Original Research
Microbiology

Initial oral microbiota and the impact of delivery mode and feeding practices in 0 to 2 month-old infants

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Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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https://doi.org/10.1590/1807-3107bor-2023.vol37.0078

Submitted: August 8, 2022
Accepted for publication: April 12, 2023
Last revision: May 5, 2023

Abstract: The aim of this study was to describe the initial oral microbiota and how delivery mode and feeding practices impact its diversity in 0-2-month-old infants. This was a cross-sectional study that consisted of one collection of saliva samples from 0-2-month infants at baseline. Ten pairs of mothers and infants were selected. Medical health history, pregnancy, birth, feeding practices (breastfeeding or milk formula), and infant health status was obtained. Pooled microbial samples were obtained from the oral surfaces using a sterile cotton swab. Infants did not receive any breast milk before sampling. After collection, each swab was analyzed through microbiological culture-based procedures, using selective mediums. Cultures were analyzed for the presence of *Streptococci*, *Lactobacillus*, *Staphylococcus*, *Enterobacterium*, and *Candida albicans*. Twenty percent of the samples were serially diluted (10-2) to assess the number of bacteria expressed as CFU. Bacillota was the leading phylogenetic group in the infant's pooled microbial sample. The most prevalent genera were *Streptococcus*, *Lactobacillus*, and *Staphylococcus*. Two participants had a positive growth of *Candida albicans*. The association between genus group, type of delivery, and feeding practices was not statistically significant ($p > 0.05$). *Lactobacillus* genus was frequently present in the cesarean delivery group but with slightly higher counts in a vaginal delivery study subject. Exclusively breastfed infants showed presence of *Streptococcus*, *Lactobacillus*, *Staphylococcus*. The oral microbiome in infants (0-2 month-old) is highly heterogeneous and dynamic. Microbiota composition seems to be impacted by mode of delivery, with slight differences among groups. Breastmilk appears as an essential factor in maintaining the oral microbiome's stability and diversity.

Keywords: Dental Caries; Microbiota; Breast Feeding.

Introduction

The first years of life are a period during which the microbiome is being established and coincide with the critical window of human development (physical and psychosocial).[1-4] The establishment of the infant microbiome during pregnancy and postpartum has been a novel subject of study, especially as its early setup lays the foundation for long-term oral health.[3] Advances in microbiological assessment

from culture-dependent and culture-independent molecular methodologies have provided details of the human-microbe relationship, allowing for increasing identification of the microbiota and its functional microbiome.[1] The majority of the scientific evidence focuses on the microbiome establishment and development at the lower intestinal tract, while information about the initial oral microbiota following delivery is still limited and poorly understood.[5,6]

Current knowledge has shown that the oral cavity has a highly complex and diverse microbiome with more than seven hundred species.[7-9] Maternal transmission is one of the primary explanation for the acquisition of oral bacteria in early childhood.[10-12] However, colonization routes are complex and not yet understood. Therefore, there are new inquiries about how coordinated interactions between external and internal factors play a role in the development of the microbiome.[13] Some external factors under study are the environment during birth, the mother's microbiota (*e.g.*, vaginal, oral, and gut microbiomes), and the infant feeding method.[14-22] Internal factors include the influences of epigenetics during infant development, which recent evidence shows that some of the epigenetic changes resulting from early nutrition and the microbiome can be inherited transgenerationally, thus having a significant impact on evolution.[3,23]

Emerging evidence has pointed to a connection between the placental environment and the oral microbiome, revealing the presence of several prominent oral commensals such as *Streptococcus*, *Fusobacterium*, *Neisseria*, *Prevotella*, and *Porphyromonas* in the human placenta.[5,6,24,25] This suggests that the early oral microbiota may have a prenatal origin given that the neonatal oral microbiota has clear associations with microbes in the placenta.[6] Other associations suggest the perinatal origin of the human microbiome, particularly the relationship between mode of delivery and specific oral microbiome patterns in three to six-month-old infants.[13] While vaginally delivered infants are exposed during passage through the birth canal, infants born by Caesarian section are exposed to the skin of parents, medical personnel, and medical equipment.[13]

