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Investigation of the effect of breast milk, probiotic supplemented and plain milk formulas on some oral bacteria in infants: an observational and in-vitro study

Investigação do efeito do leite materno, fórmulas de leite puro e suplementado com probióticos sobre algumas bactérias orais em lactentes: um estudo observacional e in vitro

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ABSTRACT

Objective: inconclusive evidence exists regarding potential cariogenicity of milk formulas compared to breast milk. The study aimed to compare Salivary *Streptococcus mutans* (*S. mutans*) and *lactobacilli* detection and counts among breastfed (B), plain formula (France Lait 1) (FL) and probiotic supplemented formula (Nan 1 optipro) (N) infants and to assess in-vitro growth of these bacteria in breast milk and milk formula samples. **Material and Methods:** salivary samples were obtained using sterile cotton swabs from 60 infants that were grouped according to nursing milk type. Samples were cultured to obtain the detection frequency and bacterial counts. For the in-vitro investigation, seven donated breast milk samples and seven freshly prepared samples of both milk formulas were inoculated by both bacteria and then cultured to assess bacterial growth. **Results:** *lactobacilli* were detected in all infants, while no significant differences were found in *S. mutans* detection among groups. Counts of both microorganisms in saliva were lowest in (B) while, insignificant difference was found between (B) and (N). Significant differences were evident in in-vitro bacterial counts being lowest in (B) followed by (N) and (FL). **Conclusion:** breast milk and probiotic supplement infants' milk formulas may have a protective role against dental caries in infants.

KEYWORDS

Breast milk; *Lactobacilli*; Milk formula; Probiotics; *Streptoccus mutans*.

RESUMO

Objetivo: existem evidências inconclusivas sobre a potencial carogenicidade das fórmulas lácteas em comparação com o leite materno. O estudo teve como objetivo comparar a detecção e contagem de *Streptococcus mutans* (*S. mutans*) e *lactobacilos* da saliva entre lactentes alimentados com leite materno (B), com fórmulas de leite puro (France Lait-FL) e suplementada com probióticos (Nan 1 optipro-N), e avaliar crescimento in vitro dessas bactérias em amostras de leite materno e fórmulas lácteas. **Material e Métodos:** amostras salivares foram obtidas com swabs de algodão estéreis de 60 lactentes que foram agrupados de acordo com o tipo de leite. As amostras foram cultivadas para obter a frequência de detecção e contagens bacterianas. Para a investigação in vitro, sete amostras de leite materno doado e sete amostras recém-preparadas de ambas as fórmulas lácteas foram inoculadas por ambas as bactérias e então cultivadas para avaliar o crescimento bacteriano. **Resultados:** *lactobacilos* foram detectados em todos os lactentes, enquanto não foram encontradas diferenças significativas na detecção de *S. mutans* entre os grupos. As contagens de ambos os microrganismos na saliva foram menores em (B), enquanto uma diferença insignificante foi encontrada entre (B) e (N). Diferenças significativas foram evidentes nas contagens bacterianas in vitro sendo mais baixas em (B) seguido por (N) e (FL). **Conclusão:** o leite materno e as fórmulas lácteas com suplementos probióticos podem ter um papel protetor contra a cárie dentária em lactentes.

PALAVRAS-CHAVE

Leite materno; *Lactobacilos*; Fórmula láctea; Probióticos; *Streptococcus mutans*.

INTRODUCTION

Multiple ecological drivers can disrupt the balanced oral microbiome shifting it into a dysbiotic, disease-causing microbial community [1]. Early childhood caries (ECC) is etiologically related to a disrupted oral microbiological ecology combined with frequent sugar intake that is not necessarily associated with nursing [2,3]. *Streptococcus mutans* (*S. mutans*) and *Lactobacillus* species, are two oral bacterial species which can colonize the oral cavity as early as 34 days after birth [4]. Yet, both species have been frequently isolated from skewed ecosystems associated with dental caries [1,4].

