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Effect of oral hygiene in infants before dental eruption on *Candida spp.* colonization and the occurrence of oral candidiasis: A randomized clinical trial

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Abstract

Background: To assess the influence of oral hygiene in infants before the primary tooth eruption on colonization by *Candida spp.* and the occurrence of oral candidiasis.

Material and Methods: Fifty-six infants were randomly selected in their first 48 hours of life and allocated into 2 groups: Group I (Mothers were instructed to sanitize the oral cavity of the infant with gauze and filtered water once a day) and Group II (Mothers were instructed not to sanitize the oral cavity of the infant before the dental eruption). Data collection was performed one month after the birth of the infant, in their residence, including saliva collection for identification and quantification of *Candida spp.*

Results: Colonization by *Candida spp.* species was found in 49.1% of the infants evaluated. There was no statistically significant difference between colonization by *Candida spp.* and intervention groups ($p=0.947$). 13.2% of the participants presented oral candidiasis during the first month of life, this prevalence was 15.4% in the control group and 11.1% in the intervention group, however, this difference was not significant ($p=0.704$).

Conclusions: The *Candida spp.* colonization and the oral candidiasis occurrence, in the first month of the life of the infant, were not influenced by oral hygiene.

Key words: Infants, oral hygiene, oral health, oral candidiasis.

Introduction

Prevention is the most effective approach to reducing the prevalence of oral diseases and their damage to the lives of affected children and their families. Keeping primary teeth healthy is essential to facilitate the proper growth and development of children. Childhood is the ideal time to establish healthy habits, which will be important for disease prevention (1).

Although oral hygiene is a preventive practice already consolidated and recommended, inconsistencies are observed concerning the time this approach is supposed to start (2,3). Some associations of Pediatrics and Pediatric Dentistry around the world recommend the oral hygiene of the infant after the eruption of the first deciduous tooth (4-6). Other associations recommend oral hygiene from the birth of the child, by cleaning the gum pads of the baby with gauze or soft tissue moistened with water (7-9). These associations claim that oral hygiene in this period is important for the child to become accustomed from an early age to oral manipulation and, when inserting foreign bodies into their mouth, they will, as a result, present a favorable behavior when starting the cleaning of the teeth (10). Additionally, caregivers would also become accustomed to the practice of oral hygiene of the infant. Additionally, caregivers would also become accustomed to the practice of oral hygiene of the infant.

Despite the different recommendations from associations in pediatric dentistry, there are still no scientific studies that support or refute oral hygiene since the birth of the infant (2). It is not clear whether these practices since birth are beneficial for the prevention of dental caries, halitosis, or behavior change (3).

It is believed that oral hygiene before tooth eruption may influence colonization by *Candida spp.* species present in the oral cavity of infants (11-13). *Candida albicans* is one of the most detected fungal species on human mucous surfaces and is considered an opportunistic pathogen (14). Thus, situations that favor the excessive growth of these species, such as the removal of defense factors present in saliva and breast milk in the oral cavity of infants, could lead to mucosal infections such as oral candidiasis.

Protective factors such as IgA and lactoferrin are important protein agents that have an antimicrobial effect and offer protection against fungi, especially in *candida spp* species (15). However, there is still no scientific evidence regarding the influence of the cleaning of the infant's oral cavity before the dental eruption, the frequency at which this hygiene is performed, and the elimination of the defense factors present in saliva and breast milk.

Identifying the benefits or impairments of oral hygiene before tooth eruption is extremely important to support the guidance of pediatricians and pediatric dentists and, consequently, promote the maintenance of good oral health and better quality of life for children and their families. Thus, this investigation aimed to evaluate, throu-

gh a randomized clinical trial, the influence of oral hygiene on colonization by *Candida spp.* and the presence of oral candidiasis in the first month of life of the infant.

Material and Methods

-Ethical Considerations

This research received approval by the Research Ethics Committee in research involving human beings of the Federal University of Minas Gerais under protocol number 3,568,480. The study was conducted according to the recommendations of the consort (Consolidated Standards of Reporting Trials) (16). The clinical trial was registered on [clinicaltrials.gov](https://www.clinicaltrials.gov) (identification number NCT04410250).

The mothers were instructed on the objectives of the study and signed a free and informed consent form agreeing with the participation of their children and themselves in the research.

