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DENTAL CARIES AND SALIVARY BACTERIA IN SCHOOL CHILDREN AT AGE OF 12 WITH PRESENT AND ABSENT DENTAL CARIES

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Abstract

A group of phenotypically similar bacteria, collectively known as mutant streptococci, are considered as the main bacterial components responsible for the onset and development of cavities. The aim of our study is to identify the salivary bacteria (*Lactobacillus* spp., *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus salivarius*, and *Streptococcus mitis*) and analyze their interdependence with the dental status.

The study included 71 children (26 female and 45 male) at the age of 12 years. According to their dental health status, they were divided into: control group - 31 examinees without caries, missing teeth (extractions) and dental fillings (DMF = 0); and experimental group - 40 examinees with caries, missing teeth (extractions) and dental fillings. In all examinees clinical and microbiological examinations were carried out. The lactobacilli in the saliva were determined with a diagnostic test CRT-bacteria (Vivadent, Schaan, Lihtenstein). Undivided sputum samples with sterile swabs were planted on Mitis Salivarius Agar (Fluka, a substrate with sucrose, glucose, trypan blue and crystal violet) which is recommended for the isolation of mixed cultures of streptococci, in particular: *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus salivarius*, *Enterococcus faecalis*, etc.

Between the detected bacteria in the saliva - *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus salivarius*, *Streptococcus mitis* and the existence of dental caries there is a significant correlation ($p < 0.001$). Between the presences of *Lactobacillus* spp. in the saliva and the existence of dental caries there is a significant correlation ($p < 0.01$).

The salivary bacterial parameters can be used as serious screening factors and can seriously participate as an instrument in the assessment of the dental caries risk.

***Key words**: Dental caries, Salivary bacterial, Lactobacillus, Streptococcus mutans, Streptococcus sobrinus, Streptococcus salivarius, Streptococcus mitis.*

1. Introduction

Keyes in the 1960's indicated for the first time that dental caries is an infectious, transmissible disease. Since then a group of phenotypically similar bacteria, collectively known as *mutans* streptococci, are considered as the main bacterial components responsible for the onset and development of cavities. The earliest time in which the cariogenic mutants streptococci can start existing and start acting is the time of the onset of tooth sprouting, for which the firm surfaces of the teeth is required in order for streptococcal colonization and their reproduction [1].

In general, all the concepts so far, about the etiology of dental caries are focused on the fermentation of carbohydrates that convert caryogenic bacteria into organic acids. The bacteria from the plaques produce a variety of end products, which may vary depending on the diet. In the presence of fermentable carbohydrates, the most common organic acids that are produced are: lactic, formic and acetic acid. These acids cause a decrease in pH in the plaque, resulting in demineralization of the tooth and the creation of an environment that is favorable for further growth of the *mutans* streptococci. In addition to the production of acid, *mutans* streptococci contain a wide variety of virulence factors that are responsible for the cariogenicity of the dental plaque [2, 3, 4, and 5].

Microorganisms play an important role in the development and progression of caries. Usually, the bacteria in the oral cavity are in balance. The rate of caries increases if the number of specific bacteria

(*S. mutans*, lactobacilli) increases significantly, while the protective factors do not function normally. It is confirmed that most caryogenic bacteria are from the group streptococci, in particular *S. mutans* and *S. sobrinus* and the lactobacilli. *Mutans* streptococci are considered to be significant determinants of plaque cariogenicity and are mainly associated with initial caries development, while the number of lactobacilli increases during the progression of cavities [6, 7].

Regardless of the metabolic method used by lactobacilli, it results in environmental acidity. Multiple studies have shown not only the acidity of the lactobacilli but also their tolerance towards acids [8]. These bacteria may cause a pH decrease of less than 4.5 [9]. Some species are able to survive in a pH of up to 2.2 [10].

Over the last fifteen years, the genus *Lactobacillus* has undergone numerous trials for verification and currently includes more than 80 species, some of which were found only in the oral cavity [11]. The use of molecular biology and its subtle tools, according to Coeuret, gave the opportunity to express doubt over conventional classifications. Taxonomy of lactobacilli is not easy because many different Gram + bacilli are grouped together under this name (GC% 32 - 53%). The analysis of the ARNr 6S sequences of different species shows that they belong to three phylogenetic individual groups, depending on their morphological and physiological characteristics [12].

Mutans streptococci play an important role in the development and progression of dental caries [13]. These bacteria are found in the carious teeth. Many studies have suggested that they also inhabit the deeper regions of the carious teeth and the surface of the teeth [14]. In contrast, lactobacilli colonize various parts of the oral cavity such as the oral mucosa, dorsum of the tongue, the saliva and the tooth surfaces, deep caries and caries in the dentine [15]. *S. mutans* and *S. sobrinus* are considered to be the main etiological agents for the appearance of dental caries in humans. It has been established that in pre-school children in whose saliva *S. mutans* and *S. sobrinus* are found, have a significantly higher incidence of dental caries than those with only the presence of *S. mutans* [16].

The aim of our study is to identify the salivary bacteria (*Lactobacillus* spp., *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus salivarius*, and *Streptococcus mitis*) and analyze their interdependence with the dental status.

2. Materials and Methods

The study included 71 children (26 female and 45 males) at the age of 12 years. We selected the 12-year-old age group based on the recommendations of the WHO, which recommends that age for global monitoring of dental caries, and refers only to children with permanent dentition [17].

According to the dental health status, the examinees were divided into. Control group - 31 examinees without caries, extractions and fillings (DMF = 0). Experimental group - 40 examinees with caries, extractions and fillings.