Human milk is identified as the ideal nutrient for infants based on an extensive body of evidence [5]. Several studies concluded that breastfeeding has a protective effect against dental caries in early childhood compared to bottle feeding [6-9]. However, increased frequency, nocturnal or prolonged breastfeeding beyond the age of 12 months were associated with an increased risk of dental caries [2,7,10].

Different approaches have been suggested to maintain or restore the homeostasis of oral microbial ecology, and thus preventing dental caries. Such approaches include sugar reduction, fluorides, salivary enhancement, and early onset biofilm engineering through pre- and probiotics to encourage the establishment of a sustainable healthy oral ecosystem [1].

The interest in bacteriotherapy to control and prevent medical and oral conditions has grown remarkably in the recent years. In dental field, bacterial interference with probiotics to support the stability and diversity of oral biofilms has gained similar interest. Although the systemic health benefits of probiotics are well established, evidence about their oral health benefits and especially dental caries is still scarce [11,12].

The currently available information about the cariogenicity of human and probiotic supplemented milk is inconclusive. Therefore, the current study investigated the association between human milk, plain and probiotic supplemented formulas and 2 types of cariogenic bacteria. The null hypothesis was that there is no difference between different tested groups.

MATERIAL AND METHODS

The study consisted of 2 phases; a cross-sectional observational investigation which followed the STROBE statement standards to detect the frequency and counts of *Streptococcus mutans (S.mutans)* and *Lactobacilli* in saliva of breastfed and formula-fed (supplemented and non-supplemented with probiotics) infants and an *in-vitro* study investigating the effect of breast milk, and commercially available probiotic supplemented and plain infants' formulas on bacterial growth.

The study complied with the ethical principles of declaration of Helsinki for medical research involving human subjects. Institutional ethical approval was obtained, and all mothers provided an informed consent prior to participation or providing breast milk samples. Power analysis using G power version 3.1.9.7. indicated that 20 infants were required per group to have a power = 80%, and an effect size (f) of (0.479) based on the results of Holgerson et al. [13]. Participants were recruited from two family health-care centers. Inclusion criteria included signing an informed consent, 2-6 months-old infants with complete gestational period who are exclusively breastfed; plain formula or probiotic supplemented formula fed without introduction of complementary food to their diet. Mother-infant pairs should have not taken antibiotics in the previous month and without any systemic disease that can affect the oral microflora.

Ninety-eight infants were screened from (May 1, 2019) till (August 30, 2019) till the required sample was fulfilled. The infants were divided into three groups; breastfed **"B"** (n=20), probiotic supplemented formula-fed **"N"** (Nestle Nan® 1 Optipro, Switzerland) (n=20) and plain formula-fed **"FL"** (France Lait® 1, France) (n=20). Each group was subdivided into two subgroups; delivered by vaginal birth or by caesarean section.

Both formulas main ingredients are Cow's milk whey protein, lactose, skimmed cow milk, soya lecithin, carbohydrates, maltodextrin, vegetables fats, minerals, and vitamins. Nan1 also contains a Bifidus culture.

Saliva samples were collected from the mouths of infants from 9:00 am -11:00 am using a sterile cotton swab, by rubbing the mucosa of the cheeks, the tongue, and the alveolar

ridge [13]. Each cotton swab was inserted into a sterile test tube containing 1ml phosphate buffer saline. The samples were vortexed for one minute and 0.01 ml of saliva was plated onto Mitis Salivarius agar (*Himedia*®, India) modified by adding 1 ml Potassium Tellurite solution and 5μg Bacitracin [14] for *S. mutans* or Man Rogosa Sharpe agar (*Himedia*®, India*)* for *Lactobacilli* using calibrated loops. The plates were incubated at 37°C anaerobically using *AnaeroGen™ (Oxoid, USA)* anaerobic sachet for 72 hours. Bacterial counts in colony forming units (CFU) were obtained based on morphological characteristics; 0.5 mm raised convex undulated colonies of light blue color with rough margins, and granular frosted glass appearance for *S. mutans* [15] and isolated single colonies, hemi-spherically round with white or yellow color for *Lactobacilli* [16].