-Sample and study design

This study is a blinded randomized controlled trial. The sample consisted of healthy infants and their mothers. These infants were selected in their first two days of life at the Maternity Hospital Nossa Senhora da Saúde, located in the city of Diamantina, Minas Gerais, Brazil, between November 2019 and January 2020.

-Sample calculation

OpenEpi (Open Source Epidemiologic Statistics for Public Health) was used to calculate the sample. The formula for randomized clinical trials was adopted. The criteria adopted: a percentage of 3.84% of non-exposed (absence of oral hygiene before tooth eruption) positive (with colonization by *Candida*) and 38.45% of exposed (presence of oral hygiene before tooth eruption) positive (13), a confidence interval of 95% and standard error of 5%. Thus, the minimum sample achieved determined a minimum sample size of 27 infants per group. To compensate for possible losses, three infants (10% of the sample) would be added to each group. Consequently, 30 infants would be selected to participate in each group study.

-Eligibility criteria

Infants who did not present any erupted teeth during selection and whose mothers agreed with their participation and the participation of their children in the study were included in the study.

Infants who presented systemic impairment in the first evaluation, such as neurological diseases, respiratory diseases, and gastroesophageal reflux disease, among others that could change the oral microbiota, were excluded from the study.

-Sample Allocation

The random allocation of the participants was performed at the maternity. All infants born in the Maternity Nossa Senhora da Saúde and their mothers, who met the eligibility criteria, were invited to participate in the study, (Fig. 1).

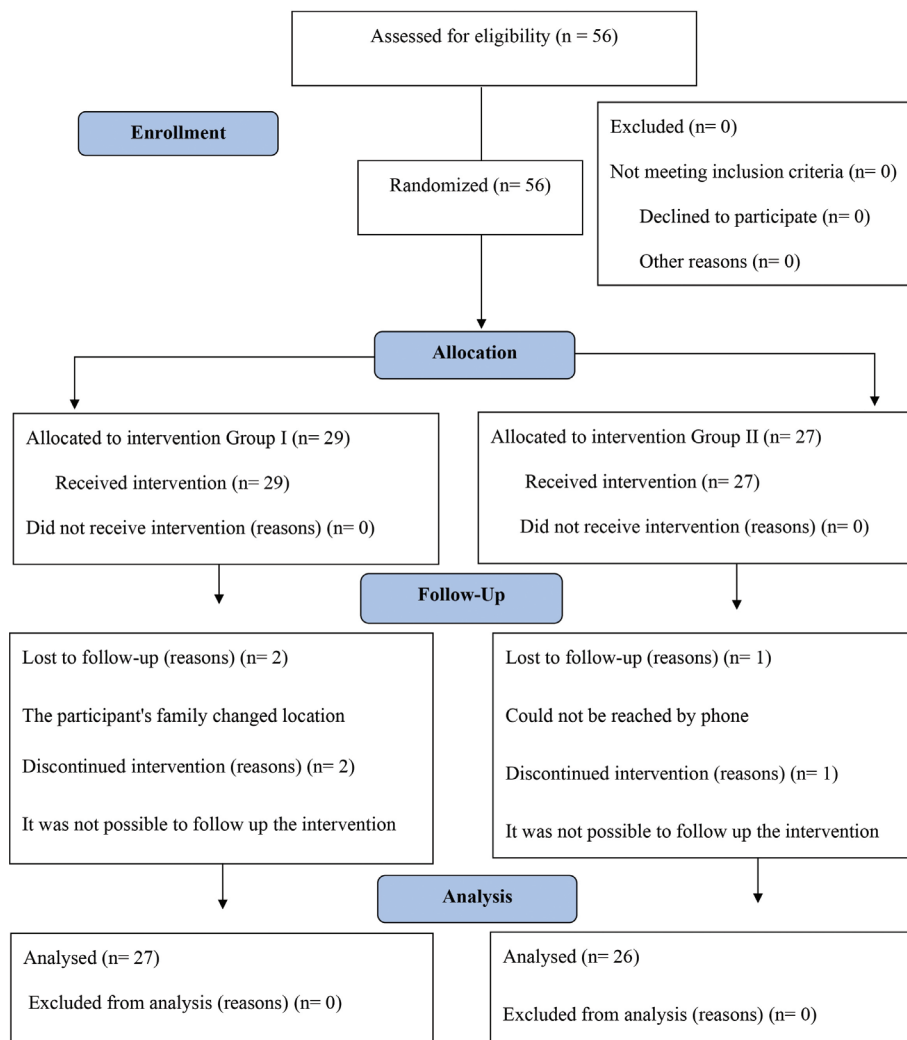


Fig. 1: Flow diagram of the inclusion, allocation, and participants analysis.

