

Detection of Bacteria in the Dental Plaque in Children with Down Syndrome

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Abstract

The purpose of this paper is to investigate the prevalence of dental caries and the types of streptococci present in the dental plaque of children with Dawn Syndrome.

The study was carried out at the Clinical Center for Pediatric and Preventive Dentistry of the University Dentistry Clinical Center of Kosovo in Prishtina and at the Department of Microbiology of the National Institute of Public Health of Kosovo in the period 2016/2017. Determination of dental status was done assessing diagnostic criteria for Decayed, Missing and Filled Teeth for permanent dentition, Oral Hygiene Index according to Greene-Vermillion and the Gingival Index according to Løe -Silness. Two groups are formed consisting of 30 patients in each group, the group with Down syndrome and the group with healthy children. Smear from the dental plaque was microbiologically analyzed using automatic VITEK 2 system and the colorimetric GP stripes.

In 50% of children involved in our research *St. myth* and *St. oral* were isolated, in 21.7% *St. parasanguinis* was isolated, and in 13.3% *St. salivarius*, in 3.3% *St. sanguinis*, in 3.3% *St. Infantarius* ssp *coli*, in 1.7% *St. pneumoniae* and *St. constellatus* ss. *Pharyngitis*. In the group suffering from Down syndrome the most frequent isolated bacteria was *St. mitis*, *St. oral* (43.3%), while at the control group *St. parasanguinis* (66.7%).

Oral health status in both groups was poor. Based on groups, in the group suffering from Down Syndrome the most frequent isolated bacteria was *St. mitis*, *St. oral*, while at the control group *St. parasanguinis*.

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Introduction

The Down Syndrome (DS), Trisomy 21 or Mongoloidism represents a chromosomal disorder as a result of genetic mutation, resulting in physical growth delay, intellectual impairment and speech disorders.^{1,2} In Kosovo there is no official statistical data regarding the persons suffering from DS. The affected community with DS in Kosovo in itself represents a specific and sensitive social category. According to the Association of Down Syndrome Kosova, the number of persons with DS in Kosovo is about

600.

Frequent oral abnormalities have been expressed in DS individuals like malformations of the small palate and maxilla, mouth breathing resulting in dry mouth, fissured tongue and lips, delayed tooth eruption, dental agenesis, low incidence of dental caries, high incidence of periodontal diseases, mucosal ulcers, candidiasis, and acute necrotizing ulcerative gingivitis, in contrast with healthy individuals.^{3,4} Patients with DS also have enlarged tongue, occlusal and soft tissues forces misbalance, open bite, impaired mastication and following difficulty in self-cleansing of teeth.^{5,6}

Dental caries represents a multifactorial diseases. A number of causal, environmental and hereditary factors influence the development of tooth decay, including nutrition habits, carbohydrate intake, oral hygiene, cariogenic microorganisms, inadequate fluoride intake,

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alveolar arch anomalies, tooth crowding and poor salivary function.⁷⁻¹¹

Low prevalence of dental caries is one of the most well-known phenomenon among DS patients, regardless of exposure to numerous risk factors, such as a caries inducing diet, impaired salivary flow, mouth breathing, deranged occlusal forces, and deprived oral hygiene. The exact mechanism of this observable fact remains unclear, regardless the reality that the etiology was verified in numerous studies. Some of the hypotheses suggested to explain the low prevalence of dental caries include the following: delayed tooth eruption in combination with an altered chronology of eruption; the high frequency of hypodontia; differences in the composition, pH, and buffering capacity of the saliva and the salivary flow and differences in the cariogenic microbiota.¹²⁻¹⁷

Variety of microorganisms consist of the number of present species (species opulence) and the number of individuals of all species (uniformity). Awareness regarding microbial variety is vital, given that a microbial population may change in terms of the number of individuals per species in response to changing conditions that favor their growth.¹⁸

Two most frequent bacterial species *Streptococcus mutans* and *Streptococcus sobrinus* are strongly related with dental caries. On the other hand, the relationship between oral streptococci and dental caries in children with DS is not well documented. Meanwhile, some studies have demonstrated that the incidence of dental caries is associated with *Streptococcus mutans* counts in children and adolescents with DS, other authors have not found such association.¹⁹⁻²²

The purpose of this paper is to investigate the prevalence of dental caries and the types of streptococci present in the dental plaque of children with DS and to compare the results with the healthy group, to evaluate the degree of oral hygiene and the gingival condition between children with DS and a group of healthy children.

