, et al. [de Carvalho et al., 2006], 2006 | Plaque: 50%; Carious dentin: 70.8%; | 14% | Fisher's or Chi-square | P<0.05 | | | The frequency of C.a in ECC was higher when compared to caries-free groups. There is a significant association between the presence of C.a and early childhood caries. | | Good |
| Rozkiewicz D et al. [Rozkiewicz et al., 2006a], 2006 | 61% | 33% | Chi-square | Didn't exam | | | Carriage of C.a 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. [Ghasempour et al., 2011], 2011 | Plaque: 80% Carious lesion: 100% | Plaque: 15% | Chi-square | Didn't exam | | | The most distribution of C. albicans in dental plaque and caries lesion was in cervical decay group. | | Fair |
| Yang, et al. [Yang et al., 2012], 2012 | Carious site: 57.1% Sound site: 14.3% | 0% | Chi-square | Didn't exam | | | This study found a high prevalence of C.a in the dental biofilm of children with S-ECC. The presence of C.a was significantly higher in carious lesions than on sound tooth surfaces of children with S-ECC, and C.a genotype A was the dominant component in both carious and sound sites. | | Fair |
| Wu et al. [Wu et al., 2015], 2015 | 49% | 7% | Pearson $\chi^2$ | P=0.000 | | | The detection rate of C.a was closely correlated to the caries filling index classification (P=0.000). There was C.a genotype distribution difference between Uyhur and Han children. | | Fair |
| Qiu et al [Qiu et al., 2015], 2015 | 44% | 19% | Chi-square | Didn't exam | | | The genotypic distribution of C.a 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. [Neves et al., 2015], 2015 | 43% | 43% | Student's t-test | Didn't exam | | | The number of Candida spp colonies did not differ between the groups (P=0.479) | | Fair |
| Moraga, et al [Lozano Moraga et al., 2017], 2017 | 63% (Moderate caries: 50%, severe caries: 72.2%) | 35.5% | Chi-square, non-parametric Kruskal–Wallis | Didn't exam | C.a was found more prevalent in the group with severe caries (P<0.05). | | | | Fair |
| Xiao, et al. [Xiao et al., 2016], 2016 | Saliva: 77% Plaque: 83% Swab: 44% | Saliva: 12% Plaque: 6% Swab: 6% | Chi-square Spearman's rank test | P<0.05 | | | Results indicated high C.a carriage rate in the oral cavity (saliva and plaque) of both S-ECC children and their mothers (>80%). Spearman's correlation coefficient also indicated a significant correlation between salivary and plaque C.a and S. mutans carriage (P<0.01) and caries severity (P<0.05). | | Good |
| Zhang et al. [Zhang et al., 2016], 2016 | 48% | 24% | Pearson $\chi^2$ | P<0.05 | | | There were significant correlations between the presence of C.a and ECC severity in terms of dmft. (Uygur children P=0.001, Han children P=0.000) | | Fair |
| Thomas et al. [Thomas et al., 2016], 2016 | 100% | 100% | 'Chi-Square', 'Mann-Whitney U' | Didn't exam | | | C. a was found in both the S-ECC group and caries free group. Median C.a of the S-ECC group was numerically greater than the caries free group and this difference was highly statistically significant (P=0,012). | | Fair |

\*

Abbreviation used in table 1: Cross-sectional (CS), Visual-tactile examination (VTE), Candida albicans (C.a); Method references used in caries diagnosis and C. albicans identification are listed in the Appendix.