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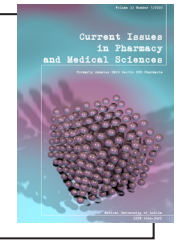

Antimicrobial efficacy of Colgate Plax Cool Mint® mouthwash – *in vivo* studies

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ABSTRACT

Hygienic procedures in oral cavity, both teeth brushing and using mouthwashes, have an influence on the composition and quantity of oral microflora. The aim of the work was to evaluate the impact of regular use of selected mouthwash on the titer of *Lactobacillus* and *Streptococcus* bacteria and *Candida* yeast-like fungi in the saliva.

The evaluation of the influence of Colgate Plax Cool Mint® mouthwash on the number of *Lactobacillus* and *Streptococcus* bacteria and *Candida* yeast-like fungi in the saliva was done. The research included 39 persons at the age of 17±3 months, out of whom 30 people constituted a study group using mouthwash and 9 people – a control group not using mouthwash. The persons from the study group used the mouthwash twice a day for 2-3 minutes for the period of 5 months. The material for the microbiological tests constituted 2 ml of saliva collected three times from the participants: at the beginning of the research – before the use of a mouthwash and respectively, after 2.5 and 5 months from the first test.

Colgate Plax Cool Mint®, the most frequently used by the youth in their daily oral hygiene and used in the present study mouthwash, showed effectiveness in reduction of *Lactobacillus* spp. and *S. mutans* bacteria, as well as *C. albicans* yeasts in the saliva of the studied persons, what is undoubtedly connected with inhibition of formation and prevention of oral plaque development.

INTRODUCTION

Human oral cavity is a favorable habitat for the microorganisms. An access to nutritional products, water, and air, provides optimal conditions for their development and is responsible for great diversification of microbiome. Directly after a baby is born, its oral cavity is aseptic, while the source of colonizing microorganisms for a newborn child is mainly mother (vertical transmission), which is proved by the presence of genotypically the same *Streptococcus mutans* strains in oral cavity of newborns and their mothers. The first microorganisms that settle oral cavity include *Streptococcus salivarius*, *Streptococcus mitis*, *Streptococcus oralis*, and during a few following months there appear *Fusobacterium nucleatum*, *Prevotella melaninogenica* and *Veillonella* spp, Gram-negative anaerobes. In young adults microbiome of oral cavity becomes very stable. It consists of the following bacteria genera: *Streptococcus*, *Veillonella*, *Fusobacterium*, *Porphyromonas*, *Prevotella*, *Treponema*, *Neisseria*, *Haemophilus*, *Eubacteria*, *Lactobacterium*, *Capnocytophaga*, *Eikenella*, *Leptotrichia*, *Peptostreptococcus* and *Propionibacterium*. Human oral cavity is inhabited by over 750 known bacterial species, while it is assumed that it constitutes only 50% of all the bacteria existing in this habitat. Great diversity of oral microorganisms is conditioned by temperature, pH, oxidation-reduction potential, salinity and saliva functioning. The quantity and content of microbiome is also largely dependent on oral hygiene, both teeth brushing and using of mouthwashes [1,2].

Dental plaque as biofilm is created by the microorganisms, cooperating with each other in the processes of organic substances decomposition, of metabolites production and generation of energy. There are two kinds of biofilm – supragingival and subgingival, differing in the composition of microorganisms. In the supragingival plaque there are mainly Gram-positive bacteria, such as: *S. mutans*, *S. salivarus*, *Streptococcus motis* and *Lactobacillus* spp.,

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while in the subgingival – Gram-negative bacteria, e.g.: *Actinobacillus* spp., *Campylobacter* spp., *Fusobacterium nucleatum* and *Porphyromonas gingivalis*. In the biofilm there are also archaea of the phylum *Euryarchaeota*, especially *Methanobrevibacter oralis* and *Methanobrevibacter smithii* [1].

Apart from the bacteria of dental plaque, on the surfaces of teeth hard tissues in human oral cavity, bacteria are also present on the surfaces of mucous membrane. Particularly favorable environment for microorganisms maturation is mucosa of the dorsal surface of the tongue. It is mainly inhabited by anaerobic species and microorganisms isolated from teeth surfaces, *Prevotella*, *Neisseria*, *Streptococcus*, *Heamophilus* and *Fusobacterium* bacteria [1-3].