In all examinees clinical and microbiological examinations were carried out.

2.1. Risk assessment for caries of permanent dentition

According to the obtained data from the clinical examination, we determined the intensity (presence / absence) of dental caries (WHO, Geneve, 1997) and we interpreted them as follows [17]:
a) 0 - 0.9 - very low dental caries risk.
b) 1 - 2.4 - low dental caries risk.
c) 2.5 - 3.8 - moderate dental caries risk.
d) 3.9 - 5.5 - high dental caries risk.
e) ≥ 5.6 - very high dental caries risk.

2.2 Microbiological analyzes

2.2.1 Assessment of the lactobacilli in the saliva

The lactobacilli in the saliva were determined with a diagnostic test CRT-bacteria (Vivadent, Schaan, Lihtenstein). The system includes a paraffin tablet to stimulate the secretion of saliva, bacitracin to prevent the growth of other bacteria, other than lactobacilli, which bacitracin is added to the saliva for at least 15 minutes before use, a selective lactobacilli agar strip, a colony evaluation scheme (*Lactobacillus* (LB) number in mL saliva), a glass, and further an incubator in the laboratory. We gently wetted the gelatin test with saliva, on both sides, without touching the surfaces so we don't contaminate. Then we put the tester in a plastic tube that is well closed and then placed in an incubator at 37 ^{0}C for 4 days. After incubation of 4 days, the density of the *Lactobacillus* LB colonies (the number of lactobacilli per milliliter of saliva) was compared to the test strip with an assessment diagram. The obtained values from the test for *Lactobacillus* spp. were interpreted by following the recommendations of the manufacturer:
0: Very low consumption of cariogenic foods and < 103 (CFU)/mL (formed colonies with the number of *Lactobacillus* spp.).
1: Low consumption of fermented carbohydrates and cariogenic diet 104 CFU/mL (formed colonies with the number of *Lactobacillus* spp.).
2: Moderate consumption of fermented carbohydrates

and cariogenic diet 105 CFU/mL (formed colonies with the number of *Lactobacillus* spp.).
3: Consuming highly fermented carbohydrates and an inadequate diet > 106 CFU/mL (formed colonies with the number of *Lactobacillus* spp.)

2.2.2 Assessment of *S. mutans*, *S. sobrinus*, *S. salivarius* and *S. mitis* in the saliva

The saliva was applied to a sterile paper, numbered 50, which we held with tweezers with sterile beaks and placed under the tip of the child's tongue for 1 minute, with a purpose to be immersed in saliva. Each paper was then transferred with sterile tweezers into sterile Eppendorf tubes, which were transported with a transportable refrigerator which had a freezer which was able to freeze to -80 ^{0}C. None of the trips exceeded 3 - 4 hours. Undivided sputum samples with sterile swabs were planted on mitis salivarius agar (Fluka, a substrate with sucrose, glucose, triptan blue and crystal violet) which is recommended for the isolation of mixed cultures of streptococci, in particular: *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus salivarius*, *Enterococcus faecalis*, etc. The plates were then incubated in microaerophilic conditions (5 - 10% CO_2) for 48 hours at 37 ^{0}C. The characteristics of the colonies and standard microbiological techniques were used to identify isolates.

To confirm the "viridans" streptococci, we used: a catalase test (negative), an optochin test (negative), an esculent reaction (negative), and a Gram culture. Specific properties of the mitis salivarius agar are following: grown colonies of this medium appear blue because of the absorption of the color triptan blue. *S. salivarius* is characterized by large, paleosins, smooth colonies, with a diameter of 1 - 5 mm, which are similar to "chewing gum", due to the production of lavan from the sucrose. *S. mutans* colonies are elevated, convex, pale blue and granulated as "frosted glass". Sometimes the appearance of the colony are "bubbly", due to the synthesis of glucan from the sucrose. *S. mitis* forms small colonies. The suspected colonies were sub-cultured on a blood agar and finally identified with the Vitek 2 system, especially *S. salivarius*. DNA isolation of *S. mutans* and *S. sobrinus* from the saliva samples were made with a genomic charge switch forensic DNA purification kit (Invitrogen).

2.3 Quantification of PCR

Quantitative detection of *S. mutans*, *S. sobrinus*, *S. salivarius* and *S. mitis* was performed with gene-specific sample pairs (200 nmM each) and probes (250 nm) for *S. mutans* and *S. sobrinus*. 1X TaqMan universal PCR master mix (Applied Biosystems) was used and 5 µL of isolated bacterial DNA in a 20 µL reactive volume. The PCR conditions were 10 min at 95 ^{0}C for activation of the enzymes, followed by 45 two-step cycles (15 sec. at 95 ^{0}C, 1 min. at 58 ^{0}C). Detection is performed using the ABI PRISM 7900 Sequence Detection System. The standard curves for each bacterium is obtained from the amplification of genomic DNA from samples containing 1 x 100 - 1 x 10^7 CFU. Each sample is analyzed in three samples and the CT value of each sample is converted into an amount of *S. mutans*, *S. sobrinus*, *S. salivarius* and *S. mitis* using standard curves and measures in the same experiment. The studies were conducted at the Institute of Microbiology and Parasitology at the Medical Faculty, UKIM, Skopje, Macedonia.

2.4 Statistical assessment of the results

The analysis of the data is performed in the statistical programs Statistica 7.1 for Windows and SPSS Statistics 17.0. The significance is set at $p < 0.05$. The data is tabulated and graphically displayed.

