Contribution of *Streptococcus Mutans* Virulence Factors and Saliva Agglutinating Capacity to Caries Susceptibility in Children: A Preliminary Study

Eloa Ramalho de Camargo*/ Jonas Bitencourt Canalle**/ Rodriguo Capozzoli***/ Tanila Wood dos Santos****/ Margareth Bulhman Ballini *****/Lucio Fabio Caldas Ferraz******/ Thaís Manzano Parisotto *******/ Michelle Darrieux********

Background: *Many factors contribute to caries development in humans, such as diet, host factors – including different saliva components – and the presence of acidogenic bacteria in the dental biofilm, particularly Streptococcus mutans (S. mutans). Despite the influence of S. mutans in caries, this bacterium is also prevalent among healthy individuals, suggesting the contribution of genetic variation on the cariogenic potential. Based on this hypothesis, the present work investigated the influence of S. mutans virulence factors and saliva agglutinating capacity on caries susceptibility in children.* ***Study design****: Saliva samples of 24 children from low income families (13 caries-free and 11 caries-active individuals) were collected and tested for their ability to agglutinate S. mutans. The bacteria were isolated from these samples and analyzed for the presence of the gene coding for mutacin IV (mut IV). Biofilm formation and acid tolerance were also investigated in both groups (caries-free and caries-active).* ***Results:*** *Saliva samples from caries-free children showed an increased capacity to agglutinate S. mutans (p=0.006). Also, bacteria isolated from the caries-free group formed less biofilm when compared to the caries-active group (p=0.04). The presence of mut IV gene did not differ between bacteria isolated from caries-free and caries-active individuals, nor did the ability to tolerate an acidic environment, which was the same for the two groups.* ***Conclusions:*** *Altogether, the results suggest that the adhesive properties of S. mutans and the agglutinating capacity of the saliva samples correlated with the presence of caries lesions in children.*

Key words: *Streptococcus mutans, caries, mutacin, biofilm.*

*Eloa Ramalho de Camargo ,PhD in Health Science, Laboratório de Biologia Molecular de Microrganismos, Universidade São Francisco, Bragança Paulista, Brazil.

**Jonas Bitencourt Canalle,Undergraduate student – Biomedicine, Universidade São Francisco, Bragança Paulista, Brazil.

***Rodriguo Capozzoli ,Undergraduate student –Universidade São Francisco, Bragança Paulista, Brazil.

****Tanila Wood dos Santos ,PhD student , Laboratório de Biologia Molecular de Microrganismos, Universidade São Francisco, Bragança Paulista, Brazil.

*****Margareth Bulhman Ballini, MS, Universidade São Francisco, Bragança Paulista, Brazil.

******Lucio Fabio Caldas Ferraz, PhD, Laboratório de Biologia Molecular de Microrganismos, Universidade São Francisco, Bragança Paulista, Brazil.

*******Thaís Manzano Parisotto, PhD,Laboratório de Biologia Molecular de Microrganismos, Universidade São Francisco, Bragança Paulista, Brazil.

********Michelle Darrieux, PhD, Laboratório de Biologia Molecular de Microrganismos, Universidade São Francisco, Bragança Paulista, Brazil.

Send all correspondence to:
Michelle Darrieux
Laboratório de Biologia Molecular de Microrganismos, Universidade São Francisco, Bragança Paulista, Brazil.
Postal code: 12916-900
Phone : 055 11 24548054
E-mail :sampaiomichelle@uol.com.br

INTRODUCTION

Dental caries is a highly prevalent, complex and multifactorial disease, influenced by socioeconomic and environmental factors, as well as the host's genetics and microbiota [1-3]. High sugar intake, associated with the presence of acid-producing bacteria in the dental biofilm lead to demineralization of the teeth, when the homeostasis in the interface is affected [2,4-6]. The dental biofilm is characterized as bacterial populations living in organized structures at a solid/liquid interface [6]. While caries can affect individuals from all age groups, it has been demonstrated that early colonization with the cariogenic mutans streptococci in infants and young children could be related to an increase in the severity of the lesions [5] and predisposes to future caries [3,7,8]. Furthermore, although caries prevalence shows a reduction trend in some countries, the global percentage of schoolchildren with this disease ranges from 60 to 90%, with the highest burden in low income countries [8].

Nowadays, despite the knowledge about the ecological and non-infectious nature of the disease, mutans streptococci still plays an important role in caries development as endogenous bacteria, outgrowing when the homeostasis in the dental biofilm is disturbed [6]. The primary source of acquisition is usually the mother or the person in closest contact

with the infant, the caretaker [9,10]. Once the bacteria reach the oral cavity, they interact with other microorganisms such as *Streptococcus mitis, Streptococcus oralis* and *Streptococcus salivarius*, forming a complex and organized structure, the dental biofilm, which is formed as soon as the teeth erupts, providing non-shedding surfaces [11]. A very interesting point is that *Streptococcus mutans (S. mutans)* are able to produce mutacins, important antibacterial proteins that kill or inhibit the growth of intimately related bacterial strains, modulating the growth of competitor microorganisms and favoring their predominance in the cariogenic biofilm [12]. In this biofilm environment, the bacteria ferment a wide variety of sugars, especially sucrose, producing large amounts of acid that are able to dissolve enamel and dentin tissues [3].