AIM

The aim of the work was assessment of the influence of regular use of selected mouthwash on the titer of *Lactobacillus* and *Streptococcus* bacteria and *Candida* yeast-like fungi in the saliva of persons using a mouthwash and those who do not use it.

MATERIAL AND METHODS

1. Analysis of the *Lactobacillus* spp., *Streptococcus* spp. bacteria and *Candida* yeast-like fungi present in human saliva

The studies concerned the influence of Colgate Plax Cool Mint® mouthwash on the number of *Lactobacillus* and *Streptococcus* bacteria and *Candida* yeast-like fungi in the saliva of the persons aged 17 years old. The choice of the mouthwash was made on the basis of the results of an anonymous survey conducted by the authors, the analysis of which is subject of a separate work [4]. It was found out that Colgate Plax Cool Mint® mouthwash is the most frequently used by 17-year-olds. The research included 39 persons at the age of 17±3 months, out of whom there were 30 people in a study group and 9 people in a control group. Each study participant and his guardian gave a written consent for taking part in the study, which was voluntary.

All the persons included in the research, after the training concerning the rules of maintaining proper oral hygiene, were given an appropriate amount of the same toothpaste (Colgate-Palmolive). It was done in order to eliminate an influence of various toothpastes on the studied microbiological parameters. Participants from the study group were also given the right amount of Colgate Plax Cool Mint® mouthwash, which was used by them twice a day for 2-3 minutes for the period of 5 months. The persons from the control group were recruited among the youth not using mouthwashes.

Colgate Plax Cool Mint® (Colgate-Palmolive) mouthwash is widely available in pharmacies, drug stores and supermarkets. It consists of: water, glycerine, sorbitol, cetylpyridinium chloride, sodium fluoride in a concentration of 0.05% (225 ppm F), menthol, propylene glycol, poloxamer 407, potassium sorbate, saccharine, patent blue (Cl 42051).

The material for microbiological analyses was saliva in the amount of 2 ml collected three times from the study participants – at the beginning of the research before the use of a mouthwash (test 1), after 2.5 months (test II) and after 5 months (test III) from the first test. In the studied material the titer of *Lactobacillus* spp., *S. mutans* bacteria and *C. albicans* yeasts was evaluated. The research began in September 2016 and finished in January 2017. There were certain inclusion criteria for the study participants (lack of systemic diseases, general good health condition, lack of braces, lack of acute periodontal conditions and inflammations of the oral mucosa, not taking antibiotics during the time of research and 4 weeks before, and state after oral sanation) and exclusion criteria (teeth crowding, acute periodontal conditions and inflammations of the oral mucosa, allergies, metabolic diseases, drug addiction, and pregnancy).

The research project had received the approval of the Bioethics Committee at the Medical University of Lublin (resolution no. KE-0254/249/2016).

2. Media for cultivation and identification of microorganisms in saliva

For cultivation and identification of microorganisms the following media were used:

- Rogosa Agar medium (Oxoid, Great Britain) – for cultivation and identification of *Lactobacillus* spp inhabiting saliva of the study participants;
  Formula (g/l):
  - agar – 20.00
  - Tryptone – 10.00
  - Tween 80 – 1.00 ml
  - ammonium citrate – 2.00
  - potassium dihydrogen phosphate – 6.00
  - yeast extract – 5.00
  - sodium acetate anhydrous – 17.00
  - glucose – 20.00
  - manganese sulphate – 0.12
  - ferrous sulphate (II) – 0.034
  - magnesium sulphate – 0.575

  pH = 5.4±0.2.
- Mitis Salivarius Agar (Fluka Analytical, Switzerland) – medium for cultivation and identification of *S. mutans* bacteria inhabiting saliva of the study participants;
  Formula (g/l):
  - agar – 15.00
  - peptic digest of animal tissue – 5.00
  - casein enzymic hydrolysate – 15.00
  - sucrose – 50.00
  - trypan blue – 0.075
  - dextrose – 1.00
  - dipotassium phosphate – 4.00
  - crystal violet – 0.0008

  pH = 7.0±0.2.
- Chromagar Candida™ (Biomaxima S.A., Poland) – ready selective-multiplying-differential medium used for cultivation and identification of *Candida* inhabiting saliva of the study participants. Yeasts synthetize specific for them enzymes which cause decomposition of particular medium ingredients and in effect they caused different colors of the isolated yeasts.