3. Results and Discussion

3.1 Results

In the experimental group from a total of 40 (56.3%) children, 18 (25.4%) children had little consumption of fermented carbohydrates and caryogenic foods (< 104 (CFU)/mL (formed colonies with the number of *Lactobacillus* spp.), 16 (22.50%) children had moderate consumption (105 CFU/mL), and 6 (8.5%) children consumed highly fermented carbohydrates and had an inadequate diet (> 106 CFU/mL) (Table 1).

Table 1. Group/*Lactobacillus* spp.

Group	No, %	*Lactobacillus*			Total
		Little consumption of CH and a cariogenic diet	Moderate consumption of CH and a cariogenic diet	CH and an inadequate diet	
Experimental	Count	18	16	6	40
	% of Total	25,4	22,5	8,5	56,3
Control	Count	20	11	0	31
	% of Total	28,2	15,5	0	43,7
Total	Count	38	27	6	71
	% of Total	53,5	38	8,5	100

In the control group from a total of 31 (43.7%) children, 20 (28.2%) children had little consumption of fermented carbohydrates and caryogenic foods (< 104 (CFU)/mL (formed colonies with the number of *Lactobacillus* spp.) and 11 (15.5%) children had moderate consumption (105 CFU/mL). In the presented distribution of data related to the assessment of lactobacilli in the saliva in children with permanent teeth, for Fisher's exact test = 6.00 and $p < 0.05$ ($p = 0.04/0.036 - 0.047$) there is a significant difference between the two groups.

Data referring to the detection of *Streptococcus mutans* in the saliva in children with permanent teeth, showed that in the experimental group from a total of 40 (56.3%) children, in 28 (39.4%) children *S. mutans* is isolated from the saliva, and in 12 (16.9%) children *S. mutans* is not isolated from the saliva. In the control group of 31 (43.7%) children, 12 (16.9%) children had the bacteria *S. mutans* isolated from their saliva, and 19 (26.8%) children did not have the bacteria *S. mutans* isolated from their saliva. In the presented distribution of data referring to the detection of *S. mutans* in the saliva from children with permanent teeth, there is a significant difference between the two groups for Pearson chi square = 6.95 and $p < 0.01$ ($p = 0.008$) (Table 2).

Data related to the detection of *Streptococcus sobrinus* in the saliva in children with permanent teeth; from the experimental group of 40 (56.3%) children, 30 (42.3%) children had the bacteria *S. sobrinus* isolated from their saliva and 10 (14.1%) did not have the bacteria *S. sobrinus* isolated from their saliva. In the control group of 31 (43.7%) children, 12 (16.9%) children had the bacteria *S. sobrinus* isolated from their saliva and 19 (26.8%) children did not have the bacteria *S. sobrinus* isolated from their saliva. In the presented distribution of data related to the detection of *Streptococcus sobrinus* in the saliva in children with permanent teeth, there is a significant difference between the two groups for Pearson chi square = 9.53 and $p < 0.01$ ($p = 0.002$) (Table 3).

Data referring to the detection of *Streptococcus salivarius* in the saliva in children with permanent teeth, showed that in the experimental group from a total of 40 (56.30%) children, 35 (49.3%) children had the bacteria *S. salivarius* isolated in their saliva, and 5 (7%) children did not have the bacteria isolated in their saliva (Table 4). In the control group from a total of 31 (43.7%) children, 10 children (10.1%), had the bacteria *S. salivarius* isolated from their saliva and 21 (29.6%) children did not have the bacterial isolated from their

Table 2. Group/*Streptococcus mutans*

Group	No, %	*Streptococcus mutans*		Total
		Yes	No	
Experimental	Count	28	12	40
	% of Total	39,4	16,9	56,3
Control	Count	12	19	31
	% of Total	16,9	26,8	43,7
Total	Count	40	31	71
	% of Total	56,3	43,7	100

Table 3. Group/*Streptococcus sobrinus*

Group	No, %	*Streptococcus sobrinus*		Total
		Yes	No	
Experimental	Count	30	10	40
	% of Total	42,3	14,1	56,3
Control	Count	12	19	31
	% of Total	16,9	26,8	43,7
Total	Count	42	29	71
	% of Total	59,2	40,8	100

Table 4. Group/*Streptococcus salivarius*

Group	No, %	*Streptococcus salivarius*		Total
		Yes	No	
Experimental	Count	35	5	40
	% of Total	49,3	7	56,3
Control	Count	10	21	31
	% of Total	14,1	29,6	43,7
Total	Count	45	26	71
	% of Total	63,4	36,6	10

saliva. In the presented distribution of data related to the detection of *Streptococcus salivarius* in the saliva in children with permanent teeth, there is a significant difference between the two groups for Pearson chi square = 22.96 and p < 0.001 (p = 0.000).

The results of the detection of *Streptococcus mitis* in the saliva in children with permanent teeth showed that in the experimental group from a total of 40 (56,3%) children, 37 (52,9%) children had the bacteria *S. mitis* isolated from their saliva and 2 (2.9%) children did not have the bacteria *S. mitis* isolated from their saliva. In the control group from a total of 31 (43.7%) children, 8 (11.8%) children had the bacteria *S. mitis* isolated from their saliva and 23 (32.9%) children did not have the bacteria *S. mitis* isolated from their saliva (Table 5).

In the presented distribution of data related to the detection of *S. mitis* in the saliva in children with permanent teeth, there is a significant difference between the two groups for Pearson chi square = 16.14 and p < 0.001 (p = 0.000) (Table 5).

3.1.1 Experimental group - DMF

The descriptive statistics on the values of the DMF index in children with permanent dentition is shown in Table 6 and Figure 1. The values of the DMF index vary in the interval 2.35 ± 1.12; ± 95.00% KI: 1.99 - 2.71; where the minimum value is 1 and the maximum value is 5.