For the in-vitro study, seven breast milk samples were obtained from mothers of six weeks-old to two years-old infants. Breast milk specimens were collected in sterile plastic containers after gentle manual expression of the breast tissue by the mother in a private room and stored on ice [17]. All milk samples were discarded after being used. No storing or genetic analyses of milk samples were performed. Formula milk samples (France Lait and Nan 1) were freshly prepared according to manufacturer instructions and used within two hours. 0.5 ml of each milk type as well as to nutrient broth (Oxoid, USA) as a negative control was inoculated by half McFarland suspension of *S. mutans* or *Lactobacilli* separately. Bacterial suspensions were obtained by adding bacterial colonies from a single plate that was cultured in the first part of the study to 0.5ml nutrient broth in test tubes. 0.01ml of the inoculated milk specimens were then added to MSB or Man Rogosa Sharpe agar and incubated anaerobically for 72 hours in 37°C and assayed for CFU [18].

Fisher's exact test and one-way ANOVA followed by Tukey's post hoc test were used for statistical analysis at $p \leq 0.05$. Odds ratios and associated 95% confidence intervals were calculated to assess the association between dependent and independent variables. Statistical analysis was performed using SPSS® (IBM, NY, USA) Version 26 for Windows.

RESULTS

Table I shows gender and age distribution of infants among the three groups.

Salivary bacterial detection

No significant differences were evident in the frequency of detection of *S. mutans* among the three feeding methods or between natural birth and C-section delivery (Table II).

Table I - Infants' demographics

Infants' Characteristics			Breast milk (B) n=20	France Lait (FL) n=20	Nan (N) n=20	p-value
Gender	Male	N	12	12	9	0.725
		%	60.0%	60.0%	45.0%	
	Female	N	8	8	11	
		%	40.0%	40.0%	55.0%	
Age	Mean ± SD		3.55 ± 1.10^{B}	4.65 ± 1.27^{A}	4.10 ± 1.44^{AB}	0.023*
	95%CI		[3.04:4.06]	[4.06:5.24]	[3.42:4.78]	

Different superscript letters indicate a statistically significant difference within the same horizontal row. *Significant ($p \leq 0.05$).

Table II - *S. mutans* detection according to feeding method and mode of delivery

S. mutans Detection		Feeding method			p-value	Delivery method		p-value
		(B) n=20	(FL) n=20	(N) n=20		Natural birth n=30	C-section n=30	
Yes	N	16	18	17	0.900	27	24	0.472
	%	80.0%	90.0%	85.0%		90.0%	80.0%	
No	N	4	2	3		3	6	
	%	20.0%	10.0%	15.0%		10.0%	20.0%	

Significant ($p \leq 0.05$).

Within each feeding group, *S. mutans* detection did not differ significantly in infants delivered by C-section compared to vaginal delivery; (p = 0.582) for breastfed infants (B), and (p=1) for both formula groups (FL), (N).

While *Lactobacilli* were detected in the saliva of all infants regardless of the feeding or delivery method.

The risks of *S. mutans* detection were lower in (B) group compared to (FL) [0.44 (CI= 0.07-2.76)] and (N) group [0.71 (CI= 0.136-3.65)]. However, the risk of *S. mutans* detection in (FL) group was 1.58 times greater than that of (N) group (Table III).

Infants' salivary bacterial counts

There was a significant difference between values of different groups (p=0.001). The highest value of *S. mutans* count was found in (FL) group (13.25±6.84) followed by (N) (8.30±6.30) while the lowest value was found in group (B) (5.45±4.56). Pairwise comparisons showed (FL) value to be significantly higher than other groups (p<0.001), while no significant difference was found between (B) and (N) groups (Table IV).