Breast milk has been considered a source of bacteria (10^6 bacterial cells/mL) that serves as inoculum for the newborn. The genus *Streptococcus* is one of the dominant bacterial groups found in human milk.[26-29] Studies performed in the last two decades with large populations of neonates aged ≥4 weeks using both culturing and molecular methods demonstrated that *Bifidobacteria* were the most represented species in both breast- and formula-fed infants.[30] Other studies suggested that *Proteobacteria* and *Actinobacteria phyla* were more abundant on the cheek of breastfed neonates. At the same time, formula-fed infants had a higher predominance of *Bacteriodetes*.[31] In contrast, *Lactobacillus* species were found in saliva culture of breastfed three-month-old infants but not of formula-fed ones.[32] A long-term effect, assessed four and twelve months after birth, reinforced the differences in oral microbiota composition between breast and formula-fed infants.[33]

As previously mentioned, the oral microbiome is considered to have exceptional functions for human health and homeostasis. Major consequences of imbalances in the oral microbiome development are dental caries and periodontal disease, both of which are mediated by the oral microbiome and host interactions (e.g., diet in dental caries and immune system in periodontal disease).[34-39] Changes in microbial community structure (*e.g.*, taxonomic composition and relative abundance) are factors to consider in the transition from health to caries disease and to periodontal disease.[39,40] Therefore, explaining the origin of the microbial attainment process can eventually enable the creation of a novel approach to induce healthy development of the oral microbiota and prevent oral microbiota-related diseases in early life. As yet, there are no published longitudinal studies that examined the initial development of the oral microbiota in early childhood with culture-dependent methods in a population of Latin American dyad (mother-infant). Therefore, this study aimed to describe the initial oral microbiota and to determine how delivery

mode and feeding practices impact its diversity in 0 to 2-month-old healthy infants.

Methods

Study design and participants

This was a cross-sectional study that analyzed data of a 2-year longitudinal research that aimed to evaluate the impact of an Educational Program on dental caries disease and its risk factors. This study was carried out at the Central University of Venezuela (UCV), Faculty of Dentistry, Institute of Dental Research, Caracas, Venezuela (2019-2022).[41] The study was approved by the Bioethical Committee of the Central University of Venezuela, Faculty of Dentistry (CB-106-2019).

The initial study had a calculated sample size of 97 participants (95% confidence, 10% margin of error, and 50% population proportion). However, the COVID-19 pandemic affected recruitment and sample size, thus retaining only a convenient sample that could travel to the clinic and follow biosafety precautions. The study population comprised 10 pairs of mothers and 0-2 month-old infants. Sample recruitment was done by one of the investigators (SF). First, participants were referred from the Department of Pediatrics at the Complejo Social Don Bosco, Chacao Municipality, and evaluated at the Pediatric Dental Department of the same institution. Then, the investigator screened patients for inclusion and exclusion criteria. The recruitment clinic is a nonprofit charity hospital that serves low-to-middle income populations from the urban capital of Caracas. Participating mothers received an oral health kit as a benefit of the study.

The infants were eligible for the study only if they met the following inclusion criteria: a) gestational age > 35 weeks, b) birth body mass ≥ 2,500 g, c) 0–2 months old, and d) parents of middle-class socioeconomic status. Infants meeting any of the following conditions were excluded from the study: a) systemic disease or immune deficiency, b) the presence of teeth c), pathological conditions in the oral cavity, d) older than two months, and e) birth body mass < 2,500 g.

Evaluations (0–2 months) were carried out during late 2019 and early 2020. Microbiological samples were collected from the oral cavity of the infants. Information including gestational age, mode of delivery, general health status, feeding practices (breastfeeding or milk formula), medical history, and antibiotic/drug use were obtained through interviews with the parents and recorded in a clinical chart designed for the study. After data collection, the educational program was implemented. All mothers had healthy pregnancies and all babies were born at term without complications.

Sample collection

Assessments and data collection were done at the Department of Pediatrics at the Complejo Social Don Bosco, and the microbiological analysis was carried out in the Microbiological Laboratory at Faculty of Dentistry, UCV. Pooled microbial samples from the oral cavity, including unstimulated saliva, were obtained from the 10 participating infants. The infants did not receive any breast milk immediately before sample collection. The collection was done using a sterile cotton swab, and the sample was collected from the dorsum and ventral surfaces of the tongue and the mucosa of the alveolar ridges (pooled microbial sample). Each swab was placed into a sterile tube containing nutritive broth (Oxoid™ Nutrient Broth), kept refrigerated for transportation, and immediately sent to the laboratory.