Materials and methods

Subjects

The study was carried out at the Clinical Center for Pediatric and Preventive Dentistry of the University Dentistry Clinical Center of Kosovo in Pristina and the Department of Microbiology of

the National Institute of Public Health of Kosovo in the period 2016/2017. The study model is prospective, comparative type.

All children aged 7 to 15 years, who participated in the study were examined with dental instruments for examination using artificial light seated in dental chair.

Determination of dental status was done according to WHO criteria.²³ For the purpose of this paper, a special study questionnaire has been created, which contains:

- The general (demographic) data of the patient (name and surname, gender, age, child's residence and complete medical history);
- Dental Status
- Gingival Index (GI)
- Dental Plaque Index according to Green-Vermilion (0 - 3)

Microbiological status for the identification of bacterial species:

We have formed 2 groups consisting of 30 patients in each group, the group with Down syndrome and the group with healthy children.

In both groups, dental plaque samples were taken and their microbiological analysis was carried out using Vitek 2 (BioMérieux Industries, 69280 Marcy-l'Étoile - France), utilizing specific method for the identification of bacterial species.

Dental Status

Determination of dental status was done according to the WHO parameters for assessing diagnostic criteria for Decayed, Missing and Filled Teeth for permanent dentition.

Gingival index (GI) according to Löe – Silness²⁴ has been used to evaluate the gingival status (changes in color, size and bleeding). The evaluation is done using exploratory probe, after drying the dental surfaces with compressed air. All necessary instruments were sterilized before the examinations. The gingival index values are marked 0-3 where: 0 - healthy sound: the gingiva has a light pink color, with grain structure, the papillas are in the interdental space and no signs of protrusion. The shape of the papilla depends on the shape of the tooth. 1- Light inflammation. The gingival lining is slightly reddish, has slight edema and increased gingival exudate. The gingiva does not bleed in probe with dental instrument; 2- Average inflammation: the gingiva is reddish, it has expressed edema and increased gingival limbus, and there is bleeding after probe, and 3- severe inflammation: the

gingiva is very reddish and grown.

The *Oral Hygiene Index* (OHI) according to Greene-Vermillion²⁵ determines the presence of dental plaque with a value of 0-3. The evaluation is done using exploratory probe. Value 0- no dental plaque in the third gingival portion of the tooth crown, 1-dental plaque covers less than 1/3 of the tooth surface. It can not be seen with the eye but only when the probe is passed through the surface, leaving a small amount of plaque on the tip of the probe. Value 2- dental plaque has covered more than 1/3, but no more than 2/3 of the tooth surface. And finally, value 3 - a large amount of dental plaque that covers more than 2/3 of the tooth surface. The interdental space is filled with dental plaque.

The index includes only 6 representative teeth:

- the vestibular surfaces of the first maxillary molars of the right and left side (in the absence of them, the second molars or the second premolars were examined), while in the primary teeth dentition vestibular surfaces of the second maxillary molars were used for examination.
- the vestibular surfaces of the permanent right maxillary central incisor and the permanent left central mandibular incisor and the primary incisors in the case of primary dentition (in their absence, the central left maxillary incisor is examined and central right mandibular incisor),
- the lingual surfaces of the first mandibular molars or the second primary molars in the primary dentition.

Microbiological methods for the identification of bacterial species

In both groups smear from the dental plaque was removed using absorbent paper points (Patterson Brand®-1031 Mendota Heights Rd, Saint Paul, MN 55120, USA) and their microbiological analysis was done using the Vitek 2 specific method (BioMérieux Industries, 69280 Marcy-l'Étoile - France) for qualitative identification of bacterial species.

Gram positive cocci are detected using the automatic VITEK 2 system and the colorimetric GP stripes. After 6-8 hours of incubation, the reaction is read automatically. Samples were taken from the dental plaque first on the buccal surfaces of the molars of the lower jaw (primary or permanent dentition), then in the incisors of the upper jaw (primary or permanent

dentition) with sterile absorbent paper point and sent for microbiological analysis.

The samples taken from the dental plaque were first diluted with 0.9% saline and then placed on the Prepared Plates with blood agar. Plates with nourishing agar were placed in the incubator for 24^h at 37°C. After bacterial cultivation, the material from the nourishing agar plate is placed in special glass cups to create a bacterial suspension. The suspension is consisted of mixed material with 0.9% saline in the amount of 2-3 ml. The glass cup consisting the suspension is then connected through a special tube with the GP stripe. Through the tube the material from the cup is transferred to the GP stripe and then the stripe kit is placed on the Vitek 2 apparatus for the identification of bacterial strains.