3. The following materials were also used:

- saline solution (0.85% NaCl) – used for diluting saliva
- Genbag anaer, Genbox anaer (bioMerieux, France)
  They are microbiological generators used to create anaerobic environment. They are produced in the form of bags filled with various chemical compounds. After putting them into the airtight containers, anaerobic atmosphere is generated, to culture microorganisms requiring anaerobic environment.
- Anaer indicator (Sigma-Aldrich, USA)
  Anaer indicator is used to evaluate whether in the environment, in which microorganisms are present, anaerobic atmosphere exists. It is a pink strip with redox indicator solution. The information whether anaerobic conditions are achieved is given by change of color of the strip to white.

4. Saliva collection

From each participant, 2 ml of resting saliva was collected to disposable sterile plastic tubes. The material was collected in the morning, about two hours after the first meal and brushing teeth.

5. Quantitative analysis of chosen bacteria and yeasts in saliva

1. *Lactobacillus* spp. – quantitative analysis in saliva

In the sterile saline solution (0.85% NaCl) the collected saliva was diluted in the range 10^{-1}-10^{-3}. From the diluted material 100 µl was plated on the Rogosa medium (selectively-multiplying medium for *Lactobacillus* spp.). *Lactobacillus* bacteria required anaerobic environment to grow. For this purpose the generators of anaerobic environment were used (Genbag anaer, Genbox anaer), which were put into the special containers together with the plates containing medium with spread saliva. Plates were incubated for 72 hours at 37°C. Next, the *Lactobacillus* spp. colonies were counted and their number was expressed as a colony forming units per milliliter of saliva (CFU/ml), taking into account dilution of saliva.

In the first stage, the morphology of bacterial colonies was assessed, initially – macroscopically and later microscopically. The colonies had either irregular or oval shapes, dull or glistening surface, and were flat or convex. In the macroscopic test, the color of bacterial colonies was determined, which was mainly cream or white. The shape of the *Lactobacillus* spp. cells was assessed microscopically and they mostly had shape of thin straight bacilli. The curved and thick bacilli were also found, however significantly less.

2. *S. mutans* – quantitative analysis in saliva

In the sterile 0.85% saline solution the collected saliva was diluted in the range of 10^{1}-10^{-3}. From the diluted material 100 µl was collected and plated with a glass cell spreader on the Mitis Salivarius Agar medium. Such medium is used for growing *S. mutans* bacteria. The seeded media were incubated at 37°C for three days under anaerobic conditions, which were ensured thanks to Genbag anaer and Genbox anaer generators. *S. mutans* colonies were counted and their number of viable bacterial cells was estimated as a colony forming units per milliliter of saliva (CFU/ml), taking into account dilution of saliva. In macroscopic observation it was found that bacterial colonies are flat or convex, circular or irregular shape and had dull surfaces. The color of the bacterial colonies was blue, as a result of absorption of trypan blue (ingredient of Mitis Salivarius Agar medium). In the Gram stain test bacteria viewed under light microscope, there were Gram-positive, round arranged in chains of varying length.

3. *Candida* spp. – quantitative and qualitative analysis in saliva

Undiluted saliva (100 µl) was thoroughly spread on the whole medium with a glass cell spreader. The material was incubated in aerobic environment for two days at 30°C. Next, the grown yeasts colonies were counted as a colony forming units per milliliter of saliva (CFU/ml). In macroscopic test, the yeasts colonies were evaluated taking into account their morphology, color and shape. In most cases yeast colonies were green and had smooth edges. Other colonies were purple, red and brown – yeasts other than *C. albicans*.

6. Statistical analysis

Two statistical tests were used: Friedman's rank test for investigating changes in the number of microorganisms with time and Mann-Whitney U test for comparing differences between the study group and control one. Means and medians were also used to depict trends and differences in the number of microorganisms. The program that was used: Statistica 13 [5].

RESULTS

1. The evaluation of the influence of Colgate Plax Cool Mint® mouthwash on the titer of *Lactobacillus* spp. in saliva

Decrease in the number of *Lactobacillus* spp. bacteria in the saliva of persons from the study group (using mouthwash) at the beginning of an experiment (0 time) and after 2.5 and 5 months were noted (p=0.0000), while in the control group not using mouthwash the number of bacteria increased with time, however it was statistically insignificant (p=0.49659) (Fig. 1). Statistically significant differences were found between both groups in the third test (5 months