Microbiological assay

Macroscopic assessment

a) Inoculation

The samples were inoculated on Petri dishes that contained the following selective culture mediums: a) Mitis Salivarius Agar (Oxoid™, UK), b) Rugosa Agar (Oxoid™, UK), c) Mannitol Salt Agar (Oxoid™, UK), d) MacConkey Agar (Oxoid, UK), e) Agar Brilliance Candida (Oxoid™, UK), and f) Columbia Base Blood Agar (Oxoid™, UK). The inoculation was done using the spread method. Ten microliters of the sample were taken with a micropipette and placed on the surface of the agar medium. The sample was evenly spread on the agar surface using a sterile glass spreader. Inoculations were done in quartets, resulting in 4 dishes for each medium. Petri dishes were labeled and dated (Figure).

b) Incubation

Two of the inoculated samples for each medium were immediately incubated in polycarbonate jars with GasPack™ system at 37°C under anaerobic conditions for 48 hours. The other two inoculated samples were placed for 48 hours in the incubator at 37°C at 1 atmosphere in aerobic conditions (Figure).

c) Identification technique

After incubation, bacteria were identified by the classical method by observation of the phenotypic characteristics of bacterial culture, including growth of *Streptococci, Lactobacillus*, *Staphylococcus*, *Enterobacterium*, *Candida albicans*, and morphologic characteristic of colonies (size, color, shape, growth pattern, and hemolytic activity) (Figure)

Counts of Colony Forming Units (CFU)

Before incubation, two samples were randomly selected and serially diluted (10^{-1} to 10^{-5}). After dilution, 10 µL of the sample culture were inoculated on Petri dishes that contained the following selective culture mediums: a) Mitis Salivarius Agar (Oxoid™, UK), b) Rugosa Agar (Oxoid™, UK), and c) Mannitol Salt Agar (Oxoid™, UK). The inoculations were done using the spread technique in duplicates. After inoculation, half of the plates were incubated in polycarbonate jars with GasPack™ system at 37C° under anaerobic conditions for 48 hours, and the other half were left for the same amount of time under aerobic conditions in the incubator at 37°C and 1 atmosphere of pressure. After incubation, the 10^{-2} dilution was selected to assess the number of detected bacteria expressed as colony forming units (CFU). Counting was done with a manual colony counting system (Boeckel Co, GmbH Co. KG) and the cultures were used for the counting of *Streptococcus*, *Lactobacillus*, and *Staphylococcus* expressed as CFU (Figure).

Statistical Analysis

The overall objective was to describe the main characteristic of the sample and the oral microbiota of infants. Descriptive statistics were performed