The descriptive statistics of the values of the DMF by surfaces in children with permanent dentition is shown in Table 7 and Figure 2. The values of the decay by surfaces vary in the range 0.98 ± 0.99; ± 95.00% KI:

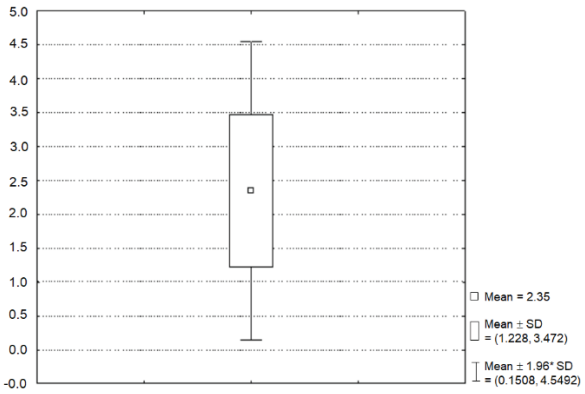

Figure 1. DMF index/Permanent dentition/ Descriptive statistics

0.66-1.29; the minimum value is 0 and the maximum value is 4. Filling values vary in the range 2.23 ± 1.62; ± 95.00% KI: 1.71-2.74; the minimum value is 0 and the maximum value is 7.

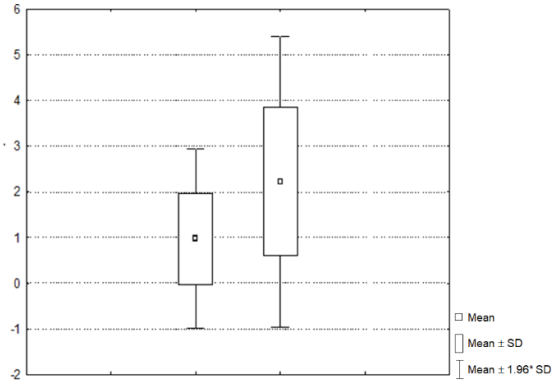

Figure 2. Decay and missing and filling (surfaces)/ Permanent dentition/Descriptive statistics

Table 5. Group/*Streptococcus mitis*

Group	No, %	*Streptococcus mitis*		Total
		Yes	No	
Experimental	Count	37	2	40
	% of Total	52.9	2.9	56.3
Control	Count	8	23	31
	% of Total	11.4	32.9	43.7
Total	Count	45	25	71
	% of Total	64.3	35.7	100

Table 6. DMF index/Descriptive statistics

Parameters	Valid N	Mean	Confidence -95%	Confidence + 95	Minimum	Maximum	Std. Dev.
DMF index	40	2.35	1.99	2.71	1	5	1.12

Table 7. Decay and missing and filling (surfaces)/descriptive statistics

Parameters	Valid N	Mean	Confidence -95,00%	Confidence +95,00	Minimum	Maximum	Std. Dev.
Decay	40	0.98	0.66	1.29	0	4	0.99
Filling	40	2.23	1.71	2.74	0	7	1.62

Data related to the intensity of dental caries in children with permanent dentition are shown in Table 8. In the experimental group of 40 (56.3%) children, 32 (45.1%) children had a low dental caries risk (1 - 2.4), 1 (1.40%) child had a moderate dental caries risk (2.5 - 3.8), and 7 (9.9%) children had a high dental caries risk (3.9 - 5.5). In the control group (without dental caries), all 31 (43.7%) children had a very low dental caries risk (0 - 0.9). The distribution of data related to the intensity of dental caries in children with permanent dentition, for Fisher's exact test = 85.30 and $p < 0.001$ ($p = 0.000/0.000 - 0.000$) there is a significant difference between the two groups.

3.1.2 DMF index/Cariogenic bacteria

The results related to the predictive values of the detected *S. mutans*, *S. sobrinus*, *S. salivarius*, *S. mitis* in the saliva from children with permanent teeth for the presence of dental caries (DMF index) are shown in Table 8. Between the detected bacteria in the saliva mentioned above, and the presence of dental caries for Pearson chi-square = 48.13 and $p < 0.001$ ($p = 0.000$) there is a significant correlation. In determining the significance of the contribution of each bacteria towards the presence of dental caries, it was found that the greatest influence had *S. mitis* (Wald = 11.36 / $p < 0.01$ ($p = 0.001$), then *S. salivarius* (Wald = 1, 76 / $p > 0.05$ ($p = 0.19$), while *S. mutans* (Wald = 0.00 / $p > 0.05$ ($p = 0.99$) and *S. sobrinus* (Wald = 0.00 / $p > 0.05$ ($p = 0.99$) did not have any predictive value for the presence of dental caries. Children in whose saliva *S. mitis* is detected in relation to children in whose sputum the bacteria *S. mitis* is not detected, have a 29.14 times (Exp (B) = 29.14) (95% CI: 4.10-207.00) significantly higher probability of having dental caries. Children in whose sputum *S. salivarius* is detected in relation to children whose saliva the bacteria *S. salivarius* is not detected have a 3.27 times (Exp (B) = 3.27) (95% CI: 0.57 - 18.82) insignificantly higher probability of having dental caries.

The results presented in Table 10, refer to the presence of *Lactobacillus* spp. in the saliva of children with permanent dentition and the presence of dental caries (DMF index).

Table 10. DMF index/*Lactobacillus* spp.