Although *S. mutans* has long been associated with caries development in all age groups [13], particularly in young children, increasing evidence suggests that the presence of the bacterium in the oral cavity *per se* is not sufficient to cause disease [14,15]. One finding that supports this affirmation is the presence of *S. mutans* in populations with low caries incidence [4,7,16] suggesting that other factors may contribute to caries establishment. An increased ability to form biofilms, high acid tolerance and the capacity to outcompete other colonizers of the oral cavity have all been correlated to an increased potential to induce caries formation in children and adults [17-20]. Analysis of the genetic diversity of mutans populations in the oral cavity of caries-free and caries-active subjects has shown a positive correlation between the number of bacterial genotypes and the presence of caries [21].

Host factors have also been associated with caries susceptibility [22,23]. Saliva contains many components that contribute to maintain tooth integrity, including antimicrobial proteins and buffering agents such as phosphate and carbonic acid-bicarbonate buffers as well as sialin, arginine and urea [5]. Moreover, salivary mucins, which play an important role in the health of the oral cavity, are able to interact with *S. mutans* by promoting their agglutination, favoring lower titers of these bacteria [5,24]. Different studies, on the other hand, have demonstrated that the presence of certain types of proteins is associated with caries increment, suggesting an important role for genetic diversity of the host in caries susceptibility [25,26].

The present work investigated the contribution of host and bacterial factors to caries susceptibility in children. The chosen parameters were biofilm formation, acidic tolerance, the presence of *mut* IV gene, and bacterial agglutination by saliva.

MATERIALS AND METHOD

The present study was approved by the Institutional Ethics Committee of São Francisco University (protocol CAAE: 0077.0.142.000-10). A convenience sample comprising 24 children, between the ages of 7 and 10 years old, were selected from two public schools in Minas Gerais, Brazil according to the following inclusion criteria: good general heath, absence of enamel hypoplasia and good behaviour during the clinical examinations. Children with comorbid conditions, syndromes or using antibiotics were excluded. Thus, with these criteria, two groups of children were formed: caries-free (13 subjects) and caries-active (11 subjects). Prior to the clinical procedures, positive written consents were signed by the responsible for each child enrolled in the study.

Children were from the same low socioeconomic status, and daily meals were also quite similar. Moreover, all of them brushed their teeth with fluoridated dentifrice (1.000 ppm) at least once a day, and lived in a fluoridated tap water area (0.6-0.8 ppm).

Caries diagnosis criteria and calibration of the examiner

Caries status was determined according to the World Health Organization criteria and the lesions activity was also recorded [27]. Cavities were classified as active when a softened floor was detected by gentle probing. Prior to the clinical exams, theoretical discussions using clinical photographs were held to give instructions to the examiner about the WHO criteria. One calibrated examiner conducted the evaluations using a focusable flashlight, a mouth mirror and a ball ended probe, to confirm questionable findings. For the calibration, about 10% of the children were re-examined with a 7-day-interval period, to avoid memorization. Then, the intra-examiner Kappa calculation was performed and the value obtained regarding the surface level was of: 0.85.

The volunteers were instructed not to eat, drink, or brush their teeth two hours before the procedure. Saliva samples were collected without stimulation and placed in sterile microtubes. For *S. mutans* isolation, 100 µL aliquots of saliva were immediately transferred to 1 mL of trypic soy broth (TSB – Oxoid, Hampshire – UK) supplemented with 10% glycerol. The samples were transported on ice and stored at -80 ºC.

Isolation of S. mutans from saliva samples

For bacterial isolation (one colony isolated per child), microtubes containing saliva in TSB were thawed and vortexed for 1 minute. The samples were plated on agar mitis salivarius medium (Himedia, Mumbai – India) supplemented with bacitracin (0.2 U/mL) (Sigma Chemical, St. Louis, Mo.) and 20% sucrose (MSB), and incubated for 72 hours at 37 ºC under anaerobic conditions. Visible colonies morphology were assessed under reflected light, and those which were firm and opaque, with a granular surface similar to grained glass, often with a polysaccharide sparkling droplet on top were identified as mutans streptococci [28]. Moreover, the colonies were submitted to catalase test, and those negative for catalase were cultured in 5 mL of brain heart infusion broth (BHI – Himedia, Mumbai – India) for 24 hours. At last, the colonies were submitted to Gram-staining and the samples were stored at -80 ºC with 10% glycerol.