Inclusion and Exclusion Criteria

In this prospective clinical research, patients with DS are included in the study group, but not with other health problems, chronic or acute diseases present. Patients in the control group - healthy subjects are also without chronic or acute illnesses.

The study did not include patients with acute health problems of the cardiovascular system, acute and chronic pulmonary disease, gastrointestinal system disorders, metabolic disease, kidney failure, blood discrepancy, and those who did not sign the patient consent form. All subjects voluntarily participated in this study and filled out the informed consent form. The study was approved by the Research Ethics Committee of the University of Prishtina.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Science SPSS 22.0. Regarding the included statistical parameters the arithmetic average values, the standard deviation (SD), and the minimum and the maximum obtained values are calculated. In the statistical analysis differences between normally distributed continuous variables were tested with the Student t-test and differences between categorical variables with the chi-square test (χ^2). For the continuous data, Fisher's exact test were applied. Level of statistical significance was set to $p < 0.05$.

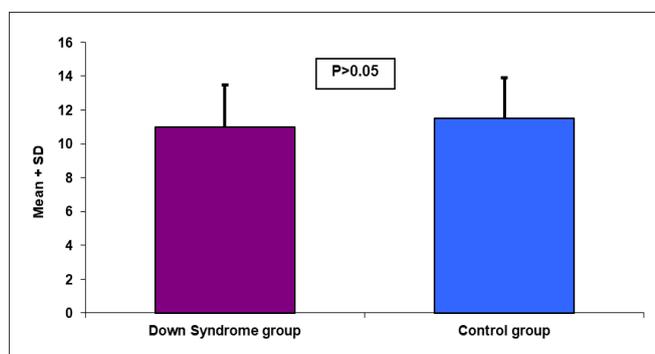
Results

The study included 60 children, of whom 34 (56.7%) were boys and 26 (43.3%) girls. From the total number of children involved in the study, 30 were children suffering from DS and 30 were healthy children as a control group. By gender, the group with DS had more boys (66.7%) compared to the control group where more than 53.3% were girls but without any important statistical significance ($P = 0.193$), (Table 1).

Gender	Down Synd. group		Control group		Total	
	N	%	N	%	N	%
Male	20	66.7	14	46.7	34	56.7
Female	10	33.3	16	53.3	26	43.3
Total	30	100.0	30	100.0	60	100.0
χ^2 -test, P-value $\chi^2=1.69$, $P=0.193$						

Table 1. Included children based on gender and groups.

The children included in our research were between ages 7 to 15 years. Those with DS were between 7 and 15 years old, while in the control group children ranged from 8 to 15 years (Table 2). The average age of children involved in the research was 11.2 years ($DS \pm 2.4$ years). The average age of children suffering from DS was 11.0 years ($DS \pm 2.5$ years), while those of the control group was 11.5 years ($DS \pm 2.4$ years). T-test did not demonstrate significant statistical significance between mean age among groups (T-test = 0.852, $P = 0.397$), (Graph 1).

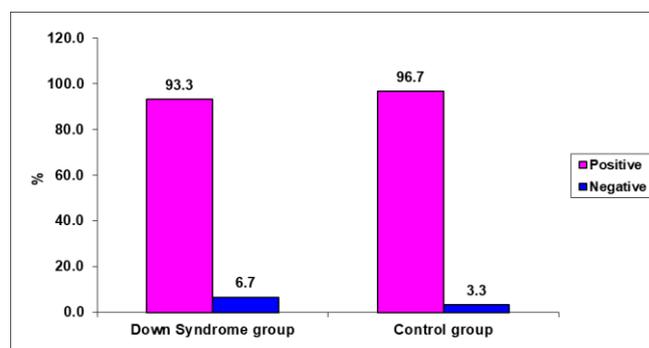


Graph 1. Average age of children included in the study based on group.

Microbiological analysis findings in 93.3% of cases was positive for the DS group, while in 96.7% of positive cases in the control group, but without significant difference ($P = 0.999$), (Table 2 and Graph 2).