			DMF index		Total
	Consumption of CH and a cariogenic diet	No, %	Yes	No	
Lactobacillus	Little consumption	Count	18	20	38
		% of Total	25.4	28.2	53.5
	Moderate consumption	Count	16	11	27
		% of Total	22.5	15.5	38
	Excessive consumption and an inadequate diet	Count	6	0	6
		% of Total	8.5	0	8.5
	Total	Count	40	31	71
		% of Total	56.3	43.7	100

Of the 38 (53.5%) children which had little consumption of fermented carbohydrates and a cariogenic diet, 18 (25.4%) children had dental caries, and 20 (28.2%) children did not have dental caries. Of the 27 (38%) children which had moderate consumption of

Table 8. Group/DMF Index - intensity

Group	No, %	*Lactobacillus*				Total
		Very low	Low	Medium	High	
Experimental	Count	0	28	4	8	40
	% of Total	0	39.4	5.6	11.3	56.3
Control	Count	31	0	0	0	31
	% of Total	43.7%	0	0	0	43.7
Total	Count	31	32	1	7	71
	% of Total	43.7	45.1	1.4	9.9	100

Table 9. DMF Index/Cariogenic bacteria

Cariogenic bacteria								95% C.I.for EXP(B)	
		B	S.E.	Wald	df	Sig.	Exp(B)	Lower	Lower
Step 1[a]	*S. mutans* (1)	18.76	28,420.71	.000	1	.999	.000	.000	34.221
	S. sobrinus (1)	20.39	28,420.71	.000	1	.999	7.180	.000	18,263
	S. salivarius (1)	1.18	.89	1.76	1	.185	3.268	.57	18.82
	S. mitis (1)	3.37	1	11.36	1	.001	29.139	4.10	207
	Constant	**-3.75**	**.99**	**14.07**	**1**	**.000**	**.024**	**/**	**/**

Legend: a.Variable (s) entered on step 1: *S.mutans*, *S.sobrinus*, S.salivarius, *S.mitis*.

fermented carbohydrates and a cariogenic diet, 16 (22.5%) children had dental caries and 11 (15.5%) children did not have dental caries. A total of 6 (8.5%) children which had dental caries consumed highly fermented carbohydrates and had a cariogenic nutrition. In the presented distribution of data relating to the presence of *Lactobacillus* spp. in the saliva of children with permanent dentition and the presence of dental caries (DMF index) for Fisher's exact test = 6.00 and $p < 0.05$ ($p = 0.04/0.037 - 0.048$) there is a significant difference.

Results related to the predictive values of *Lactobacillus* spp. in the saliva of children with permanent dentition for the presence of dental caries (DMF index) are shown in Table 11.

Between the presence of *Lactobacillus* spp. in the saliva and the presence of dental caries for Pearson chi-square = 11.88 and $p < 0.01$ ($p = 0.003$) there is a significant correlation. In determining the significance of the contribution of each component to the presence of dental caries, it was found that a greater insignificant influence has the moderate consumption of fermented carbohydrates and a cariogenic diet (Wald = 0.89 / $p > 0.05$ ($p = 0.35$) and the consumption of highly fermented carbohydrates and a cariogenic diet (Wald = 0.00 / $p > 0.05$ ($p = 0.99$) / has an insignificantly predictive effect/ (Exp (B) = 1.996) (95% CI: 0.000). Little consumption of fermented carbohydrates and a cariogenic nutrition is taken as a reference category. Children who have a moderate consumption of fermented carbohydrates and a cariogenic nutrition in relation to children who have a low consumption of fermented carbohydrates and cariogenic food have a 1.62 times (Exp (B) = 1.62) (95% CI: 0, 60 - 4.38) greater chance to have dental caries, but it is not significant for $p > 0.05$ ($p = 0.35$).

3.2 Discussion

There is numerous information on the incidence and prevalence of dental caries, from which we can conclude that it is relatively higher in children from developing countries, in comparison to the children of the same age in developed countries, where in the last ten years a significant decline was observed, especially in children from more developed countries in Europe and the United States [18, 19]. The examination of the values of the DMF index in our examinees vary in the interval 2.35 ± 1.12; and for each component independently - the caries component varies in the interval 0.68 ± 0.69; the values for extracted teeth vary in the interval 0.25 ± 0.44 and for filled teeth they vary in the interval 1.43 ± 1.11.

Data about the intensity of dental caries in children with permanent dentition showed that from a total of 40 (56.3%) children in the experimental group, 32 (45.1%) children had low caries risk (1 - 2.4), 1 (1.4%) child had moderate caries risk (2.5 - 3.8) and 7 (9.9%) children had a high caries risk (3.9 - 5.5) and compared to the control group where examinees were without dental caries and with a very low caries risk, there is a significant difference between the two groups ($p < 0.001$). The results obtained by Nurelhuda *et al.*, [20], indicate a low prevalence of dental caries among 12-year-olds from Khartoum (24% of children) and emphasize the impact of the socio-economic status as an indicator of the risk to the oral health, then on the improvement of the diet and an increased awareness of the importance of oral hygiene which together improved the dental health in children, which is not the case with our examinees. Epidemiological studies of risk factors for dental caries mainly focus on the salivary levels of cariogenic bacteria as direct causes. It is proven that salivary levels of *mutans* streptococci and *Lactobacillus* spp. are related to the number of carious teeth (DMF). Most commonly referred bacteria in the MS group, which are responsible for the occurrence for dental caries are Streptococcus *mutans* and *S. sobrinus*, and their mechanisms of virulence are well known because they are found in the oral biofilm of the tooth surface. Key negative factors for mutant streptococci are acidosis, acid tolerance, and synthesis of insoluble glucose from sucrose. Lactobacilli colonize the surfaces of the teeth, and their number in the saliva reflects the consumption of carbohydrates by the host-tooth [21].