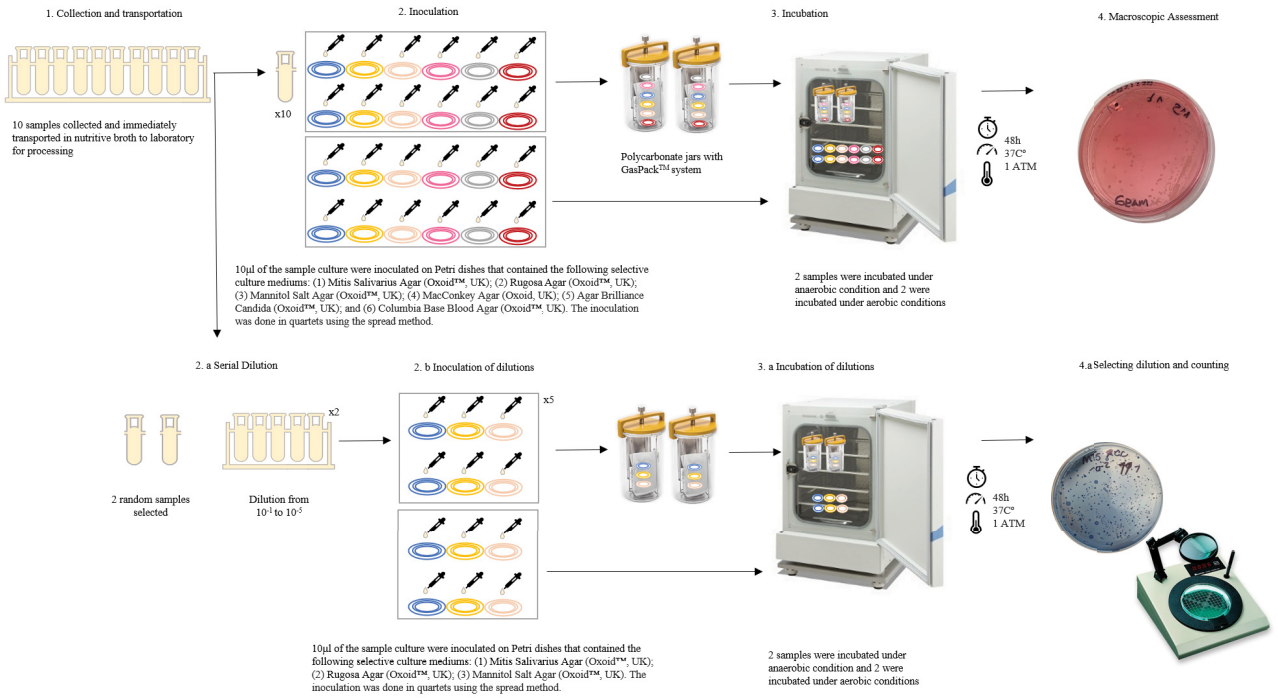

Figure. Schematic representation of the microbiological assay.

with measures of center (means, medians) and spread (percentiles and SD). The chi-Square test was used to asses associations between categorical variables, and analytic statistics were performed to assess independence between microbiota at genus level and the two external factors that potentially influence its development (mode of delivery and feeding practices). STATA software version 17.0 was used to run the statistical analysis, with a significance level of 0.05.

Results

Characteristics of the mother-infant pairs are depicted in Table 1. Mothers' mean age was 29.0 ± 7.36 years. The majority of the sample belonged to the middle-high socioeconomic group (40%), while the other categories were represented in equal proportions, except for middle-low category, in which no one was classified. Only two mothers (20%) reported having used antibiotics during pregnancy.

All of the infants in this study were born after 37 weeks of gestation with low variation (38.6 ± 0.97). The mean age of infants was 1.2 ± 0.79 months and the gender ratio was 1:1. Four infants were delivered vaginally and six by caesarean section. Six of the infants were exclusively fed with breastmilk directly from the mother's breast (EB), three were fed using a combination of breastmilk and formula using a bottle (C), and only one was exclusively fed with breastmilk, both by bottle and at the mother's breast (EBB). The infants' weight 50th percentile was 3,240 grams (range 3,000 to 4,200 grams) with an IQR=330g. From birth to the date of the study, half of the infants (50%) had not received any drug, two (20%) had received antibiotics, and 2 (20%) had received other medications.

Table 1. Characteristics of mother-infant pairs.

Maternal Characteristics	n = 10
Age ([years) mean (SD)	29.3 (7.36)
Socioeconomic Status[a] [n (%)]	
I (high)	2 (20)
II (middle-high)	4 (40)
III (middle)	2 (20)
IV (middle-low)	0 (0.0)
V (low)	2 (20)
Gestational age (weeks) mean (SD)	38.6 (0.97)
Antibiotic use during pregnancy [n (%)]	
Yes	2 (20)
No	8 (80)
Newborn characteristics	
Age (months) mean (SD)	1.2 (0.79)
Gender	
Male	5 (50)
Female	5 (50)
Birth weight* (g) mean (IQR)	3,240 (330)
Type of birth [n (%)]	
Cesarean (CS)	6 (60)
Vaginal delivery (VD)	4 (40)
Feeding Practices* [n (%)]	
EB	6 (60)
EBB	1 (10)
Combined	3 (30)
Medication [n (%)]	
Antihistamine	1 (10)
Antibiotic	2 (20)
Other	2 (20)
No medication	5 (50)

*g (grams); EB: exclusive breastmilk from mother's breast; EBB: exclusive breastmilk from mother's breast and bottle; C: breastmilk from breast mother and formula in bottle.
[a]Graffar-Mendez Castellano Scale.

Infant oral microbiota

The distribution of the sample regarding the presence of microorganisms by genus in relation to mode of delivery is shown in Table 2. All the infants had a relatively even distribution of different bacterial genera. Overall, *Streptococcus*, *Lactobacillus*, and *Staphylococcus* were the most common genera in both modes of delivery. Notably, *Streptococcus* was the only genera present in all infants of both delivery mode groups. In contrast, *Candida* was infrequently seen and *Enterobacter* did not occur in any infant. Association between genus

Table 2. Proportions (%) of two-month-old infants with a positive selective culture medium by genus level in relation to mode of delivery.