53 infants and their mothers, who agreed to participate in the study, were divided into 2 groups (Group I or Group II):

I. Group whose mothers received the guidance to perform the oral hygiene of the infants with gauze and filtered water, once a day. This hygiene should preferably be performed during the bath of the infant and consists of cleaning the palate area, gingival rodetes, oral mucosa, and tongue.

II. Group whose mothers received the guidance not to perform oral hygiene of the infant.

The mothers received a leaflet with the type of intervention according to the allocation group. In addition, oral cleaning was demonstrated, and gauze was given to the infants selected in the intervention group (I).

The random allocation of the participants was performed at the maternity through simple randomization. When the mother agreed to participate in the study, lots had to be drawn by the researcher to select the intervention

group (I or II) utilizing opaque envelopes and informed the mother about the allocation group. The orientations were conducted by the same researcher, the one who did not participate in the subsequent evaluations.

During the selection of the study participants at the maternity, the mothers were asked for their names, addresses, and telephone for subsequent data collection, which was performed in their own homes. The examiner responsible for data collection in the residence of the participants was blind to the group in which the infants were allocated.

-Training and calibration

Before data collection, the researchers underwent a training and calibration process. The researcher responsible for the guidelines on oral hygiene, which were transmitted to the mothers of the infants, received theoretical training.

The examiner received training for the diagnosis of oral candidiasis through theoretical explanation and the projection of images of clinical aspects related to the patho-

logy. This examiner also underwent a calibration process in two moments, through the evaluation of images of different clinical situations for oral candidiasis, with a one-week interval between evaluations. This evaluation was also performed by an experienced researcher for comparison ($Kappa \geq 0.93$).

Laboratory training was performed to verify and quantify the growth of *Candida spp.* colonies. A third researcher was trained by an experienced researcher in the field of microbiology. Saliva collections were performed from infants who were not included in the sample to perform laboratory analysis and verify *Candida spp.* growth.

-Data collection

30 days after the intervention, the participants included in the study were visited in their homes for data collection. The mothers were asked to answer a questionnaire regarding the socio-environmental, behavioral, and general health data of the participants. The information collected included the type of delivery (cesarean or normal), gestational age (in weeks), gender of the infant, weight (in grams) and length (in centimeters) of the infant at birth, use of pacifier, thumb-sucking, bottle use, breastfeeding, and health status of the infant during the follow-up period. During the home visit, an oral clinical examination of the infant was also performed along with saliva sample collection to evaluate colonization by *Candida spp.*

Assessment of colonization by *Candida spp.* species.

The sample was collected using a sterile swab and was transported in Stuart medium (Olen, Jiangsu Province, China). A sterile swab was rubbed into the mucosa in the oral cavity of the infant, in the palate, tongue, oral mucosa, and upper and lower gums. After collection, the material was stored in the sterile tubes in Stuart medium, identified according to the name of the participant, and transported in a refrigerated thermal box between 275.15 and 276.15 Kelvin, to the Bioprocess Laboratory of the Federal University of Vale do Jequitinhonha e Mucuri (UFVJM).

The saliva sample collected was plated on CHROMagar Candida culture medium (Difco CHROMagar Candida, Paris, France) and incubated in an oven at 310.15 K. The plates were analyzed after 48 hours to verify the growth of the colonies, and when they occurred, the colony-forming units (CFU) were quantified. The culture plates that were negative for the growth of *Candida spp.* species were left in the greenhouse for another 24 hours and then examined within 72 hours before being discarded as negative.

CHROMagar Candida is a selective medium used for the growth and differentiation of *Candida spp.* species. With the inclusion of chromogenic substrates, it is possible to differentiate the colonies of *Candida spp.* according to their colors, thus allowing the direct detection of yeast species on the isolation plate. The colonies of *C. albicans* appear with a light green to medium green

color, those of *C. tropicalis* appear blue greenish to metallic blue and those of *C. krusei* appear light pink with a whitish edge. Other yeast species could develop their natural color (cream) or appear pink or light to dark, such as *Candida glabrata* and other species (17).