Ayna *et al.*, [22], proved that the formation of the biofilm by lactobacilli in a monoculture is poor, and some of them which are involved in the appearance of dental caries are very complex and variable and have not yet been fully identified, although key lactobacilli are usually associated with progression of the disease. In children, the presence of lactobacilli in the appearance of caries is unquestionable but are found in smaller number than *S. mutans* and not in the initial carious lesion. The presence of lactobacilli also depends

Table 11. DMF index/*Lactobacillus* spp.

Lactobacillus								**95% C.I. for EXP(B)**	
		B	**S.E.**	**Wald**	**df**	**Sig.**	**Exp(B)**	**Lower**	**B**
Step 1[a]	Moderate consumption of CH (1)	.48	,.51	.890	1	.346	1.616	.60	.48
	Excessive consumption of fermented CH	21.31	16408.71	.000	1	.999	1.795	.00	21.31
	Constant	**-.10**	**.325**	**.105**	**1**	**.746**	**.900**		

Legend: a.Variable(s) entered on step 1: *Lactobacillus* spp.

on the size of the cavity and are more numerous in medium and large cavities. The obtained results from our research on the estimation of lactobacilli in the saliva in children with permanent teeth from the experimental group of 40 (56,3%) children in 18 (25,4%) children we registered little consumption of fermented carbohydrates and caryogenic foods < 104 (CFU) / ml (formed colonies with *Lactobacillus* spp. number), moderate consumption of fermented carbohydrates and cariogenic foods (105 CFU/mL) was noted in 16 (22.5%) of the children and 6 (8.5%) children consumed highly fermented carbohydrates and had an inadequate diet (> 106 CFU/mL). In the control group from a total of 31 (43.7%) children, in 20 (28.2%) children there is a small consumption of fermented carbohydrates and cariogenic foods (<104 CFU/mL (formed colonies with *Lactobacillus* spp. number) and 11 (15.5%) children had a moderate consumption of fermented carbohydrates and cariogenic foods (105 CFU/mL). In the presented distribution of data related to the assessment of lactobacilli in the saliva in children with permanent teeth, there is a significant difference between the two groups ($p > 0.001$). The 2008 study by Gudkina, [23], carried out on children from 6 - 12 years from Riga indicates the absence of a positive correlation between dental caries and saliva levels of *S. mutans* and *Lactobacillus* spp. which is partly correlated with our results. Epidemiological studies of risk factors for dental caries mainly focus on the salivary levels of cariogenic bacteria as a sign that the salivary levels of *mutans* streptococci (MS) and *Lactobacillus* spp. (LB) are related to the number of DMFs [24]. Although there is a strong correlation between lactobacilli and caries, little is known about the ratio of the level and the lactobacilli species identified, because of the conventional methods used in studies, emphasizes Teanpaisan, [25]. If their adherence properties are known, then the determinant of lactobacilli cariogenicity is their capacity to produce acids and their ability to grow and survive in acidic environment. These bacteria have fermentable metabolism and according to the species there are two metabolic types, some species use only homo-milk fermentation and produce only lactic acid, and others use a hetero-milk fermentation, where the result is the production of lactic acid, CO_2, acetic acid or ethanol [26]. The number of bacteria in the saliva and the amount of plaque is directly related to the appearance of caries in each individual. Particularly strong indicators are the number of pathogenic *mutans* streptococci mobilized in the plaque, saliva and fissures of the occlusal surfaces of the teeth, which is a high risk for the onset of dental caries in patients. Apart from *mutans* streptococci and *Lactobacillus* spp. related to caries, *Actinomyces odontolyticus* is also mentioned, originally isolated from the carious lesions, and colonizes the babies before the eruption of their teeth [25]. Other significant strains of *Streptococcus* involved in the emergence of caries are *S. mitis* and a group of acidophilic streptococci that act only in "low pH" such as Bifidobacterium isolated from the white tooth spotting [27].

The data we got on the detection of *S. mutans* in the saliva in children with permanent teeth showed that from the experimental group of 40 (56,3%) children, in 28 (39,4%) children *S. mutans* was isolated from the saliva and in 12 (16.9%) children *S. mutans* was not isolated, and in the control group from 31 (43.7%) children, in 12 (16.9%) children *S. mutans* was isolated from the saliva and in 19 (26.8%) of the children *S. mutans* was not isolated from the saliva. In the presented distribution of data relating to the detection of *S. mutans* in the saliva in children with permanent teeth, there is a significant difference between the two groups ($p < 0.01$).

Data referring to the detection of *S. sobrinus* in the saliva in children with permanent teeth, show that in the experimental group from a total of 40 (56.3%) children, in 30 (42.3%) children *S. sobrinus* was isolated from the saliva and in 10 (14.1%) of the children *S. sobrinus* was not isolated from the saliva and in the control group of 31 (43.7%) children, in 12 (16.9%) children *S. sobrinus* was isolated from the saliva and in 19 (26. 8%) children it is not isolated from the saliva. The distribution of data on the detection of *S. sobrinus* in saliva in children with permanent teeth indicated that there was a significant difference between the two groups ($p < 0.01$).

Data referring to the detection of *S. salivarius* in the saliva in children with permanent teeth, show that in the experimental group from a total of 40 (56,3%) children, in 35 (49,3%) children *S. salivarius* was isolated from the saliva and in 5 (7%) of the children *S. salivarius* is not isolated from the saliva and in the control group from a total of 31 (43.7%) children, in 10 (14.1%) children *S. salivarius* is isolated from the saliva and in 21 (29.6%) children *S. salivarius* is not isolated from the saliva [47]. The detection of *S. salivarius* in the saliva in children with permanent teeth showed that there was a significant difference between the two groups ($p < 0.001$).