Phylogenetic group and genus	Vaginal delivery (n = 4)	Cesarean delivery (n = 6)	p-value
	n (%)	n (%)	
Ascomycota			
Candida[c]	1 (25)	1 (16.7)	0.747[a]
Firmicutes			
Streptococcus[b,c]	4 (100)	6 (100)	1.000[a]
Lactobacillus[b,c]	3 (75)	6 (100)	0.197[a]
Staphylococcus[c]	4 (100)	5 (83.3)	0.389[a]
Proteobacteria			
Enterobacter	0 (0%)	0 (0)	1.000[a]

[a]Chi-square statistical test ($p > 0.05$); [b]Positive Growth in anaerobic condition; [c]Positive Growth in aerobic condition.

Table 3. Proportions (%) of two-month-old infants with a positive selective culture medium by genus level in relation to feeding practice.

Phylogenetic group and genus	Feeding practice* (n = 10)			p-value
	EB	C	EBB	
	(n = 6)	(n = 3)	-1	
Ascomycota				
Candida[c]	2 (33.3%)	0 (0.0%)	0 (0.0%)	0.435[a]
Firmicutes				
Streptococc[usb,c]	6 (100%)	3 (100%)	1 (100%)	0.189[a]
Lactobacillu[sb,c]	6 (100%)	2 (66.6%)	1 (100%)	0.274[a]
Staphylococcus[c]	6 (100%)	2 (66.6%)	1 (100%)	0.274[a]
Proteobacteria				1.000[a]
Enterobacter	0 (0%)	0 (0.0%)	0 (0.0%)	

*EB: exclusive breastmilk from mother's breast; EBB: exclusive breastmilk from mother's breast and bottle; C: breastmilk from breast mother and formula in bottle. aChi-square statistical test ($p > 0.05$) verified with Mantel-Haenszel; bPositive growth in anaerobic condition; cPositive growth in aerobic condition.

group and mode of delivery was not statistically significant ($p > 0.05$).

The distribution of the sample regarding the presence of microorganisms, by genus, in relation to feeding practice is shown in Table 3. The EB group showed the highest frequencies (60%) of *Streptococcus*, *Lactobacillus*, and *Staphylococcus*. In contrast, the EBB group showed low frequencies of this genus. The association between genus group and feeding practices was not statistically significant ($p > 0.05$).

Counts of Colony Forming Units (CFU)

Two individual samples (ID1 and ID2) were selected to assess the CFU of oral microbiota and its association with mode of delivery and feeding practices (Table 4). The genera that dominated the infants' microbiota composition were *Streptococcus* and *Lactobacillus*. *Streptococcus* was the predominant genus in the microbiota of the CS infants, with 1.5 times more CFU compared with the VD infants. Similarly, infants from the CS group had 3.2 times

Table 4. Oral bacterial genera counts in two case studies of Venezuelan infants.

Phylogenetic group and genus	Bacterial counts CFU (%)	
	ID1 (m, VD, EBB)*	ID2 (m, CS, EB) *
Firmicutes		
Streptococcus	2,1 x10^5 CFU (41,98%)	3,2 x10^5 CFU (54,2%)
Lactobacillus	2,9 x10^5 CFU (57,8%)	2,7 x10^5 CFU (45,7%)
Staphylococcus	1,7 x10^2 CFU (0,03%)	5,4 x10^2 CFU (0,09%)

*M: male; VD: vaginal delivery; CS: cesarean section; EB: exclusive breastmilk from mother's breast; EBB: exclusive breastmilk from mother's breast and bottle.

more *Staphylococcus* CFU than the infants born by VD.