-Evaluation of the presence of oral candidiasis

The mothers were instructed to contact the researcher if they observed any alteration in the oral mucosa of the infant in the period before or after the evaluation. In situations in which the mothers came into contact with the researcher reporting alterations in the oral cavity of their children, a home visit with an evaluation of the oral cavity was performed to observe alterations with suspected oral candidiasis infection, characterized by white, soft, and detachable plaques on an erythematous surface (18). In these cases, samples were also collected to identify and quantify *Candida spp.* species in order to confirm the presence of infection by these species, regardless of the age of the infant (less than or greater than 1 month of age) and the follow-up period (before or after the first evaluation).

Oral clinical examinations were performed under artificial lighting Forclaz - Onnight head lamp 50 - 30 lumens) and the infants were examined sitting on the lap of their mothers, in a knee-to-knee position (19).

During the clinical examination, wooden spatulas and sterile clinical mirrors were used (Golgran, São Caetano-do-Sul, Brazil). National biosafety standards and recommendations were followed for both infection control and waste disposal. The researchers performed oral examinations wearing white clothing, a cap, a mask, an apron, and disposable latex gloves.

In situations where oral candidiasis was confirmed in the infant, the mothers were instructed to use Nystatin (aqueous solution/ 100,000 IU/ml), topical use, four times a day for 7 days. The drug was administered with the aid of a cotton swab and spread throughout the oral cavity (oral mucosa, tongue, palate, and gingival).

In the oral clinical examination form of the infant, the presence or absence of oral candidiasis was recorded, and its location (palate, tongue, gingival rodete, or oral mucosa).

-Statistical analysis

The data were entered and organized in the Statistical Package for the Social Sciences (version 22.0; SPSS Inc. Chicago, IL, USA). Descriptive data analysis and Kolmogorov-Smirnov test were performed for quantitative variables. The Mann-Whitney test, independent sample T-test and Spearman correlation test were used to verify the association between the independent variables (type of delivery, gestational age, infant gender, weight and length of the infant at birth, pacifier use, thumb sucking, breastfeeding, bottle use and occurrence of some diseases in the first month of life) and dependent variables (colonization by *Candida spp.*, and Presence of oral candidiasis).

Results

In total, fifty-six infants were initially included in the study. Of these, 53 were followed up until the end of the study, 27 infants in Group I and 26 infants in Group II (Response rate = 94.6 %). Three infants could not be reached for the collection of saliva samples in the first month of life, since two participants had moved to another city. Most participants (56.6%) were male. The frequency distribution of social, behavioral, and general health factors of infants is presented in Table 1. The UFC median was 71.85 and 13.2% of the participants presented oral candidiasis. Among the infants evaluated, 17% were colonized by *Candida albicans* species, followed by *Candida krusei* (15.1%), *Candida glabrata* (7.5%) and *Candida tropicalis* (1.9%).

The Mann-Whitney test (Table 2) showed no significant difference between colonization by *Candida spp* species. and the intervention groups I and II ($p=0.947$). The test revealed that the colony count of *Candida spp* in the entire sample was associated with pacifier use ($p=0.036$) and the occurrence of some diseases in the first month of life of the infant ($p=0.003$).

The Spearman correlation analysis (Table 3) revealed that there was no association between colonization by *Candida spp.* species and gestational age ($p=0.320$), birth weight ($p=0.252$) and infant length ($p=0.996$).

Table 4 shows that there was no statistically significant difference between the presence of oral candidiasis and the intervention groups I and II ($p=0.704$) and neither with the other independent variables evaluated ($p>0.05$).

Discussion

The present study investigated the influence of infants oral hygiene before dental eruption on colonization by the *Candida spp.* species in the first month of life.

Our main results showed that oral hygiene before dental eruption in the first month of life of infants did not influence the outcomes evaluated. However, the use of pacifiers and the presence of some diseases in the first month of life of the infant were associated with higher colonization by the *Candida spp.* species.

The colonization by the *Candida spp.* species was verified in 49.1% of infants in their first month of life. The prevalence of *Candida spp.* colonization in our study was high compared to most studies conducted in healthy infants during the first year of infant life, where the prevalence ranged from 4% to 20% (20-23). The *Candida albicans* was the species of *Candida spp.* most commonly found in the oral cavity. This finding is in agreement with other studies that have also found *Candida albicans* as the most common and prevalent species on oral colonization in infants (20-22). The prevalence of

Table 1: Frequency distribution of social and behavioral factors and infant’s general health.