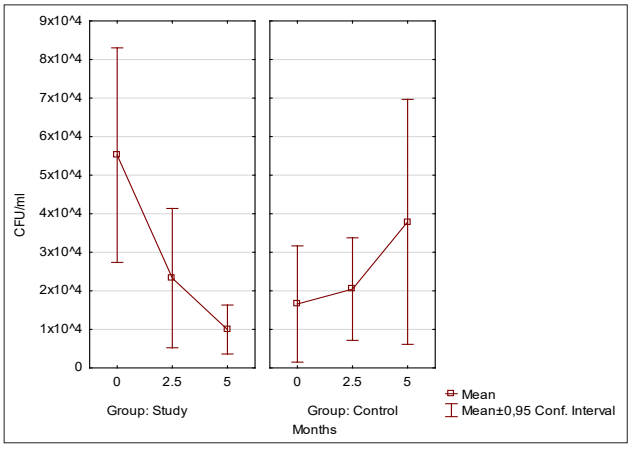

Figure 1. *Lactobacillus* spp. titer (CFU/ml) in saliva of persons from the study and control groups at the beginning and after 2.5 and 5 months of experiment

from the beginning of experiment), i.e. p=0.002206, while in the tests I (beginning of experiment) and II (2.5 months from the beginning of experiment) no differences concerning bacterial colony number were observed.

In the conducted tests concerning the influence of Colgate Plax Cool Mint® mouthwash on the number of *Lactobacillus* spp. in saliva, certain increasing tendency in the control group (Fig. 1, 2, 4) was found, while in the study group significant decrease (Fig. 1, 3, 4) could be noted. Therefore, effectiveness of the assessed Colgate Plax Cool Mint® mouthwash in reduction of the number of *Lactobacillus* spp. bacteria in saliva was proved. Thus, it can be assumed that if the studies were conducted for a longer period of time, the obtained results would show higher decrease in *Lactobacillus* spp. number in persons belonging to the study group using mouthwash.

2. The evaluation of the influence of Colgate Plax Cool Mint® mouthwash on the count of *S. mutans* bacteria in saliva

In the case of evaluation of the influence of the mouthwash on *S. mutans* number in saliva, slightly higher values of these bacteria strains were noted in the study group than in the control one (Fig. 5, 6, 7, 8), however this tendency was statistically insignificant. Significant differences concerning decrease in bacteria number in the consecutive periods in the saliva of persons from the study group (p=0.00001) were found. Slight decrease in bacteria number in saliva of persons from the control group was also found although it was not statistically significant (p=0.06081).

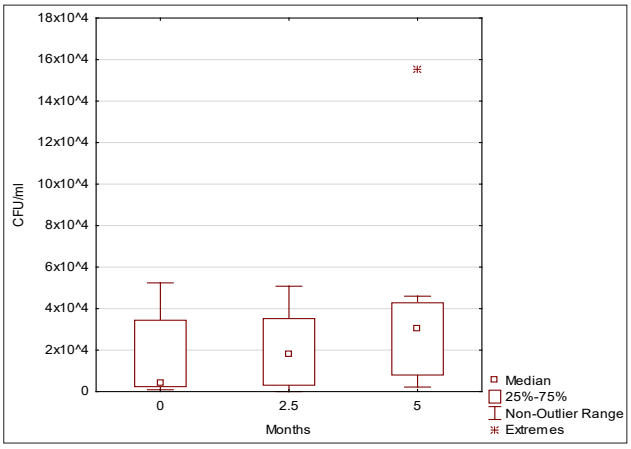

Figure 2. *Lactobacillus* spp. titer (CFU/ml) in saliva of persons from the control group at the beginning and after 2.5 and 5 months of experiment

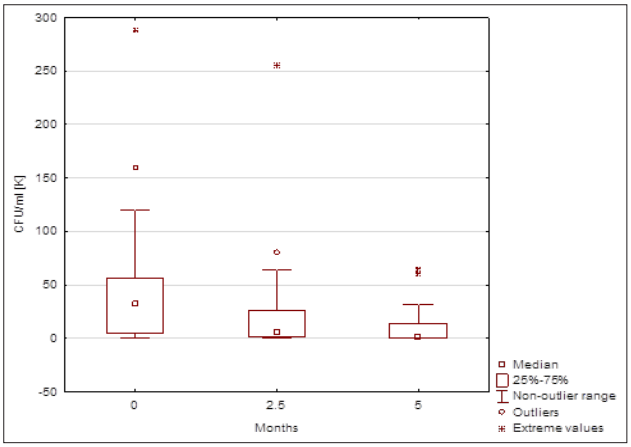

Figure 3. *Lactobacillus* spp. titer (CFU/ml) in saliva of persons from the study group at the beginning and after 2.5 and 5 months of experiment