Extraction of bacterial chromosomal DNA

Chromosomal DNA was isolated according to the method described by Welsh and McClelland [29] and adapted by Kamiya *et al* [30]. *S. mutans* strains obtained from saliva samples were thawed and grown in BHI for 24 hours at 37 ºC. The bacteria were pelleted by centrifugation, washed and

boiled in TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) for 10 minutes. The bacterial debris were pelleted by centrifugation, and the DNA at the supernatant, quantified through spectrophotometry and stored at -20 ºC for PCR analysis.

PCR screening of mut IV and gtfB genes

Detection of *mut* IV gene was performed by PCR using primers designed by Qi *et al*. [31]. The sequences of the primers were 5'-ATGGGATATTTAAAGGGAAA-3' and 5'-TCAGAGCAGCTACAAAAACT-3'. 1 µL (100 ng) of chromosomal DNA of *S. mutans* isolated from caries-free and caries-active children was added to a 50 µL mixture containing 1X PCR buffer with 2,5 mM MgCl2, 1 mM of each desoxynucleotide, 2 mM of each oligonucleotide primer and 5 units of *Taq* DNA polymerase (Fermentas, Burlington – Canada). The reaction was performed in a Gene Amp PCR 9700 (Applied Biosystems, Carlsbad – US) as follows: an initial denaturation at 94 ºC for 5 minutes, 30 cycles of denaturation at 94 ºC for 30 seconds, annealing at 52 ºC for 1 minute and extension at 72 ºC for 2 minutes, and a final extension at 72 ºC for 10 minutes. The final PCR products were analyzed by electrophoresis in 1% agarose gels stained with ethidium bromide, under ultraviolet light.

An internal fragment of the *gtfB* gene was used as a positive control for each sample, to confirm the identity of the *S. mutans* isolates. Amplification of this fragment was performed using a set of primers designed by Oho *et al* [32], and the same conditions described for *mutacin* IV. The primers used for amplification of *gtf*B were 5'-ACTACACTTTCGGGTGGCTTGG-3' and 5'-CAGTATAAGCGCCAGTTTCATC-3'. The PCR reaction was repeated twice in order to confirm the results. Comparison between the caries-free and caries-active groups was performed using chi-square test, and p values ≤ 0.05 were considered significant.

Biofilm formation by S. mutans isolates

Biofilm formation was determined according to the protocol adapted from Banu *et al* [33]. Frozen bacterial stocks were plated on BHI and incubated at 37 ºC overnight in anaerobic conditions. Colonies were transferred to BHI broth and grown to an optic density at 600 nm ($O.D._{600\ nm}$) of 0.4-0.5. After reaching mid log phase, the samples were diluted in BHI supplemented with 20% sucrose to an $O.D._{600\ nm} < 0.1$, transferred to microtiter plates and incubated at 37 ºC for 24 hours without agitation. The medium was transferred to a new microtiter plate, and the $O.D._{600\ nm}$ was measured. The adhering bacteria were fixed with 99% methanol for 15 minutes, and stained with 0.1% fuchsine for 5 minutes. The plate was washed twice with phosphate buffered saline (PBS) and dried at 50 ºC for 30 minutes. The absorbance of the biofilms was measured at 492 nm using a microplate reader (Asys). Statistical analysis was determined using the Mann-Whitney test, and p values ≤0.05 were considered significant.

Acid Tolerance assay

The ability of *S. mutans* isolates to resist a sharp reduction in pH was analyzed using the protocol described by Hana *et al*. [34] and modified by Nakano *et al*. [35].

The bacterial frozen stocks were thawed, grown in Todd Hewett medium plus 0,3% yeast extract (THY–Himedia) at 37 ºC overnight, diluted to an $O.D._{600\ nm}$ of 0.1 and cultivated until they reached mid log phase ($O.D._{600\ nm}$ 0,4-0,5). The samples were centrifuged, and the pellets, re-suspended in THY with pH adjusted to 7.5 or 5.0 using HCl. After two hours, the cultures were centrifuged once more, and the bacteria transferred to THY medium with pH adjusted to 3.5 and incubated for another 3 hours at 37 ºC. The control group was maintained in THY pH=7.5 throughout the experiment. Aliquots of the bacteria before and after incubation in low pH were plated in BHI (pH=7.5) in triplicates and incubated overnight at 37 ºC in anaerobiosis. The results are shown as the percent survival in comparison with the control (prior to incubation in pH=3.5). Statistical analysis was conducted using Student t test, with a significance level of 5%.

S. mutans agglutination by saliva

One milliliter of non-stimulated whole saliva was collected in disposable plastic cups and stored at -80 ºC. Analysis of the agglutinating potential of the saliva samples over *S. mutans* was performed using a protocol adapted from Soukka *et al*. [36]. *S. mutans* isolated from a caries-active child and confirmed by PCR was grown in BHI to an $O.D._{600\ nm}$ of 0.5 and washed with sterile PBS. Aliquots of the bacterial culture (100 µL) were transferred to microtubes containing 100 µL of saliva, which had been previously clarified by centrifugation, and incubated for 1 hour at 37 ºC. Absorbance at 600 nm was measured at time points 0 and 1 h. Statistical significance was determined using Mann-Whitney test.