Regarding *Lactobacilli* counts, there was a significant difference between values of different groups (p<0.001). The highest value of bacterial count was found in (FL) group (107.25±44.23) followed by (B) (68.75±17.61) while the lowest value was found in group (N) (59.25±14.53). Pairwise comparisons showed (FL) mean value to be significantly higher than other groups (p<0.001) (Table III).

Salivary bacterial counts in different sub-groups

There was no significant difference between children delivered by natural birth and C-section for both bacterial types, Table V.

In-vitro bacterial growth

There was a significant difference between values of different groups (p<0.001). The highest

Table III - Odds ratio of *S. mutans* detection according to feeding method

S. mutans Detection		Feeding method: Breast milk (B)	Feeding method: France Lait 1(FL)	Odds ratio (95%CI)	p-value
Yes	N	16	18	0.44 (0.07-2.76)	0.661
	%	80.0%	90.0%		
No	N	4	2		
	%	20.0%	10.0%		
Detection		**Breast milk (B)**	**Nan 1 (N)**	**Odds ratio (95%CI)**	**p-value**
Yes	N	16	17	0.71 (0.136-3.65)	1
	%	80.0%	85.0%		
No	N	4	3		
	%	20.0%	15.0%		
Detection		**France Lait 1 (FL)**	**Nan 1 (N)**	**Odds ratio (95%CI)**	**p-value**
Yes	N	18	17	1.58(0.23-10.70)	1
	%	90.0%	85.0%		
No	N	2	3		
	%	10.0%	15.0%		

Significant ($p \leq 0.05$).

Table IV - Salivary Bacterial count (CFU^2) for different groups

Salivary counts		Breast milk (B) n=20	France Lait 1 (FL) n=20	Nan 1 (N) n=20	p-value
S. mutans	(mean ±SD)	5.45±4.56^{B}	13.25±6.84^{A}	8.30±6.30^{B}	0.001*
	95%CI	[3.32:7.58]	[10.05:16.45]	[5.35:11.25]	
Lactobacilli	(mean ±SD)	68.75±17.61^{B}	107.25±44.23^{A}	59.25±14.53^{B}	<0.001*
	95%CI	[60.51:76.99]	[86.54:127.95]	[52.44:66.05]	

Different superscript letters indicate a statistically significant difference within the same horizontal row. *Significant ($p \leq 0.05$).

Table V - Salivary bacterial counts (CFU^2) for different delivery methods

Salivary counts		Natural birth n=30	C-section n=30	p-value
S. mutans	(mean ± SD)	10.47 ± 6.36	7.53 ± 6.85	0.091
	95%CI	[8.09:12.84]	[4.97:10.09]	
Lactobacilli	(mean ± SD)	81.67 ± 36.70	75.17 ± 33.85	0.479
	95%CI	[67.96:78.15]	[62.53:87.81]	

Significant ($p \leq 0.05$)

Table VI - *In-vitro bacterial* count (CFU^3) for different groups

In-Vitro bacterial growth		Breast milk (B)	France Lait 1 (FL)	Nan 1 (N)	Control	p-value
S. mutans counts	(mean ± SD)	7.86 ± 3.34^{C}	65.71 ± 11.34^{A}	42.86 ± 13.80^{B}	3.40 ± 1.67^{C}	<0.001*
	95%CI	[1.07:14.64]	[55.23:.76.20]	[30.09:55.62]	[1.32:5.48]	
Lactobacilli counts	(mean ± SD)	32.29 ± 9.22^{C}	112.86 ± 18.17^{A}	77.86 ± 5.67^{B}	31.00 ± 7.42^{C}	<0.001*
	95%CI	[5.27:59.31]	[77.55:.148.16]	[72.61:83.11]	[21.79:40.21]	

Different superscript letters indicate a statistically significant difference within the same horizontal row. *Significant ($p \leq 0.05$).

value of bacterial count was found in (FL) group (65.71±11.34) followed by (N) (42.86±13.80) and (B) (7.86±3.34) while the lowest value was found in the control group (3.40±1.67). Pairwise comparisons showed (FL) and (N) groups to be significantly different from each other and significantly higher in value than other groups ($p<0.001$) (Table VI).