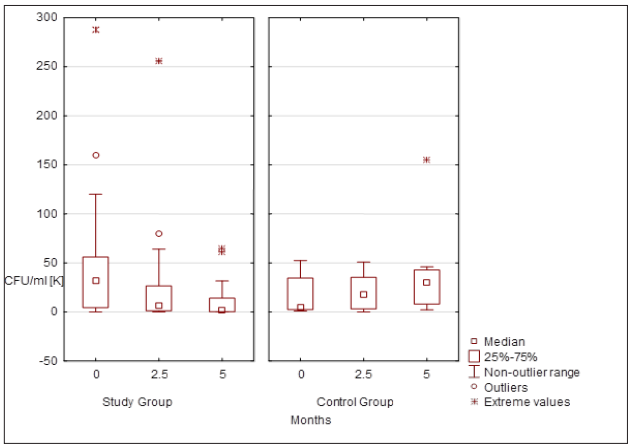

Figure 4. *Lactobacillus* spp. titer (CFU/ml) in saliva of persons from the study and control groups at the beginning and after 2.5 and 5 months of experiment

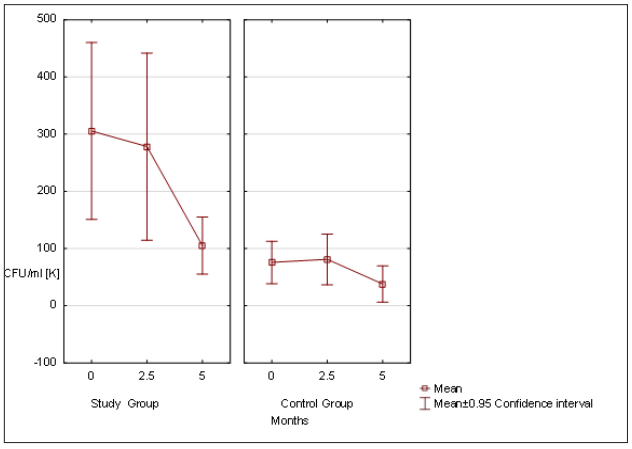

Figure 5. *S. mutans* titer (CFU/ml) in saliva of persons from the study and control groups at the beginning and after 2.5 and 5 months of experiment

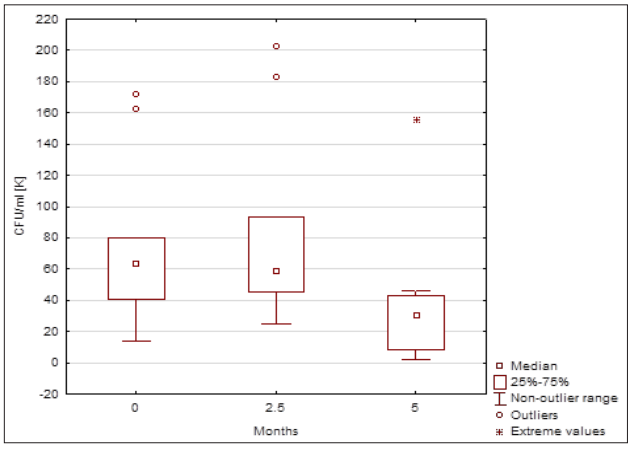

Figure 6. *S. mutans* titer (CFU/ml) in saliva of persons from the control group at the beginning and after 2.5 and 5 months of experiment

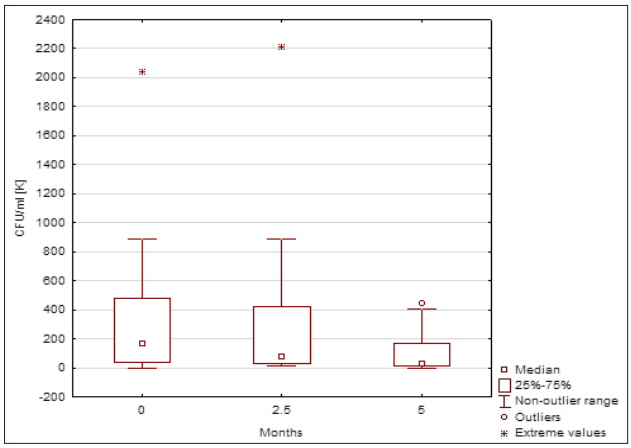

Figure 7. *S. mutans* titer (CFU/ml) in saliva of persons from the study group at the beginning and after 2.5 and 5 months of experiment