RESULTS

Isolation of S. mutans from saliva samples

Bacterial colonies with typical morphology of *S. mutans* were detected in the saliva cultures of all twenty-four children, regardless of their caries status. One colony was isolated from each saliva sample, and submitted to PCR (for amplification of a *gtf*B gene fragment) in order to confirm their identity (data not shown).

PCR screening of mutacin IV gene

Chromosomal DNA extracted from *S. mutans* belonging to caries-free and caries-active children (and previously identified by PCR) was used as template for amplification of the *mutacin* IV gene (*mut* IV) by PCR. Figure 1 shows the analysis by gel electrophoresis of the amplicons. From a total of 24 DNA samples, 16 were positive for *mut* IV, being nine strains derived from caries-free children and six strains belonging to children with caries. No significant differences were found between the two groups, indicating that *mut* IV prevalence was similar on *S. mutans* isolated from caries-free and caries active subjects. Table 1 summarizes the overall frequency of the *mut* IV gene in the bacterial samples.

Biofilm formation by S. mutans isolates

Biofilm formation was evaluated by a microplate adhesion assay in presence of sucrose. The final absorbance of the sample reflects the amount of bacterial cells that have

attached to the microplate during growth, and is considered to be indicative of biofilm formation. Figure 2 shows the normalized absorbance measures of bacteria from caries-free and caries-active children. *S. mutans* isolated from children with caries exhibited a significantly higher adherence to the microplate during growth (p=0.04), when compared with bacteria from children who had no caries history. This can be interpreted as an increased ability to form biofilms in bacteria originated from children with caries.

Figure 1–Amplification of the *mut* IV gene from *Streptococcus mutans* isolates. Chromosomal DNA from 24 bacterial strains belonging to caries-free and caries-active children was amplified by PCR using primers specific for *mut* IV. The DNA bands around 1344 bp correspond to the *mut* IV gene. 1. Ladder; 2-7 (upper panel) and 1-6 (lower panel) isolates from caries-free children. 8-13 (upper panel) and 7-13 (lower panel), isolates from caries-active children.

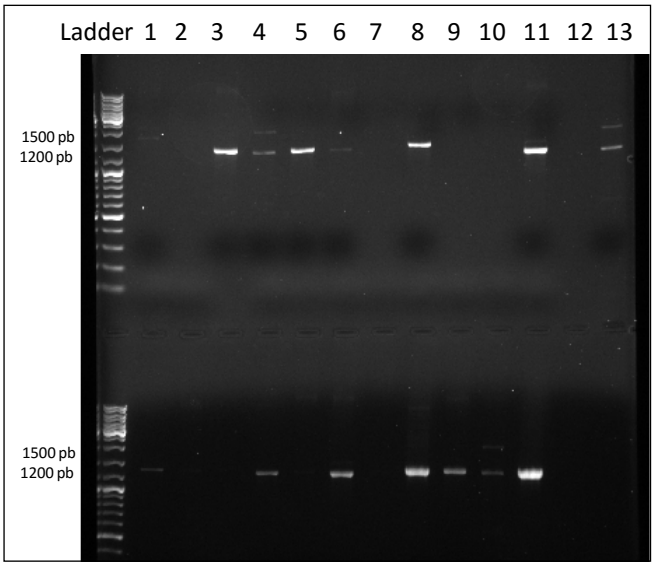


Table 1–Frequency of *mut* IV gene among *Streptococcus mutans* isolated from caries-free and caries-active children

	mut IV$^+$	%	*mut* IV$^-$	%
Caries-free	9	56,25	4	50
Caries-active	7	43,75	4	50
Total	16		8	

mut IV$^+$ Number of isolates positive for *mutacin* IV gene

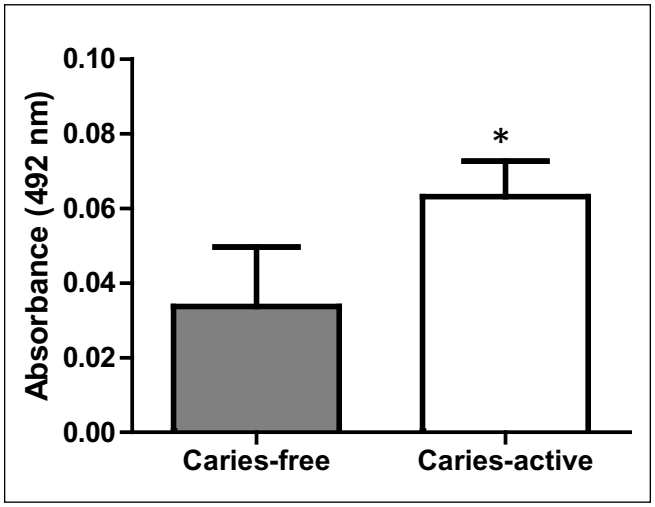
mut IV$^-$ Number of isolates negative for *mutacin* IV gene

The total number of positive and negative isolates is shown, as well as the relative frequency.