Data relating to the detection of *S. mitis* in the saliva in children with permanent teeth showed that in the experimental group, from a total of 40 (56.3%) children, in 37 (52.9%) children *S. mitis* was isolated from the saliva, and in 2 (2.9%) children *S. mitis* was not isolated from the saliva and in the control group, from a total of 31 (43.7%) children, in 8 (11,4%) children *S. mitis* was isolated from the saliva, and in 23 (32,9%) children *S. mitis* was not isolated from the saliva. The data related to the detection of *S. mitis* in the saliva in children with

permanent teeth indicated a significant difference between the two groups of examinees ($p < 0.001$).

When determining the significance of the contribution of each bacteria toward the presence of dental caries, it was found that the greatest influence had the bacteria *S. mitis* $p < 0.01$, then *S. salivarius* $p > 0.05$, while *S. mutans* $p > 0.05$, and *S. sobrinus* $p > 0.05$ have no predictive value for the presence of dental caries. Children in whose sputum *S. mitis* is detected in relation to children in whose sputum *S. mitis* has not been detected have a 29.14 times significantly higher probability of presence of dental caries. The children in whose saliva *S. salivarius* is detected in relation to children in whose saliva *S. salivarius* is not detected have a 3.27 times insignificantly greater probability of dental caries. Del Rio Gomez, [28], in children aged 12 - 14 noted the DMF and salivary mutant streptococci, and obtained a statistically significant difference in the presence of mutant streptococci between the city and rural specimens, and *S. sobrinus* was present in particular, which is in correlation to our results.

Mutans streptococci (*S. mutans* and *S. sobrinus*) are considered to be the major etiological factor for dental caries. Oda, [29], using the polymer chain reaction method made a comparison of the levels of those bacteria in 145 patients from 12 to 20 years and compared them to the onset of caries, and found that in children where *S. mutans* and *S. sobrinus* were isolated had a significantly higher incidence of dental caries than those with just *S. mutans*. The detection of *S. sobrinus* along with *S. mutans* showed a high association with caries, which is consistent with studies that associate this combination with the development of caries and a higher prevalence of cavities than when *S. mutans* is isolated alone. These bacteria have the ability to multiply and adhere to the smooth surfaces of the teeth and produce organic acids resulting in dental caries [30, 31]. An analysis conducted by the World Health Organization found that inaccurate data on caries distribution in many countries is displayed, which is significantly accented in 12 year olds in which there is higher or even very high DMF values. In the world, about 2.43 billion people (36% of the population) have dental caries on their permanent teeth. This disease is most common in Latin American countries, the Middle East and South Asia, and it is least reported in China, and the examinees which we included in the research unfortunately are not amnestied from this data. What we can say for determining caries risk is the possibility that in the period that follows, new caries can occur, but in unchanged conditions and factors that have been responsible for its occurrence, and it is of great importance that the results obtained can be used as guidelines for the need for additional diagnostic procedures, determining the time intervals for visiting the dentist and for the application of specific and appropriate preventive measures that will directly act on the etiological factors responsible for the emergence of dental caries.

4. Conclusions

- The realization of our work on the association of dental caries and salivary parameters can be used as serious screening factors and seriously participate as an instrument for caries risk assessment.
- Decades of research to evaluate and identify children at high risk of caries and to find the cause are of paramount importance, and it has been shown that multiple risk factors often act simultaneously on the onset of caries and instead of treating all patients equally. Traditional treatment and the application of preventive procedures for all patients equally and which certainly reduce the risk of caries in only a part of the population, however we believe that such empirical measures are not equally effective in patients at high risk of caries. Because of that fact our results offer data that can guide physicians to apply specific preventative measures to reduce the risk of caries.

5. References

[1] Loesche W. J. (1986). *Role of Streptococcus mutans in human dental decay*. Microbiol. Rev., 50, pp. 353-380.
[2] Geddes D. A. M. (1981). *Studies on metabolism of dental plaque: Diffusion and acid production in human dental plaque*. Front. Oral. Physiol., 3, pp. 78-87.
[3] Nyvad B., Fejerskov O. (1994). *Development, structure, and pH of dental plaque*. In: Thylstrup A., Fejerskov O. (Eds.), Textbook of clinical cariology, Munksgaard, Copenhagen, Denmark, pp. 89-110.
[4] Brandtzaeg P. (1989). *Salivary immunoglobulins*. In: Human saliva: Clinical chemistry and microbiology, (Vol. II), Tenovuo O. J. CRC Press, Boca Raton, USA, pp. 1-54.
[5] Dashper S. G., Reynolds E. C. (2000). *Effects of organic acid anions on growth, glycolysis and intracellular pH of oral streptococci*. Dent. Res., 79, pp. 90-96.
[6] Zickert I., Emilson C. G., Krasse B. (1982). *Streptococcus mutans, lactobacilli and dental health in 13-14-year-old Swedish children*. Community Dent. Oral Epidemiol., 10, pp. 77-81.
[7] Granath L., Cleaton-Jones P., Fatti L. P., Grossman E. S. (1994). *Salivary lactobacilli explain dental caries better than salivary mutants streptococci in 4-5-year-old children*. Scand J. Dent. Res., 102, (6), pp. 319-323.
[8] Borgstrom M. K., Sullivan A., Granath L., Nilsson G. (1997). *On the pH-lowering potential of lactobacilli and mutans streptococci from dental plaque related to the prevalence of caries*. Community Dent. Oral. Epidemiol., 25, (2), pp. 165-169.
[9] Badet M. C., Richard B., Dorignac G. (2001). *An in vitro study of the pH-lowering potential of salivary lactobacilli associated with dental caries*. J. Appl Microbiol., 90, (6), pp. 1015-1018.
[10] Svensater G., Larsson U. B., Greif E. C., Cvitkovitch D.