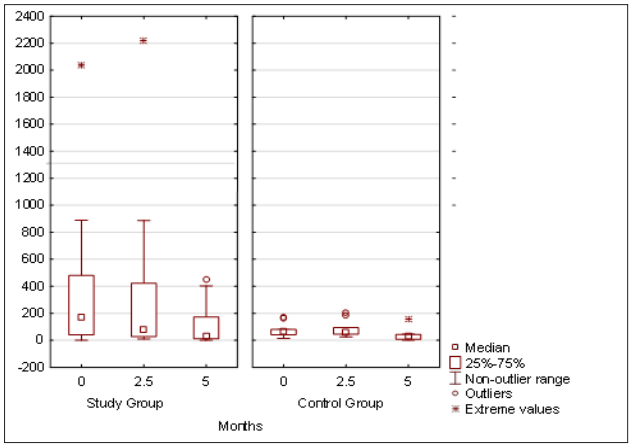

Figure 8. *S. mutans* titer (CFU/ml) in saliva of persons from the study and control groups at the beginning and after 2.5 and 5 months of experiment

The studies of *S. mutans* number in saliva of persons using Colgate Plax Cool Mint® mouthwash for 5 months showed its decrease (Fig. 5, 7, 8). In the control group, however, no statistically significant changes in the number of bacteria were observed (Fig. 5, 6, 8). The influence of the assessed mouthwash on the reduction of *S. mutans* number in saliva of studied persons was proved.

3. The evaluation of the influence of Colgate Plax Cool Mint® mouthwash on the number of *C. albicans* in saliva of the studied persons

In the case of evaluation of *C. albicans* number in saliva, significantly higher number of *C. albicans* was found in the group of persons using Colgate Plax Cool Mint® mouthwash than in the control group (I test: p=0.000732; II test: p=0.014210; III test: 0.049144) (Fig. 9). In the saliva of persons from the study group *C. albicans* number was statistically significantly differed in the consecutive tests and statistically significant decrease in yeasts presence was observed (p=0.00001). The titer of *C. albicans* in the control group did not significantly differ in the consecutive studies (p=0.96923).

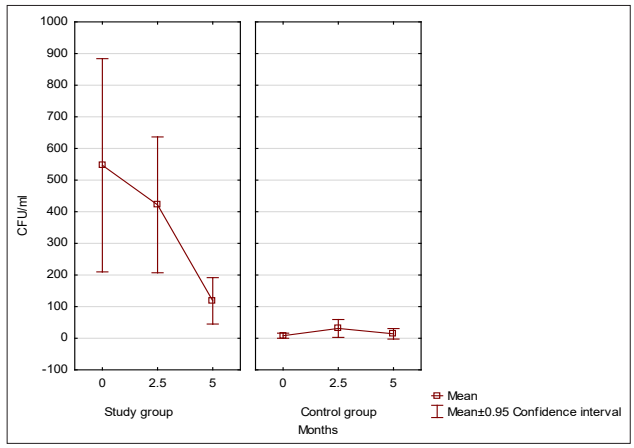

Figure 9. *C. albicans* titer (CFU/ml) in saliva of persons from the study and control groups at the beginning and after 2.5 and 5 months of experiment

Microbiological tests concerning *C. albicans* prevalence showed its decrease in the study group (Fig. 9, 11, 12), however no significant change in the presence of yeasts in the control group was found (Fig. 9, 10, 12). The influence of the assessed mouthwash on the reduction of *C. albicans* prevalence in saliva was proved.