Acid Tolerance by S. mutans

The ability of *S. mutans* isolates to resist a sharp decrease in pH was evaluated in comparison with a gradual decrease in pH. Figure 3 shows the percentage of surviving bacteria after treatment. The decrease in pH promoted a reduction on bacterial loads in both caries-free and caries-active groups. Furthermore, a significant reduction in bacterial numbers was observed in *S. mutans* submitted to a brisk reduction in pH, from neutrality to pH=3.5 (unadapted condition), when compared to the samples cultured in pH=5.0 and then transferred to pH=3.5 (adapted condition). Around thirty-five percent of the bacteria in the caries-active group survived a slow decrease in pH, against 20% in the caries-active group. When the bacteria were submitted to a brisk drop in pH, the percent survival of *S mutans* decreased to around 5% in both groups (p=0,0039 in the caries active group and 0,0017 in the caries-free group). This effect demonstrates that, despite the ability of *S mutans* to tolerate acidic conditions, a sudden drop in pH greatly impacts bacterial fitness. However, no differences in bacterial loads were observed for the same condition (unadapted or adapted), between bacterial samples from caries-free and caries active individuals, indicating that these two sets of bacteria have the same tolerance to acid challenge.

Figure 2–*In vitro* biofilm formation by *Streptococcus mutans* in presence of sucrose. Biofilm formation was evaluated in a microplate assay. The absorbance corresponds to the amount of bacterial cells adhered to the plate after 24 hour incubation. The analyses were performed in triplicates, and each value plotted corresponds to the arithmetic mean of these replicas. *p<0.05 for comparisons between caries-free (13 strains) and caries-active (11 strains) samples.



S. mutans agglutination by saliva

The potential of saliva samples to agglutinate *S. mutans* was determined by spectrophotometry, as bacterial agglutination leads to a gradual reduction in the optical density of the sample over time. Figure 4 shows the percentage of reduction in $O.D._{600nm}$ of *S. mutans* after treatment with saliva from caries-free and caries-active children. The saliva from caries-free individuals induced a significantly more pronounced reduction in bacterial density when compared with saliva from caries-active children (p=0.006). The saliva samples from the caries-free group promoted 20% reduction in absorbance, while only 5% of the bacteria were agglutinated in the caries-active group. This indicates that saliva from children with no caries history is four times more effective in promoting *S. mutans* agglutination than saliva from children with caries.

Figure 3–Acid tolerance of *Streptococcus mutans* isolates. Acid tolerance was determined as the percentage of surviving bacteria after acid challenge (pH=3.5). Bacteria isolated from caries-free (13 strains) and caries-active (13 strains) children were compared for acid tolerance after a sharp decrease in pH (from neutral pH to 3.5 – unadapted) in relation to a gradual decrease in pH (from neutral to pH=5.0 and then to pH=3.5 – adapted). **p<0,005.

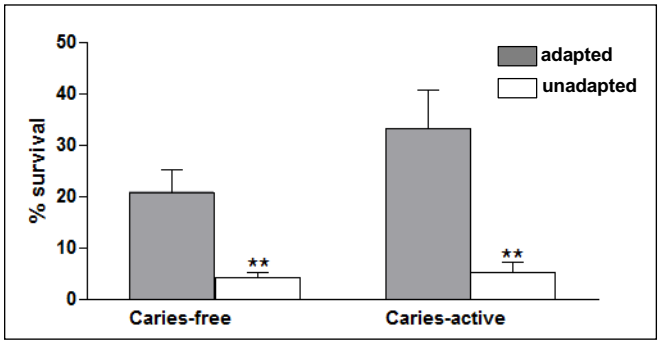
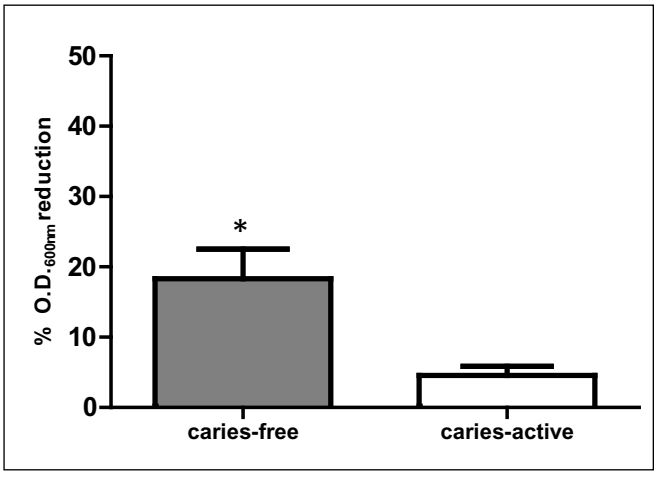


Figure 4–Agglutination of *Streptococcus mutans* by saliva. Saliva samples from children with and without caries were compared for their ability to agglutinate *Streptococcus mutans*. The results are shown as the percentage in $O.D._{600nm}$ reduction in relation to untreated controls. *p≤0.05 between saliva from caries-free and caries-active children.