G., Hamilton I. R. (1997). *Acid tolerance response and survival by oral bacteria*. Oral Microbio.. Immunol., 12, (5), pp. 266-273.
[11] Coeuret V., Dubernet S. Isolation (2003). *Characterization and identification of Lactobacilli focusing mainly on cheeses and other dairy products*. Lait, 83, pp. 269-306.
[12] Collins M. D, Rodrigues U., Ash C., Aguirre M., Farrow E. A. J., Martinez-Murcia A., Phillips A., B. Williams M. A., Wallbanks S. (1991). *Phylogenetic analysis of the genus Lactobacillus and related lactic acid bacteria as determined by reverse transcriptase sequencing of 16S rRNA*. FEMS Microbiol. Lett., 77, pp. 5-12.
[13] Zickert I., Emilson C. G., Krasse B. (1982). *Streptococcus mutans, lactobacilli and dental health in 13-14-year-old Swedish children*. Community Dent. Oral Epidemiol., 10, pp. 77-81.
[14] Kohler B., Bjarnason S. (1987). *Mutans streptococci, lactobacilli and caries prevalence in 11- and 12-year-old Icelandic children*. Community Dent. Oral Epidemiol., 15, pp. 332-335.
[15] Crossner C. G. (1981). *Salivary lactobacillus counts in the prediction of caries activity*. Community Dent. Oral Epidemiol., 9, pp. 182-190.
[16] Marsh A., Feathe.rstone, A. S., McKee A. (1989). *Microbiological study of early caries of approximal surfaces in schoolchildren* J. of Dental Research, 68, 7, pp. 1151-1154.
[17] World Health Organization (1997). *Oral Health Surveys: Basic* methods (4th Ed.). WHO, Geneva, Switzerland.
[18] Marthaler T. M., O'Mullane D. M., Vrbic V. (1996). *The prevalence of dental caries in Europe 1990-1995*. Caries Res., 30, (4), pp. 237-255.
[19] Nowak A. J., Warren J. J. (2000). *Infant oral health and oral habits*. Pediatr. Clin. N. America, 47, pp. 1043-1066.
[20] Nurelhuda M., Tordis A. T., Wahab A., Faisal A. (2009). *Oral health status of 12-year-old school children in Khartoum state, the Sudan: A school-based survey*. BMC Oral Health, 9, (15), pp. 167-178.
[21] de Soet J. J., Weerheijm K. L., van Amerongen W. E., de Graaff J. A. (1995). *Comparison of the microbial flora in carious dentine of clinically detectable and undetectable occlusal lesions*. Caries Res., 29, (1), pp. 46-49.
[22] Ayna B., Celenk S., Atakul F., Sezgin B., Ozekinci T. (2003). *Evaluation of clinical and microbiological features of deep carious lesions in primary molars*. J. Dent. Child., 70, (1), pp. 15-18.
[23] Gudkina J., Brinkmane A. (2008). *Caries experience in relation to oral hygiene, salivary cariogenic microflora, buffer capacity and secretion rate in 6- year olds and 12 year olds in Riga*. Stomatologija, 10, pp. 76-80.
[24] Roeters F. J. M. Van Der Hoeven J. S, Burgersdijk R. C. W., Schaeken M. J. M. (1995). *Lactobacilli, mutans streptococci, and dental caries: A longitudinal study in 2-year-old children up to the age of 5 years*. Caries Res., 29, pp. 272-279.
[25] Teanpaisan R., Dahlen G. (2006). *Use of polymerase chain reaction techniques and sodium dodecyl sulfate-polyacrylamide gel electrophoresis for differentiation of oral Lactobacillus species*. Oral Microbiol. Immunol., 21, pp. 79-83.
[26] Borgstrom M. K., Sullivan A., Granath L., Nilsson G. (1997). *On the pH-lowering potential of lactobacilli and mutans streptococci from dental plaque related to the prevalence of caries*. Community Dent. Oral Epidemiol., 25, (2), pp. 165-169.
[27] Fejerskov O. (1997). *Concepts of dental caries and their consequences for understanding the disease*. Community Dent. Oral Epidemiol., 25, (1), pp. 5-12.
[28] Del Rio Gomez I. (1991). *Dental caries and mutans streptococci in selected groups of urban and native Indian schoolchildren in Mexico*. Community Dentistry and Oral Epidemiology, 19, 2, pp. 98-100.
[29] Oda Y., Hayashi F., Okada M. (2015). *Longitudinal study of dental caries incidence associated with Streptococcus mutans and Streptococcus sobrinus in patients*. BMC Oral Health, 2, 15, pp. 102.
[30] Gregory R. L, Kindle J. C, Hobbs L. C., Filler S. J., Malmstrom H. S. (1990). *Function of anti-streptococcus mutans antibodies: Inhibition of virulence factors and enzyme neutralization*. Oral Mirobial Immonol., 5, pp. 181-188.
[31] McGhee J. R., Michaler S. M. (1981). *Immunology of dental caries. Microbial aspects and local immunity*. Ann. Rev. Microbiol., 35, pp. 595-638.