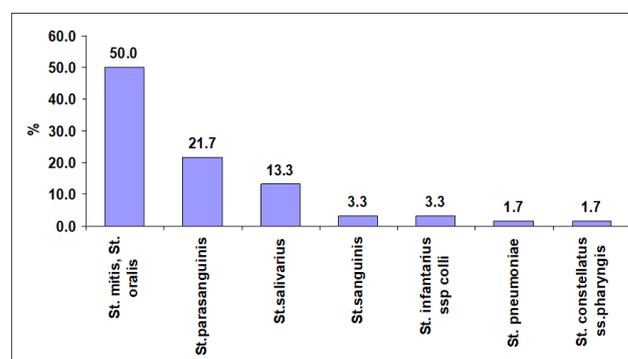
Microbiologic Analysis	Down syndrome group		Control group		Total	
	N	%	N	%	N	%
Positive	28	93.3	29	96.7	57	95.0
Negative	2	6.7	1	3.3	3	5.0
Total	30	100.0	30	100.0	60	100.0
Fischer test, P-value $P=0.999$						

Table 2. Microbiologic test results based on groups



Graph 2. Microbiologic analysis results of the subjects based on the group

In 50% of children involved in our research *St. myth* and *St. oral* were isolated, in 21.7% *St. parasanguinis* was isolated, and in 13.3% *St. salivarius*, in 3.3% *St. sanguinis*, in 3.3% *St. Infantarius ssp coli*, in 1.7% *St. pneumoniae* and *St. constellatus ss. Pharyngitis* (Graph 3).

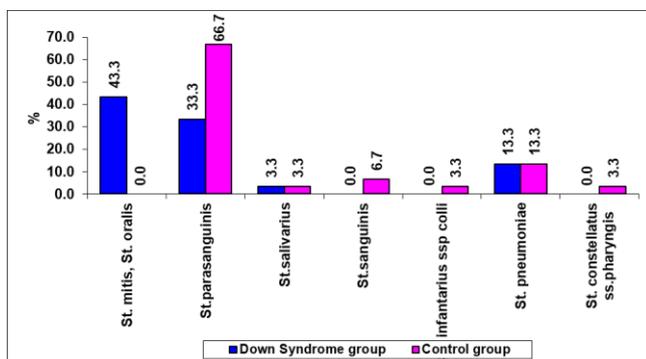


Graph 3. Microorganisms ranking isolated in all study subjects .

Based on groups, in the group suffering from DS the most frequent isolated bacteria was *St. mitis*, *St. oralis* (43.3%), while at the control group *St. parasanguinis* (66.7%), (Table 3 and Graph 4).

Microbiologic analysis	Down syndrome group		Control group		Total	
	N	%	N	%	N	%
<i>St. parasanguinis</i>	13	43.3	-	-	13	21.7
<i>St. mitis</i> , <i>St. oralis</i>	10	33.3	20	66.7	30	50.0
<i>St. sanguinis</i>	1	3.3	1	3.3	2	3.3
<i>St. infantarius</i> <i>ssp colli</i>	-	-	2	6.7	2	3.3
<i>St. pneumoniae</i>	-	-	1	3.3	1	1.7
<i>St. salivarius</i>	4	13.3	4	13.3	8	13.3
<i>St. constellatus</i> <i>ss. pharyngis</i>	-	-	1	3.3	1	1.7
None	2	6.7	1	3.3	3	5.0
Total	30	100.0	30	100.0	60	100.0

Table 3. Types of isolated bacteria between children's groups.



Graph 4. Isolated Microorganisms Structure based on groups.

Discussion

Microbiological analysis findings of our cases was positive for the DS and control healthy group, but without significant difference ($P = 0.999$),

The results of our study are in contradiction with the results of the other two studies²⁶⁻²⁸ from the literature that report DS children present a lower salivary density of *S. mutans* and a lower dental caries experience than non-Down controls.

Scalioni F et al.²⁶ in their representative study aimed to assess the salivary densities of *S.*

mutans, *S. sobrinus*, and streptococci and dental caries experience, in a group of DS children and adolescents. Authors found that DS children and adolescents present a lower dental caries experience and a lower salivary density of *S. mutans* than non-Down controls.

Meanwhile, in another systematic review and meta-analysis carried out by Deps et al.²⁷ demonstrated that based on scientific evidence, individuals with DS have fewer dental caries than individuals without DS.²⁷

In our study, we analyzed the types of streptococci in the dental plaque and find out that the most often detected bacteria was *St. Myths*, *St oralis* (43.3%) in the Down syndrome group whereas *St. parasanguinis* (66.7%) in the healthy control group.