DISCUSSION

The present preliminary study has demonstrated that the ability of *S. mutans* to form dental biofilm *in vitro* and the agglutination capacity of saliva influenced caries susceptibility in school children.

Susceptibility to dental caries is dependent on a multitude of factors, including diet, oral hygiene, host factors and the oral microbiota [37]. The presence of acidogenic bacteria in the mouth has long been associated with caries development, and increasing evidence suggests that variations on genetic and metabolic traits of these microorganisms play an important role on this process [15,21,31,38-43].

In our study, we observed that mutans isolates derived from children with caries produced more biofilm *in vitro* (as determined by a higher absorbance in microplate assay) then bacteria isolated from children with no caries experience. This data suggests that bacteria from children with caries have an enhanced ability to produce biofilms in presence of sucrose. Given the major importance of bacterial attachment and biofilm formation to caries development, an increased ability to produce biofilm in an inert surface suggests a higher fitness of mutans isolated from children with caries. However, our results contrast previous findings from a similar study with preschool children in Iran, which did not show a correlation between biofilm production and the presence of caries in the subjects [41]. This could be because dental biofilm formation is just one of the characteristics of the bacteria that favor their maintenance in the oral environment; however it's a very significant property, as dental biofilm is a key point for carious lesions development, and it is in the interface dental biofilm/dental surface that the demineralization takes place.

Analysis of the acid tolerance by *S. mutans* isolates demonstrated a similar ability to survive a brisk acidification of the culture medium in both groups (caries-free and caries-active). This effect has been investigated by other study, which showed an increased acid tolerance of bacteria derived from children with caries [41]. The ability to survive the rapid pH changes that frequently occur during caries challenge in the mouth is crucial for *S. mutans* selection in the cariogenic biofim. For this reason, the bacterium has evolved many mechanisms to regulate internal H^+ levels [44].

Alaluusua *et al* (1996) were the first to show that genetic variations in *S. mutans* were related to differences in their cariogenic potential [40]. A few years later, a study evaluating the genotypic variability of *S. mutans* in young adults found a positive association between the bacterial genetic diversity and the presence of carious lesions [21]. One bacterial gene that has been implicated in mutans colonization rate is the *mut* IV, which encodes the non-lantibiotic bacteriocin, mutacin IV [30,31]. PCR analysis of the *mut* IV in *S. mutans* isolated from caries-free and caries active adults revealed a higher presence of this gene in the latter group [30]. Further studies evaluating mutacin production [43] and the expression of genes involved in mutacin biosynthesis [42] confirmed these results. Since mutacin IV exerts antimicrobial activity against members of the mitis group of oral streptococci [31], the presence of this gene (and consequent expression of the protein) could help *S.*

mutans outcompete the early colonizers of the oral biofilm, thus allowing a more effective colonization of the tooth surfaces [20,30,43]. However, in the present work, the prevalence of *mut* IV gene did not differ among isolates derived from caries-free and caries-active children. Actually, the gene was present in around half of the bacteria in each group. This result suggests that, at least in children, other factors may have greater influence over *S. mutans* survival and caries establishment.

The present study evaluated the ability of the saliva samples to agglutinate *S. mutans in vitro*, considering that saliva contains many components that display antimicrobial activity and therefore can influence caries development [22,45]. Saliva from caries-free children displayed a higher agglutinating ability when compared to saliva from children with caries, suggesting that saliva from children with no caries experience is able to aggregate *S. mutans* more efficiently. This result has been previously described in a longitudinal study evaluating the contribution of many salivary components to caries development, however, their sample comprised adolescents instead of younger children [22]. The agglutinating effect of saliva has been attributed to the presence of low-molecular-weight components – like β2-microglobulin [46], or to other components such as lactoferrin [36] and IgA antibodies [32]. Furthermore, since the parameter used to measure bacterial aggregation was the reduction in absorbance, the lytic effect of antimicrobial proteins present in saliva (such as antimicrobial histatins, and beta defensin 1 [25] may play a role in the observed reduction in bacterial loads. In order to confirm that bacterial lysis by saliva contributes to the reduction in absorbance, it would be important to determine the total number of colony forming units before and after treatment. This could be a limitation of the present study, which is preliminary, together with the number of children assessed.

Additionally, increasing evidence suggests that the genetic background of the human host can also impact caries development. For example, the presence of basic proline-rich proteins (PRPs) in saliva has been associated with an increased capacity to neutralize the acids produced during bacterial metabolism in dental biofilms, while acid PRPs contribute to caries establishment [25]. Therefore, while the present study found important correlations of host's and microbe's components with caries status in children, further studies are necessary in order to generate an accurate panel of factors influencing caries.