DISCUSSION

In the present study, 2-6 months old infants were enrolled to preclude the effect of teeth presence and introduction of complementary food which are known to affect oral bacterial colonization [5]. Breast milk specimen donors were chosen to be lactating mothers of infants with age ranging from 6 weeks to 2 years. Human breast milk undergoes 3 stages of maturation; colostrum is the first fluid produced by mothers after delivery, then breast milk undergoes several changes in its composition going through a transitional stage until reaching the final mature stage which develops after 4-6 weeks postpartum. Thereafter, milk composition usually stays the same till the age of 2 years [19].

Although there was no significant difference in *S. mutans* detection among feeding groups, the risk of *S. mutans* detection was lower in breastfed infants compared to both formula types. Moreover, *S. mutans* counts were lowest in breastfed infants whether delivered vaginally or by C-section compared to both types of formulas. The interaction between neonatal saliva and breast milk was found to inhibit the growth of multiple pathogenic bacteria possibly due to the release of antibacterial compounds as hydrogen peroxide. This might explain the difference between oral microflora of breastfed and formula-fed infants [20].

A review on the health benefits of breast milk to intestinal health during early life revealed that breast milk has a plenty of bioactive components that interact with intestinal micro-organisms to regulate the immune system of infants. These include immunoglobulins, essential microbes, lactoferrin, fatty acids and oligosaccharides. Milk microbiome includes more than 700 bacterial species to which infants are exposed daily. These species furnish nearly 25% of infants' intestinal microbiota and affect the acquisition of bacteria in early infancy and thus play a key role in infants' innate immunity. Yet, genetic, geographic, dietary, and health status variations among mothers may affect the type and counts of milk bacteria [21]. Early acquisition of beneficial bacteria may also warrant a lifelong healthy bacterial community according to the principle of "first come, first served" [1]. Thus, a similar role of breast milk may also exist when it comes to oral health.

Breast milk and formulas supplemented with either *Bifidobacterium* or *lactobacilli* probiotic species were also found to decrease *S. mutans* growth significantly compared to breast milk. This was related to lactoferrin, IgA, and IgG in breast milk. Whereas probiotic strains may have inhibited bacterial adhesion to saliva coated hydroxyapatite [22]. Other possible reported probiotic actions include immunomodulation, competitive inhibition, and bacteriocin like compound production [10,11].

Interestingly, *Bifidus* bacteria in Nan 1 was also found in breast milk and guts of breastfed infants. *Bifidus* bacteria is a strain that belongs to the aneorobic Gram positive Bifidobacterium genus that normally inhabits the gastrointestinal tract of mammals and helps maintain its homeostasis, thus, it is added to several dairy products [23].

In the present study, the counts of *S. mutans* in breastfed children (B) did not differ significantly from those of probiotic supplemented formula (N). However, (FL) had a significantly higher *S. mutans* count compared to (N) and (B). The odds of *S. mutans* detection was also 1.58 times greater in (FL) compared to (N). This supports the protective role of probiotics against dental caries development and goes in line with Duse et al. [22]. Salminen et al. [24], also found that the addition of probiotics to formula milk resulted in production of gut microbiota mimicking that of breastfed infants in terms of beneficial microorganisms.

Sandoval et al. [25], supported the anticariogenic role of probiotics. In their randomized clinical trial, they demonstrated that preschool children with a high caries risk who were given plain bovine milk for 10 months displayed a significant increase in dental caries increment and a lower concentration of h D-3 (a salivary antimicrobial peptide) compared to children that consumed probiotic supplemented bovine milk.