	Group 1	Group 2	Total
Gestational age (weeks) (Median/DP)	39,04 (1,126)	38,92 (0,98)	38,9 (1,0)
Birth weight (grams) (Median/DP)	3166,93 (505,32)	2938,73 (419,78)	3054,9 (475,0)
Birth length (centimeters) (Median/DP)	48,46 (2,02)	47,48 (1,94)	47,9 (2,02)
Gender			
Male	17 (56,7%)	13 (43,3%)	30 (56,6%)
Female	10 (43,5%)	13 (56,5%)	23 (43,4%)
Types of birth			
Normal birth	18 (52,9%)	16 (47,1%)	34 (64,2%)
Cesarean birth	9 (47,4%)	10 (52,6%)	19 (35,8%)
Pacifier			
No	17 (51,5%)	16 (48,5%)	33 (62,3%)
Yes	10 (50%)	10 (50%)	20 (37,7%)
Thumb sucking			
No	16 (48,5%)	17 (51,5%)	33 (62,3%)
Yes	10 (52,6%)	9 (47,4%)	20 (37,7%)
Breastfeeding			
Yes	25 (49%)	26 (51%)	51(96,2%)
No	2 (3,7%)	0 (0%)	2 (3,7%)
Baby bottle			
No	20 (53,3%)	16 (46,7%)	36 (67,9%)
Yes	7 (37,5%)	10 (62,5%)	17 (32,1%)
Diseases in infants during the first month of life			
No	19 (43,2%)	25 (56,8%)	44 (83%)
Yes	8 (88,9%)	1 (11,1%)	9 (17%)

Table 2: Association between the social, behavioral factors and general health of infants and colonization with *Candida spp.*

	Colonization with <i>Candida spp.</i> (UFC) Median (25p-75p)	<i>p</i> *
Intervention group Control Group (II) Group Test (I)	0,50 (0,0-32,0) 0,50 (0,0-144,5)	0,947
Gender Male Female	0,0 (0,0-132,0) 1,0 (0,0-26,0)	0,765
Types of birth Normal birth Cesarean birth	0,0 (0,0-16,0) 1,0 (0,0-168,0)	0,596
Pacifier No Yes	0,0 (0,0-6,0) 8,0 (0,0-300,0)	0,036
Thumb sucking No Yes	0,00 (0,0-32,0) 2,00 (0,0-193,5)	0,338
Breastfeeding Yes No	0,00 (0,00-24,0) 265,00 (230,0-300,0)	0,063
Baby bottle No Yes	0,0 (0,0-11,0) 9,0 (0,0-230,0)	0,085
Diseases in infants during the first month of life No Yes	0,0 (0,0-36,0) 3,5 (0,0-70,5)	0,003

* Mann-Whitney test $p < 0,05$

Table 3: Association between factors related to the birth of infants and colonization with *Candida spp.*

	<i>r</i>	<i>p</i> **
Gestational age	0,141	0,320
Birth weight	0,162	0,252
Birth length	-0,001	0,996

**Spearman correlation

oral candidiasis occurred in 13.2% of the participants during the first month of life. Other studies conducted in healthy children up to one year of age found a prevalence between 1% and 12.5% (18, 20). Differences in the prevalence of colonization by *Candida spp.* and oral candidiasis among the studies could be explained by the different methodologies and age groups of the children evaluated.

Oral hygiene before dental eruption with gauze and filtered water, once a day, did not influence colonization by *Candida spp.* and candidiasis in the oral cavity in the first month of life of healthy infants. Corroborating our findings, other studies have also shown that infant oral

hygiene before dental eruption does not influence the development of oral candidiasis (11,12). Nevertheless, these studies did not evaluate colonization by *Candida spp.* and were performed through observational designs (11,12).

On the other hand, recent research has demonstrated that infant oral hygiene before dental eruption represented 3.68 times more chance of colonization by *Candida spp.* in the first four months of infant life (13). It is believed that this difference is related to the frequency of oral hygiene performed by the intervention group. In our study, parents were instructed to sanitize the oral cavity of infants only once a day, while in the study by Vicente *et al.* they instructed guardians to perform the oral hygiene of infants three times a day (13). There is a replacement of important protective agents present in saliva and breast milk during successive feedings. The protein agents responsible for the antifungal factor of breast milk and saliva are mainly lactoferrin and IgA (15). Studies demonstrate a protective effect of lactoferrin on colonization by *Candida spp.* species and development of candidiasis in the oral cavity of infants (15,21). It is possible that oral hygiene before dental eruption, when performed regu-

Table 4: Association among social and behavioral factors, children general health and presence of oral candidiasis.