CONCLUSIONS

Data from the present study reinforce the multifactorial nature of dental caries, with the contribution of both host factors and microbial traits. In particular, *S. mutans* ability to form biofilms and the agglutinating potential of the saliva have shown a strong correlation with the presence of caries lesions in children, with the first leading to an increase in caries susceptibility and the latter inhibiting bacterial attachment.

REFERENCES

1. Bowen WH. Dental caries–not just holes in teeth! A perspective. Mol Oral Microbiol 31: 228-33, 2016.
2. Caufield PW. Dental caries – a transmissible and infectious disease revisited: a position paper. Pediatr Dent 19: 491-498, 1997.
3. Islam B, Khan SN, Khan AU. Dental caries: from infection to prevention. Med Sci Monit 13: 196-203, 2007.
4. Alaluusua S, Kleemola-Kujala E, Nystrom M, Evalahti M, Gronroos L. Caries in the primary teeth and salivary Streptococcus mutans and lactobacillus levels as indicators of caries in permanent teeth. Pediatr Dent 9: 126-130, 1987.
5. Guo L, Shi W. Salivary biomarkers for caries risk assessment. J Calif Dent Assoc 41: 107-109, 2013.
6. Fejerskov O. Changing paradigms in concepts on dental caries: consequences for oral health care. Caries Res 38: 182-191, 2004.
7. Ingemansson Hultquist A, Lingstrom P, Bagesund M. Risk factors for early colonization of mutans streptococci–a multiple logistic regression analysis in Swedish 1-year-olds. BMC Oral Health 14: 147, 2014.
8. Lagerweij MD, van Loveren C. Declining Caries Trends: Are We Satisfied? Curr Oral Health Rep 2: 212-217, 2015.
9. Kishi M, Abe A, Kishi K, Ohara-Nemoto Y, Kimura S, Yonemitsu M. Relationship of quantitative salivary levels of Streptococcus mutans and S. sobrinus in mothers to caries status and colonization of mutans streptococci in plaque in their 2.5-year-old children. Community Dent Oral Epidemiol 37: 241-249, 2009.
10. Lapirattanakul J, Nakano K. Mother-to-child transmission of mutans streptococci. Future Microbiol 9: 807-823, 2014.
11. Marsh PD. Dental plaque: biological significance of a biofilm and community life-style. J Clin Periodontol 32: 7-15, 2005.
12. Hale JD, Ting YT, Jack RW, Tagg JR, Heng NC. Bacteriocin (mutacin) production by Streptococcus mutans genome sequence reference strain UA159: elucidation of the antimicrobial repertoire by genetic dissection. Appl Environ Microbiol 71: 7613-7617, 2005.
13. Yang Y, Li Y, Lin Y, Du M, Zhang P, Fan M. Comparison of immunological and microbiological characteristics in children and the elderly with or without dental caries. Eur J Oral Sci 123: 80-87, 2015.
14. Law V, Seow WK. A longitudinal controlled study of factors associated with mutans streptococci infection and caries lesion initiation in children 21 to 72 months old. Pediatr Dent 28: 58-65, 2006.
15. Saarela M, Hannula J, Matto J, Asikainen S, Alaluusua S. Typing of mutans streptococci by arbitrarily primed polymerase chain reaction. Arch Oral Biol 41: 821-826, 1996.
16. Beighton D, Rippon HR, Thomas HE. The distribution of Streptococcus mutans serotypes and dental caries in a group of 5- to 8-year-old Hampshire schoolchildren. Br Dent J 162: 103-106, 1987.
17. Law V, Seow WK, Townsend G. Factors influencing oral colonization of mutans streptococci in young children. Aust Dent J 52: 93-100, 2007.
18. Shen S, Samaranayake LP, Yip HK. In vitro growth, acidogenicity and cariogenicity of predominant human root caries flora. J Dent 32: 667-678, 2004.
19. Beighton D. The complex oral microflora of high-risk individuals and groups and its role in the caries process. Community Dent Oral Epidemiol 33: 248-255, 2005.
20. Kamiya RU, Taiete T, Goncalves RB. Mutacins of Streptococcus mutans. Braz J Microbiol 42: 1248-1258, 2011.
21. Napimoga MH, Kamiya RU, Rosa RT, et al. Genotypic diversity and virulence traits of Streptococcus mutans in caries-free and caries-active individuals. J Med Microbiol 53: 697-703, 2004.
22. Kirstila V, Hakkinen P, Jentsch H, Vilja P, Tenovuo J. Longitudinal analysis of the association of human salivary antimicrobial agents with caries increment and cariogenic micro-organisms: a two-year cohort study. J Dent Res 77: 73-80, 1998.
23. Parisotto TM, King WF, Duque C, et al. Immunological and microbiologic changes during caries development in young children. Caries Res 45: 377-385, 2011.