The current results also showed that there was a statistically significant difference in the counts of *Lactobacilli* between breastfed and formula-fed infants, being highest among the (FL) group, followed by breastfed group (B) and lowest among (N) group. However, a limitation of the current study is that total oral *Lactobacilli* were assessed without differentiation of probiotic and cariogenic strains. This may be the reason why *Lactobacilli* were detected in all children. Our findings disagreed with those of Vestman et al. [26], who stated that *Lactobacilli* colonized the oral cavity of breastfed infants significantly more than formula-fed infants. However, the authors detected that the dominant *Lactobacilli* strain in saliva of breastfed infants was *Lactobacillus gasseri* which has an inhibitory effect on *S. mutans* and acts as a probiotic.

Considering the mode of delivery, non-significant differences were found in the mean values of *S. mutans* and *Lactobacilli* counts between natural delivery and C-section delivery within each group or collectively regardless feeding types. Although vaginal delivery is thought to be protective against acquisition of harmful microbiota [27], some researchers stated that mode of delivery did not affect the development of ECC and that other factors such as oral health related habits and social determinants play a more important role in the development of ECC [28,29].

The current study did not assess several maternal or infants' confounders that might have affected the acquisition or counts of oral bacteria in infants. One reason was that infants were enrolled from two different healthcare centers: one in a low-middle socioeconomic area and the other in a middle-high socioeconomic area. This is because probiotic supplemented formula is higher in cost compared to plain formula (France Lait 1), and the latter is supplied for free by the Government for those who are financially disadvantaged. Thus, the second phase of the study was employed to assess the role of each milk type as a single variable.

There was a statistically significant difference in bacterial counts among the three groups being highest in the (FL) group, followed by (N) group then breast milk group (B). The current findings support observational studies which reported that children who were solely bottle-fed had a higher risk of developing ECC when compared to breastfed children [30,31]. Aly et al. [32] also emphasized the protective role of breast milk, where it was found that incubating primary incisors infected with *S. mutans* in breast milk resulted in a significant elevation of enamel calcium content and no change in enamel phosphorous content. Whereas a significant reduction in calcium and phosphorus contents was evident in teeth immersed in plain and probiotic supplemented formulas.

A recent in-vitro study disclosed a low cariogenic potential of both breast milk and bovine milk. Saliva coated bovine enamel slabs were infected with *S. mutans* biofilm for 120 hours. Results showed that intermittent exposure to both milk types resulted in comparable % surface hardness loss (%SHL), biofilm pH, viable bacterial counts and amounts of formed extracellular polysaccharides (EPS) mean values which were lower than sucrose or solutions of different lactose concentrations as controls. The authors stated that lactose can not be rapidly fermented by several oral bacteria including *S. mutans* as they require the expression of *Lac* operon genes for lactose breakdown. Additionally, glucosyltransferases can not synthesize EPS from lactose. EPS aid in

bacterial adhesion to enamel and create a porous plaque matrix which facilitates carbohydrates diffusion and retention and inhibits outwards diffusion of acids. The calcium, phosphate, and protein contents of milks aid in enamel remineralization which accounts for the lower % SHL in milk groups. Moreover, milk protein (casein) maintains the availability of calcium and phosphate ions and inhibits their precipitation. Proteins also provide an acid buffering capacity. But in this study, milk was pasteurized before use, thus, possibly eliminating any additional protective effects of milk bacteria [33].