	Oral Candidiasis		p*
	No n (%)	Yes n (%)	
Intervention group			
Control group (II)	22 (84,6)	4 (15,4)	0,704*
Group Test (I)	24 (88,9)	3 (11,1)	
Gender			
Male	26 (86,7)	4 (13,3)	0,651*
Female	20 (87,0)	3 (13,0)	
Types of birth			
Normal birth	30 (90,9)	3 (9,1)	0,400*
Cesarean birth	15 (78,9)	4 (21,1)	
Pacifier			
No	30 (90,9)	3 (9,1)	0,405*
Yes	16 (80,0)	4 (20,0)	
Thumb sucking			
No	27 (82,4)	6 (17,6)	0,516*
Yes	16 (78,9)	4 (21,1)	
Breastfeeding			
Yes	42 (82,4)	9 (17,6)	0,345*
No	1 (50,0)	1 (50,0)	
Baby bottle			
No	32 (88,9)	4 (11,1)	0,667*
Yes	14 (82,4)	3 (17,6)	
Diseases in infants during the first month of life			
No	39 (88,6)	5 (11,4)	0,588*
Yes	7 (77,8)	2 (22,2)	
Gestational age (Mean-25p-75p)	39,0 (38,0-40,0)	39,0 (38,5-39,0)	0,779**
Birth weight grams (Mean-DP)	3028,53 (69,71)	3240,00 (208,70)	0,282***
Birth length (Mean-DP)	47,93 (0,29)	48,43 (0,98)	0,553***

*Chi-Square test $p < 0,05$ **Mann-Whitney test $p < 0,05$ ***Independent Samples T-test $p < 0,005$

larly, may contribute to the removal of these agents, reducing the protection offered in the oral mucosa of the infant. However, future experimental studies are needed to investigate the relationship between the frequency of infants oral hygiene before dental eruption and the protective effect of salivary components and breast milk on colonization by *Candida spp* species and development of candidiasis in the oral cavity of the infant.

In our study, colonization by *Candida spp.* was associated with the pacifier sucking habit in the first month of life of the infant. Other studies that investigated the relationship between colonization by *Candida spp.* and pacifier use have shown that this habit contributes to the colonization and proliferation of these and other microorganisms (24,25), being considered a risk factor for the development of diseases such as oral candidiasis (24) and intestinal infections (25). Laboratory studies have shown that *Candida albicans* is the predominant species in pacifier colonization, being able

to grow and adhere to the silicone surface even in the absence of nutrients (26). Another reason for the increased frequency of *Candida spp.* in children who use pacifiers can also be explained due to the change in environmental conditions in the oral cavity, which occurs through the continuous maceration process of the oral mucosa before the suction of the pacifier, resulting in small ruptures in the oral epithelium that predispose to colonization by *Candida spp.* (27). Based on these findings, parents and caregivers should be warned emphatically of the need for good sterilization and cleaning to prevent the pacifier from becoming a vehicle for transmission and proliferation of *Candida spp.* species and other microorganisms.

The occurrence of diseases in the first month of life of the infant was associated with higher rate of colonization by *Candida spp.* The disease most reported by parents/caregivers was acute diarrhea. Among the factors implicated in the detection of acute diarrhea in the child is the un-

favorable immunological condition. Disturbances in the immune status of the host favor colonization by *Candida spp.* Although the treatment of this disease involves the use of antibiotics in those more severe situations, and the use of antibiotics is associated with higher colonization by *Candida* and occurrence of oral candidiasis (27), we believe that this could not be a justification for this association, since the use of antibiotics was not associated with the outcomes evaluated, probably due to the small number of infants who used antibiotics.

Some limitations of the study deserve to be discussed. Data collection was interrupted due to the establishment of the COVID-19 Pandemic and performed only in the first month of life of infants, which made it impossible to monitor the sample for a longer period, in which it would be possible to perform new microbiological collections. In addition, blinding was only possible in relation to the examiner, considering the clear differences in the intervention protocols.

Investigating the advantages and negative consequences of oral hygiene before dental eruption is extremely important, since it is through these results that it is possible to conduct the guidelines of health professionals. The outcomes evaluated in the present study have a great clinical relevance, since evidence demonstrates the role of *Candida spp.* species in the formation of cariogenic biofilms (28). In addition, the occurrence of oral candidiasis has impacts on the quality of life of the infant and their mother, which may result in pain and difficulties in breastfeeding, both for the mother and the infant (20). Future investigations should be conducted with a longer follow-up period, in addition to investigating other clinically important outcomes, such as infant behavior, the incidence of caries during early childhood, among others.