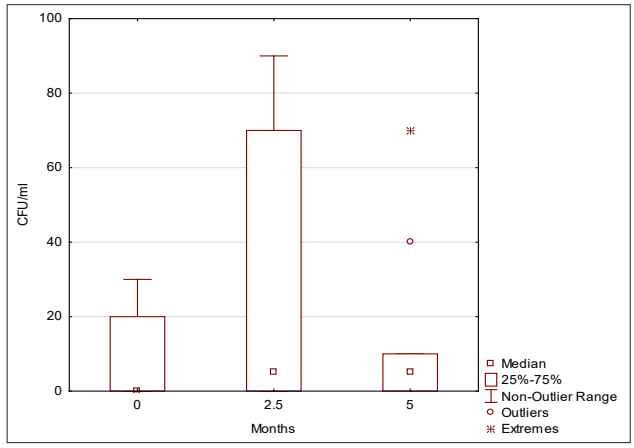

Figure 10. *C. albicans* titer (CFU/ml) in saliva of persons from the control group at the beginning and after 2.5 and 5 months of experiment

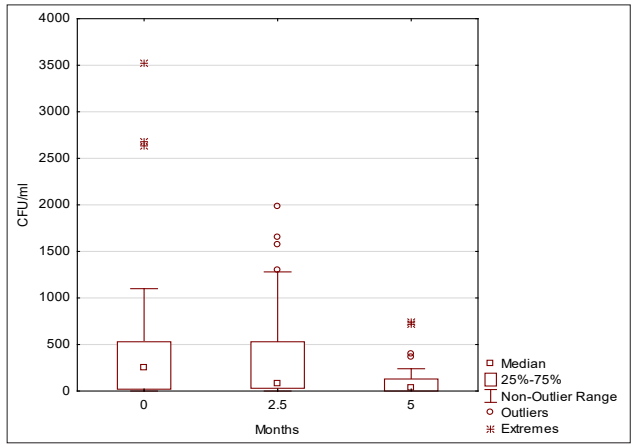

Figure 11. *C. albicans* titer (CFU/ml) in saliva of persons from the study group at the beginning and after 2.5 and 5 months of experiment

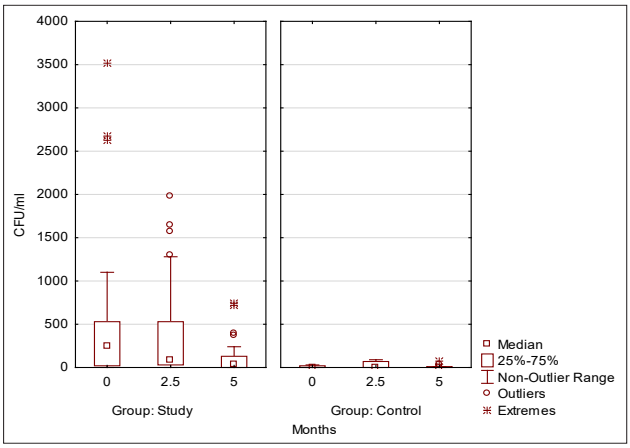

Figure 12. *C. albicans* titer (CFU/ml) in saliva of persons from the study and control groups at the beginning and after 2.5 and 5 months of experiment

DISCUSSION

In the own studies a statistically significant decrease of the number of *S. mutans* bacteria in saliva of persons using Colgate Plax Cool Mint® mouthwash was found, whereas in the Dutch studies no significant differences in the count of *S. mutans* bacteria in saliva and supragingival dental plaque of persons using this mouthwash were noted. In these studies an influence of, among others, a widely available mouthwash – Meridol GABA was also assessed. This mouthwash has in its content amine fluoride and stannous fluoride, while it does not include cetylpyridinium chloride. The mouth rinse did not have any significant influence on decrease in quantity of *S. mutans* in saliva, and similar effect was achieved in participants washing their mouth with distilled water [6]. Jothika *et al*. evaluated an impact of mouthwashes on the titre of *S. mutans* in the sample of bacterial biofilm from the surface of cheek premolars or maxillary molars four times. Each participant used the mouthwash only at the beginning of the study. The samples were collected at intervals of 7, 14, and 30 days from the first use of mouthwash. Similarly to own studies, decrease in the amount of *S. mutans* bacteria after the use of mouthwash with fluorine was noted. Fluorine turned out to be as effective as chlorhexidine. The researchers found significantly slower growth of the number of microorganisms after the use of mouth rinse with chlorhexidine and fluorine compared to water. The number of *S. mutans* bacteria increased 30 days after the use of mouthwash containing chlorhexidine from 1.14 to 10.38 CFU/ml, while after the use of mouth rinse with sodium fluoride, the number of these bacteria increased from 1.9 to 19.76 CFU/ml. The increase in the number of bacteria after 30 days from washing mouth with water was significantly higher – from 1.9 to 70.33 CFU/ml. The results of the mentioned studies, similarly to own research, prove that widely available mouthwashes show a positive influence on the reduction of *S. mutans* bacteria, when they are used in everyday oral hygiene procedures for a longer period of time [7].