In two independent studies Shapira J et al.⁶ and Stabholz A et al.⁹ showed that *Streptococcus mutans* counts, expressed as number of colony-forming units on mitis salivarius agar plates among the Down syndrome group, were the lowest, although not statistically significant compared with the counts of the healthy children.^{6,9}

Martinez - Martinez RE et al.²⁹ analyzed the periodontal biofilm in DS patients, with and without periodontitis, *Tannerella forsythia* was the most frequent bacteria detected in the group with and without periodontitis (95.5 and 63.3%) followed by *Treponema denticola* (88.8 and 50%) and *Porphyromonas gingivalis* (53.3 and 25% respectively). There were statistical differences between groups ($p < 0.05$).²⁹

In a five-year longitudinal study of dental caries risk related to *Streptococcus mutans* and *Streptococcus sobrinus* in subjects with intellectual disabilities Oda Y et al.³⁰ calculated the proportion of each of these strains to total bacteria, and compared dental caries incidence over 5 years, the proportion of *S. mutans* to total bacteria was moderately correlated with DMFT in year 2, Δ DMFT in years 2 and 5, and Δ SNAC in years 2 and 5 (correlation coefficient = 0.470, $P < 0.001$), while the proportion of *S. sobrinus* to total bacteria was moderately correlated with DMFT in years 2 and 5, Δ DMFT in years 1, 2, and 5, and Δ SNAC in years 2 and 5 (correlation coefficient = 0.695, $P < 0.001$). Individuals with ID who harbored both bacterial strains had a higher risk of dental caries and a significantly higher proportion of *S. sobrinus* to total bacteria.³⁰

Study carried out by Kishi et al.³¹ examining 54 mother-and-child pairs, they collected saliva samples from the mothers and the plaque samples from the children, to assess the relationships of quantitative salivary levels of *Streptococcus mutans* and *S. sobrinus* in mothers with the colonization of *mutans streptococci* (MS) in plaque and caries status in their 2.5-year-old children. Results from this study showed that the maternal salivary levels of *S. mutans* and *S. sobrinus* determined by real-time PCR were significantly related to MS colonization in plaque as well as dental caries in their children at 2.5 years of age.³¹

The relationship between oral streptococci and dental caries in children with SD is not well elaborated.²⁶

In the current literature, there have been many researches on oral health and prevalence of caries in children with DS, particularly in comparison with children suffering from various disabilities (mental retardation, cerebral palsy, musculoskeletal disorders, blindness, deafness, ect).

In the research, carried out by Shyama M. et al.³², in a study population comprised 832 disabled children and young adults (3-29 years; mean age 12.1 years), the mean DMFT was 5.4, and DMFS 15.2, being highest in the Down's syndrome and lowest in the blind. The proportion of caries-free subjects in permanent dentition, over 5 years of age was 24.2%. The smallest percentage of caries-free subjects was found in the hearing impaired (16.4%) and highest percentage in the blind (35.5%). The mean DMFT was 4.5 and the DMFS 8.7, being highest in the Down's syndrome and lowest in the blind.³²

In a cross-sectional study that included 33 DS individuals were included, aged 19 - 45 years, from Sarajevo and Tuzla Canton, Bosnia and Herzegovina conducted by Porović et al.³³ showed that the mean DMFT index is $15,96 \pm 8,08$; the analysis of oral hygiene of Down syndrome children by using the debris index, is found that 42,4% have very good oral hygiene, 21,2% respondents have good oral hygiene, 27,3% are with poor oral hygiene, while the very poor hygiene have 9,1% subjects, whereas CPI index value was 0,82.³³

This study had several strengths, e.g. it is a controlled design. According to subjects' characteristics at baseline, obtained results corresponded to those of similar studies.

A few limitations should be noted. First, this study utilized a small sample, did not provide power calculations and may have been underpowered. Second, we did not investigate the correlation between microbiological analyzes with other indices (oral hygiene index, gingival index).

Conclusions

Our results show that oral health condition was poor in both groups. Based on groups, in the group suffering from Down Syndrome the most frequent isolated bacteria was *St. mitis*, *St. oral*, while at the control group *St. parasanguinis*. Follow-up examinations as well as preventive approaches should be utilized for such individuals.

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Conflicts of interest

The authors declare no conflict of interest.

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