Conclusions

Oral hygiene did not influence colonization by *Candida spp.* and the occurrence of oral candidiasis during the first month of life of the infant. Scientific investigations with long-term follow-up time through randomized clinical trials are necessary to verify the influence of oral hygiene before primary tooth eruption and the identification and quantification of *Candida spp.* species in the oral cavity of infants.

References

- Brecher EA, Lewis CW. Infant oral health. *Pediatric Clinics*. 2018;65:909-921.
- Gomes Silva Cerqueira A, Barauna Magno M, Barja-Fidalgo F, Vicente-Gomila, J, Cople Maia L, Fonseca-Gonçalves, A. Recommendations from paediatric dentistry associations of the Americas on breastfeeding and sugar consumption and oral hygiene in infants for the prevention of dental caries: A bibliometric review. *International Journal of Paediatric Dentistry*. 2021;31:664-675.
- dos Santos APP, Nadanovsky P, & de Oliveira BH. Inconsistencies in recommendations on oral hygiene practices for children by profes-

- sional dental and paediatric organisations in ten countries. *International journal of paediatric dentistry*. 2011;21:223-231.
4. abodontopediatria.org.br [Internet]. Brazil: Brazilian Association of Pediatric Dentistry. c2021-2023 [cited 2023 Jun 1]. Guidelines for parents on oral health care for babies and children [about 2 screens]. Available from: <https://abodontopediatria.org.br/orientacoes-ao-pais-sobre-cuidados-com-a-saude-bucal-do-bebe-e-das-criancas/>
5. gcdfund.org [Internet]. London: Global Child Dental Fund. Oral Health and Your Baby. [cited 2023 Jul 3]; [about 12 p.]. Available from: <https://www.gcdfund.org/publications>
6. American Academy of Pediatric Dentistry. Periodicity of examination, preventive dental services, anticipatory guidance/ counseling, and oral treatment for infants, children, and adolescents. The Reference Manual of Pediatric Dentistry. Chicago, Ill.: American Academy of Pediatric Dentistry; 2022:253-65.
7. American Dental Association [Internet]. Mouthhealthy ADA; c.2014-2023 [cited 2023 18 Jul]. 6 Things Nursing Moms Should Know About Dental Health; [about 3 screens]. Available from: Breastfeeding | MouthHealthy - Oral Health Information from the ADA.
8. American Academy of Pediatrics [Internet]. [healthychildren.org](https://www.healthychildren.org); c.2015-2023. [Updated 2016 Jun 6] [cited 2023 Jul 3]. Teething: 4 to 7 months; [about 3 screens]. Available from: <https://healthychildren.org/English/ages-stages/baby/teething-tooth-care/Pages/Teething-4-to-7-Months.aspx>
9. Asociación Latinoamericana de Odontopediatria [Internet]. Guía de salud bucal infantil para pediatras; c.2017-2023. [cited 2023 Jun 4] [about 13 screens]. Available from: <https://www.revistaodontopediatria.org/index.php/alop/guias>
10. Fernandez Rodriguez B, Pena Gonzalez L, Calvo MC, Chaves Sanchez F, Pallas Alonso CR, de Alba Romero C. Oral care in a neonatal intensive care unit. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2017;30:953-957.
11. Yared FN, Araújo ED, Barroso JA, Cunha RC. Oral candidosis: Influence of oral hygiene in infants before tooth eruption [Internet] 2001 Mar [cited 2023 Jun 6] 4(18): [about 7 p.] *Jornal Brasileiro de Odontopediatria e Odontologia do Bebe*. Available from: <https://pesquisa.bvsalud.org/portal/resource/pt/biblio-852034>
12. Scherma AP, de Oliveira Santos DV, Jorge AOC, da Rocha RF. Avaliação de fatores predisponentes à candidose bucal em recém-nascidos. *Brazilian Dental Science*. 2010;7.
13. Vicente RMB, Echeverria S. Avaliação da Relação entre Higienização Bucal de Neonatos e *Candida spp.*: Estudo Piloto de um Ensaio Clínico Randomizado. passgroup.com.br [Preprint]. 2017 [Cited 2023 Feb 10]. Available from: <https://www.passgroup.com.br/hotsite2/site/default.asp?TroncoID=518080&SecaoID=905380&SubSecaoID=&Template=../asp/hotsite2/AnaisTrabalhoArquivo.asp&iid=150/1159-0&Formato=Resumo>
14. Samaranyake L, Matsubara VH. Normal oral flora and the oral ecosystem. *Dental Clinics*. 2017;61:199-215.
15. Chheda S, Keeney SE, Goldman AS. Immunology of human milk and host immunity. *Fetal and neonatal physiology*. 2004;1610.
16. Moher D, Hopewell S, Schulz KF, Montori V, Gotzsche PC, Devereaux PJ, Altman DG. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *BMJ*. 2010;340:c869.
17. Odds FC, Bornaerts RIA. CHROMagar *Candida*, a new differential isolation medium for presumptive identification of clinically important *Candida* species. *Journal of clinical microbiology*. 1994;32:1923-1929.
18. Vainionpää A, Tuomi J, Kantola S, Anttonen V. Neonatal thrush of newborns: Oral candidiasis? *Clin Exp Dent Res*. 2019;5:580-582.
19. Fux N, Shmueli A, Halperson E, Ram D, Moskovitz M. "Knee-To-Knee" Position for Minor Procedures in Infants and Toddlers- Dentists Attitudes. *J Clin Pediatr Dent*. 2019;43:86-90.
20. Morrill JF, Heinig MJ, Pappagianis D, Dewey KG. Risk factors for mammary candidosis among lactating women. *Journal of Obstetric, Gynecologic, & Neonatal Nursing*. 2005;34:37-45.
21. Stecksén-Blicks C, Granström E, Silfverdal SA, West CE. Prevalence of oral *Candida* in the first year of life. *Mycoses*. 2015;58:550-556.