Discussion

The first 1,000 days of life are a critical period for human development, thus it is an important period in the early microbiome establishment. Exposures during this period could unleash metabolic programming that can modify the structure and function of organs and systems, impacting health status later in life (The Developmental Origins of Health and Disease theory).[2] Hence, to characterize a health status of an individual, and most specifically that of the oral cavity, the microbiome must follow a fundamental system of microbiological dynamics, biochemical and metabolomic processes, with early establishment considered to be helpful and necessary for preserving functional stability and homeostasis.[2]

The oral cavity is one of the first ports of entry for colonization of the oral and gut microbiota. Consequently, the oral microbiota influences infant health, with early life dysbiosis being related to the progression of both systemic (*e.g.*, obesity)[23] and oral conditions (e.g., dental caries, periodontitis, and oral mucosal diseases).[22,25-27]

Bacterial colonization of the gastrointestinal tract (including the oral cavity), is influenced by multiple factors. Upon birth and in the first few months of life, major factors influence the oral microbial succession, including the environment (*e.g.*, mode of delivery, person-to-person transmission, the composition of infant saliva, feeding practice, and microbial cross-talk)[28-30] and epigenetics, which are decisive in the balance between health and disease.[30] These influences could favor a shift in the commensal dental biofilm microbiota later in life, leading to the establishment of a modified bacterial ecosystem (dysbiosis) that is the driving force behind negative health outcomes, such as dental caries.[31] As such, it is important to characterize the oral temporal development, such as the relationship between oral microbiota, type of delivery, and feeding practices.

The purpose of this study, rather than to provide confirmatory results, was to offer an exploratory understanding of early microbiota composition, depict early patterns and characteristics of the infant oral microbiome at an early stage of life, and describe how delivery mode and feeding practices might impact the diversity of the oral microbiota in 0-to-2-month-old infants.

The results are presented under limitations such as small sample sizes, culture-dependent methods, and study methodology. The study was initially designed (2019) to include a larger sample; however, the unprecedented COVID-19 pandemic limited the study execution and only a small sample could be selected (n = 10). Nonetheless, we consider that the results from this sample, under the scope of a pilot study, could have provided relevant results for understanding the impact of mode of delivery and feeding practices on early establishment of the oral microbiome in populations with similar characteristics. Lack of resources restricted the application of updated technology for microbial assessment (molecular sequencing approaches), so culture-dependent methods were used to evaluate

the microorganisms at the phyla and genus level. Due to funding limitations, only two samples could be processed for counting, which impacted the scope of these results and were not considered in the discussion.

Due to its ease of collection, saliva is the most frequently collected sample in oral microbiome studies. However, because of the young age of the study participants, it was not possible to collect saliva samples using standard sampling protocols such as unstimulated saliva by drooling. Instead, a pooled sample was obtained from all available surfaces of the oral cavity.

Several studies have addressed the development of the oral microbiome during the first years of life.[22,32] To the authors' knowledge, this is the first study to assess and characterize the oral microbiota of 0-2-month-old infants.

The conditions of the studied microbiome consist of a microbial system in a highly variable maturation state. Some studies agree that several perinatal and postnatal factors influence the general patterns of diversity and stability in early microbial communities.[22,32,33] The time period of this study (0-2 months old) corresponds to changes in the environment (*e.g.*, feeding practice)[20] and a highly variable time window for human development.[2] Thus, understanding when and how the early microbiome stabilizes is crucial. As the infant grows, microbial communities continue to evolve and microbial diversity increases.[8] During the first months of life, the oral microbiota is characterized by high variability and, according to current knowledge, it reaches adult-like stability around two years of age.[3] Moreover, one study[22] also revealed that the oral microbiota is acquired in an orderly sequence. This pattern of increasing phylogenetic diversity has previously been observed in the human infant gut, where the microbiota arranges itself over time in an orderly succession that ultimately resembles the profile of the adult gastrointestinal tract.[34]

Other studies[22,35,36] showed that diversity significantly increased from 2–5 days to 1–4 months, highlighting the continuous development and maturation of the oral microbiota in infancy. Consistent with this, another research reported that both salivary and biofilm microbial composition underwent significant changes from 1 to 2.5 y of age, followed by less pronounced microbial shifts by 4 y of age.[4]

Hence, our results are limited to this period. However, the patterns of microbial composition and the influence of perinatal and postnatal factors on these factors could serve as indicators for dental caries disease onset later in life.