Earlier, Signori et al. [34] showed that exposure of bovine enamel slabs coated with human saliva and a multispecies biofilm to unpasteurized breast milk that was stored at -20 °C resulted in a lower total bacterial, aciduric bacteria, and *Lactobacilli* counts compared to bovine milk. In this latter study, breast milk was also found to cause a biofilm pH drop and loss of enamel surface microhardness greater than that of bovine milk, though the differences were not significant. These finding were attributed by the authors to the higher lactose and lower mineral contents of breast milk compared to bovine milk, and the smaller volume of culture media that may have concentrated the effect of produced acids. Additionally, the investigators noted that artificial saliva with 1% sucrose caused a significant reduction in biofilm pH compared to breast milk and that enamel demineralization caused by breast milk mixed with artificial saliva did not differ significantly from that of artificial saliva, suggesting that human milk is not acidogenic if used without other sugar sources.

Neves et al. [35] also highlighted the protective role of breast milk, when they showed that breast milk did not reduce pH of biofilms collected from children with and without ECC, while sucrose induced a significant drop in dental plaque pH of both children.

By comparing the composition of the two tested formulas to human breast milk, the findings of the current study could be partly explained. Both tested formulas had a higher carbohydrates content than human breast milk such that the total carbohydrates content in both formulas (lactose and other carbohydrates) is 56.5g/ 100gm for France Lait 1 and 57.8 g/100gm for Nan 1. On the other hand, human breast milk carbohydrates content is in the form of 6.4 to 7.6 g/100gm lactose, and 1 g/100gm oligosaccharides. Oligosaccharides in breast milk have a non-nutritive effect and act as a prebiotic [19].

The cariogenic potential of maltodextrins and other carbohydrates in milk formulas is unclear. Maltodextrins are oligosaccharides derived from cornstarch hydrolysis and are used as thickeners in formulas. They were found to cause a drop in plaque pH in vivo, though not as much as sucrose [36]. In another study, maltodextrins were not found to increase the cariogenecity of sucrose in situ. But the authors stated that the extent of starch hydrolyses as well as starch type determine the solubility, molecular weight, and viscosity of maltodextrins and thus their cariogenic potential [37].

Peres et al. [38], demonstrated that milk formula caused as much as dental caries as sucrose in rats infected with *Streptococcus sobrinus* and that breast milk was more cariogenic than bovine milk but not as much as formula milk. Moreover, formula milk cariogenicity decreased with the addition of fluoride. The authors also related these findings to the high total carbohydrates content in formula milk, the higher lactose content and lower mineral and protein contents in breast milk compared to bovine milk.

Other authors demonstrated that two probiotic formula types significantly reduced *S. mutans* suspension pH possibly due fermentation of their carbohydrates content such as lactose and, maltodextrins. Yet, pH did not go below 6.86. They also noted that formula's mineral content could also influence cariogenecity [39]. Another study showed that maltodextrin containing milk-based formula increased *S. mutans* biofilm formation in-vitro compared to soy-based formula containing sucrose. The authors related this finding to the higher total carbohydrates content in milk-based formula [40]. Thus, existing evidence suggests that multiple factors can affect the cariogenecity of milk formulas.

It is worth mentioning that recent evidence suggests that dental caries results from the interactions of multiple bacterial species in a dysbiotic oral biofilm. These interactions may promote bacterial virulence or resistance to antimicrobials. Thus, studies investigating the association of specific bacterial species with dental caries may not accurately represent the clinical pathogenesis of dental caries [41,42].

CONCLUSION

It can be inferred from the results of the current study that both breast milk and probiotic supplemented formula may have a protective role

against infant's oral colonization by pathogenic bacteria such as *S. mutans* and *Lactobacilli.* Based on the current results and the existing evidence of their additional benefits for general health, the use of breast feeding and/or probiotic supplemented formulas should be encouraged by all health care providers.

Authors' Contributions

All authors shared in conceiving the idea and designing the research, MMA collected data. MMA, MOW and NSK interpreted and analyzed the data. MSN carried out all the microbiological analysis. MOW wrote the manuscript. All authors revised and approved the final manuscript.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of: Research Ethics Committee Faculty of Dentistry Ain Shams University. The approval code for this study is: FDASU-Rec M041606.

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