22. Zisova LG, Chokoeva AA, Amaliev GI, Petleshkova PV, Miteva-Katrandzhieva T, Krasteva MB, Ivanova ZV. Vulvovaginal Candidiasis in pregnant women and its importance for Candida colonization of newborns. *Folia medica*. 2016;58:108.
23. Al-Rusan RM, Darwazeh AMG, Lataifeh IM. The relationship of Candida colonization of the oral and vaginal mucosae of mothers and oral mucosae of their newborns at birth. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2017;123:459-463.
24. Mattos-Graner RO, de Moraes AB, Rontani RM, Birman EG. Relation of oral yeast infection in Brazilian infants and use of a pacifier. *ASDC J Dent Child* 2001;68:33-10.
25. Comina E, Marion K, Renaud FN, Dore J, Bergeron E, Freney J. Pacifiers: a microbial reservoir. *Nursing & health sciences* 2006;8:216-223.
26. Lopes DF, Fernandes RT, Medeiros YDL, Apolonio ACM. Disinfection of pacifier focusing on *Candida albicans*. *Clinical Pediatrics* 2019;58:1540-1543.
27. Majorana A, Bardellini E, Flocchini P, Amadori F, Conti G, Campus G. Oral mucosal lesions in children from 0 to 12 years old: ten years' experience. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* 2010;110:e13-e18.
28. Xiao J, Huang X, Alkhers N, Alzamil H, Alzoubi S, Wu TT, Koo H. *Candida albicans* and early childhood caries: a systematic review and meta-analysis. *Caries research* 2018;52:102-112.

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Ethics

This research received approval by the Research Ethics Committee in research involving human beings of the Federal University of Minas Gerais under protocol number 3,568,480.

We would like to highlight that this study was conducted in accordance with the ethical principles outlined in the Helsinki Declaration of 1964. We obtained written informed consent from all participants. Measures were taken to protect the privacy and confidentiality of study participants. We believe that adherence to the Helsinki Declaration underscores our commitment to ethical and responsible research practices and ensures that the rights and well-being of our patients were safeguarded.

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Authors' contributions

Ana Beatriz Silva Lopes and Luana Viviam Moreira: acquisition of the data, analysis, and interpretation of data, have been involved in drafting the manuscript and performed a critical review of the manuscript. Izabella Barbosa Fernandes and Valeria Macedo Cardoso: study design, statistical analysis, and interpretation of the data, have been involved in drafting the manuscript and performed a critical review of the manuscript. Maria Leticia Ramos Jorge and Joana Ramos Jorge: interpretation of data, have been involved in drafting the manuscript and performed a critical review of the manuscript. All authors contributed to the revision of the manuscript and approved the final version for submission.

Conflict of interest

All authors disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) our work.