Bacillota was the leading phylogenetic group in the infant's pooled microbial sample. The most prevalent genera were *Streptococcus*, *Lactobacillus*, and *Staphylococcus*. These results agree with other studies[22,34] reporting that oral bacterial colonization in the neonate is dominated by bacterial groups ("early colonizers") that inhabit the oral cavity during the first 3–6 months and include *Streptococcus*, *Veillonella*, *and Lactobacillus spp.*

Microbial communities in this study were composed of bacterial groups that have been reported to be prevalent in healthy individuals during the same time frame.[4] The biodiversity in this initial stage has been hypothesized to be determined and modulated by the maternal microbiome (gut, vagina, and oral microbiota),[37-39] and that the extent to which these microbiomes are healthy depends on the mother's health status. As in this study, the mothers met the parameters of a healthy pregnancy; hence, we hypothesize that the microbial communities are in homeostatic balance at a dynamic phase of stability (Integrated Ecological Hypothesis of Dental Caries).[38] However, this statement could vary during microbiome maturation depending on external factors such as environmental and behavioral influences.[40]

Regarding yeast, two participants had a positive growth of *Candida albicans* (20%). Yeasts, such as *Candida albicans*, are frequently associated with early childhood caries;[11] however, their roles in health and shift to a disease stage remain unclear. Similarly, one study found fungal communities (*e.g.*, *Candida albicans*) at the earliest time point, yet in contrast they reported a high prevalence of fungal DNA in their sample.[11] Researchers have tried to explain the presence of these microbes, and

some reasons include low complexity of bacterial communities and a relatively immature immune system during the first year of life.[11] Nonetheless, given the controversy regarding the presence of yeasts in dysbiotic conditions, this result can provide an insight into the existence of dental caries disease at different stages of severity at the end of the longitudinal study.[26]

Concerning the factors that might impact the composition of early microbiota, this study considered type of delivery and feeding practices as factors that influence biodiversity composition at early life stages. In this study, differences in some genera were found between study groups; however, the data did not suggest a significant impact on the microbiota diversity of 0-2-month-old infants. Nonetheless, some patterns could be seen on a small scale and in individual comparison.

There were slight differences between infants born vaginally and by cesarean section in the presence of *Lactobacillus* and *Staphylococcus*, both in the frequency of infants with positive growth and in bacterial CFU in individual cases. It was observed that the genus *Lactobacillus* was frequently present in the cesarean delivery group but with slightly higher counts in the vaginal delivery subjects. In contrast, *Staphylococcus* was frequently present in both modes of delivery but in higher counts (3.2 times higher) in the cesarean delivery group. Differences in the microbial composition of oral and gut microbiota by delivery mode have been reported by other studies.[22,28]

On the other hand, exclusively breastfed infants (EB and EBB) showed the presence of *Streptococcus*, *Lactobacillus*, and *Staphylococcus*. This result seems to suggest that breastmilk, either directly from the mother's breast or by bottle, provides some stability to the microbial composition and biodiversity with dominant genera related to health. In contrast, the introduction of formula milk seems to slightly impact the microbiota diversity. Similarly, differences in the oral microbiota have been reported between breastfed and formula-fed infants,[3,20,29] with *Streptococcus* being one of the dominant bacterial groups in human breast milk.[39] Moreover, this pioneer bacterium is often found in the infant's oral cavity because of its ability to adhere to and colonize mucosal surfaces.[22] The products derived from the catabolism of dietary oligosaccharides (in human milk) might pave the way for favoring symbiotic conditions in the oral cavity.[29]

The proposed statements indicate possible trends in the characteristics, composition, and diversity of the early oral microbiota. These patterns are limited to the population under study and previously discussed biases. Thus, to reach definitive conclusions supporting these statements, it is necessary to conduct very well-designed clinical studies with a representative sample and multiple confounding variables. However, we believe that the information obtained in this pilot study could provide important insights for the development of such clinical studies.

Finally, these baseline results could become more relevant once the clinical data from the longitudinal pilot study are analyzed. Hence, changes during the first 1000 days of life and the impact of multiple factors during this period (*e.g.*, environment, behaviors, biochemical process) will help to understand the complex etiology of the dental caries disease and its clinical expression in severe stages.

Conclusion

To achieve a deep understanding of the factors that impact initial oral microbiota, researchers must consider the time frame in which the study is conducted. The oral microbiome of infants 0-2 months old is highly heterogeneous and dynamic. Microbiota composition seems to be impacted by mode of delivery, with slight differences among groups. Breastmilk appears to be an essential factor in the stability of the oral microbiome and determining its diversity.

Acknowledgements

The authors would like to thank the IADR Venezuelan Division for encouraging the research team. Carolina Guilarte, Chief of The Microbiological Laboratory at UCV, Faculty of Dentistry, Institute of Dental Research for enabling the execution of the experimental analysis. IADR David Scott Award Fellowship 2019.

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