24. Levine MJ, Herzberg MC, Levine MS, et al. Specificity of salivary-bacterial interactions: role of terminal sialic acid residues in the interaction of salivary glycoproteins with Streptococcus sanguis and Streptococcus mutans. Infect Immun 19: 107-115, 1978.
25. Levine M. Susceptibility to dental caries and the salivary proline-rich proteins. Int J Dent 2011: 953412, 2011.
26. Yu PL, Bixler D, Goodman PA, Azen EA, Karn RC. Human parotid proline-rich proteins: correlation of genetic polymorphisms to dental caries. Genet Epidemiol 3: 147-152, 1986.
27. Oral Health Surveys – Basic Methods. World Health Organization; 1997.
28. Hardie JM. Bergey's Manual of Systematic Bacteriology. Ed Williams and Wilkins, Baltimore, 1986.
29. Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18: 7213-7218, 1990.
30. Kamiya RU, Napimoga MH, Hofling JF, Goncalves RB. Frequency of four different mutacin genes in Streptococcus mutans genotypes isolated from caries-free and caries-active individuals. J Med Microbiol 54: 599-604, 2005.
31. Qi F, Chen P, Caufield PW. The group I strain of Streptococcus mutans, UA140, produces both the lantibiotic mutacin I and a nonlantibiotic bacteriocin, mutacin IV. Appl Environ Microbiol 67: 15-21, 2001.
32. Oho T, Yu H, Yamashita Y, Koga T. Binding of salivary glycoprotein-secretory immunoglobulin A complex to the surface protein antigen of Streptococcus mutans. Infect Immun 66: 115-121, 1998.
33. Banu LD, Conrads G, Rehrauer H, Hussain H, Allan E, van der Ploeg JR. The Streptococcus mutans serine/threonine kinase, PknB, regulates competence development, bacteriocin production, and cell wall metabolism. Infect Immun 78: 2209-2220, 2010.
34. Hanna MN, Ferguson RJ, Li YH, Cvitkovitch DG. uvrA is an acid-inducible gene involved in the adaptive response to low pH in Streptococcus mutans. J Bacteriol 183: 5964-5973, 2001.
35. Nakano K, Fujita K, Nishimura K, Nomura R, Ooshima T. Contribution of biofilm regulatory protein A of Streptococcus mutans, to systemic virulence. Microbes Infect 7: 1246-1255, 2005.
36. Soukka T, Tenovuo J, Rundegren J. Agglutination of Streptococcus mutans serotype C cells but inhibition of Porphyromonas gingivalis autoaggregation by human lactoferrin. Arch Oral Biol 38: 227-232, 1993.
37. Johansson I, Witkowska E, Kaveh B, Lif Holgerson P, Tanner AC. The Microbiome in Populations with a Low and High Prevalence of Caries. J Dent Res 95: 80-6, 2016.
38. Mattos-Graner RO, Li Y, Caufield PW, Duncan M, Smith DJ. Genotypic diversity of mutans streptococci in Brazilian nursery children suggests horizontal transmission. J Clin Microbiol 39: 2313-2316, 2001.
39. Mattos-Graner RO, Napimoga MH, Fukushima K, Duncan MJ, Smith DJ. Comparative analysis of Gtf isozyme production and diversity in isolates of Streptococcus mutans with different biofilm growth phenotypes. J Clin Microbiol 42: 4586-4592, 2004.
40. Alaluusua S, Matto J, Gronroos L, et al. Oral colonization by more than one clonal type of mutans streptococcus in children with nursing-bottle dental caries. Arch Oral Biol 41: 167-173, 1996.
41. Ghasempour M, Rajabnia R, Irannejad A, Hamzeh M, Ferdosi E, Bagheri M. Frequency, biofilm formation and acid susceptibility of streptococcus mutans and streptococcus sobrinus in saliva of preschool children with different levels of caries activity. Dent Res J 10: 440-445, 2013.
42. Kamiya RU, Hofling JF, Goncalves RB. Frequency and expression of mutacin biosynthesis genes in isolates of Streptococcus mutans with different mutacin-producing phenotypes. J Med Microbiol 57: 626-635, 2008.
43. Kamiya RU, Napimoga MH, Rosa RT, Hofling JF, Goncalves RB. Mutacin production in Streptococcus mutans genotypes isolated from caries-affected and caries-free individuals. Oral Microbiol Immunol 20: 20-24, 2005.
44. Matsui R, Cvitkovitch D. Acid tolerance mechanisms utilized by Streptococcus mutans. Future Microbiol 5: 403-417, 2010.
45. Malcolm J, Sherriff A, Lappin DF, et al. Salivary antimicrobial proteins associate with age-related changes in streptococcal composition in dental plaque. Mol Oral Microbiol 29: 284-293, 2014.
46. Ericson D. Agglutination of Streptococcus mutans by low-molecular-weight salivary components: effect of beta 2-microglobulin. Infect Immun 46: 526-530, 1984.