Latimer *et al*. evaluated an impact of mouthwash with fluoride and cetylpyridinium chloride used for a longer period of time in everyday oral hygiene procedures on *S. mutans* titer. The samples of saliva were collected and treated with various antimicrobial substances in laboratory conditions. One of the mouthwashes contained fluoride and cetylpyridinium chloride. The researchers observed significant differences in the number of *S. mutans* reduction after the use of mouthwash including fluoride, as well as fluoride and cetylpyridinium chloride compared to water used as a mouthwash [8]. Similarly to own studies, it was proved that mouthwash containing both fluoride and cetylpyridinium chloride contributed to decrease in the amount of *S. mutans* bacteria, and reduction, as in the case of own study.

Positive influence of fluoride mouthwash on *S. mutans* reduction was proved in the studies of Jauhari *et al*., which were conducted among children aged 6-12 years, evaluating impact of selected mouthwashes on *S. mutans* number before and after washing mouth twice a day for two weeks. The first of the mouthwashes contained fluoride, the second – herbal substances, the third – natural oils. The number of bacteria amount was assessed using Dentocult SM Strip test at the beginning of the study and after two weeks. Decrease in the number of *S. mutans* colonies from 2.62 to 0.31 CFU/ml was observed after the use of mouthwash with sodium fluoride, and from 2.61 to 0.31 CFU/ml after the use of herbal mouthwash. Reduction of the number of *S. mutans* colonies after washing mouth with water was insignificant [9]. In Hindu studies the young people aged 6-14 years washed their mouths with four different fluoride mouthwashes for two weeks. The first mouthwash contained sodium fluoride at concentration 0.2%, the second – 0.5% sodium fluoride solution and 0.03% triclosan solution, the third – sodium fluoride at concentration 0.05%, and the fourth one – 0.2% sodium fluoride solution and 0.3% triclosan solution [10]. Similarly to own studies, the samples of saliva were collected from the participants. It was done twice, at the beginning of the study and after 14 days of daily use of selected mouthwash. Each of the used mouthwashes showed positive influence on reduction of *S. mutans* bacteria. Likewise, Perala and Bhupathiraju found reduction of *S. mutans* bacteria by averagely 54% after using each of the mouthwashes containing sodium fluoride. In own studies reduction of *S. mutans* colonies was also observed after the use of Colgate Plax Cool Mint® mouthwash, which contained sodium fluoride at concentration 0.05%.

In own studies, Colgate Plax Cool Mint® mouthwash was used twice a day. Similarly, in the Iranian studies assessing effectiveness of mouthwashes with fluoride, a group of the youth aged 12-14 years washed mouths twice a day for two weeks. One of the evaluated mouthwashes contained fluoride, while another – fluoride and chlorhexidine. In the first case, slight reduction of *S. mutans* bacteria was observed. After using a mouthwash including fluoride and chlorhexidine, reduction was significant and was found in 50% of respondents [11].

The comparison of an influence of mouthwashes, one with sodium fluoride (0.05%) and another with chlorhexidine (0.2%), was made by the researchers from India. They collected plaque swab from the cheek surface of an upper right molar, from the tongue surface of upper and lower incisor and from the tongue surface of a lower left molar.

In the mentioned studies, similarly to own research, decrease in *S. mutans* titer was observed after using mouthwash containing sodium fluoride. However, an effectiveness of the mouthwash including chlorhexidine was slightly higher. *S. mutans* titer, after the mouthwash containing only chlorhexidine was used, was reduced from 2.61 to 1.61 CFU/ml of saliva. After the use of mouthwash including sodium fluoride, *S. mutans* number decreased from 2.4 to 1.41 CFU/ml of saliva [12]. In turn, Sundas and Rao compared an influence of mouthwashes having in their content chlorhexidine and sodium fluoride on *S. mutans* number in saliva. The study group consisting of the youth aged 8-14 years, who washed mouths with the assessed mouthwashes with chlorhexidine as well as with sodium fluoride twice a day for 15 days exhibited decrease of *S. mutans* cells in saliva. *S. mutans* titer in saliva before the use of the mouthwash with chlorhexidine amounted 586.80 CFU/ml, while after its use it was 88.80 CFU/ml. In turn, after the use of mouthwash containing sodium fluoride, reduction of *S. mutans* titer was from 699.20 to 130 CFU/ml [13]. In the own studies the reduction of *S. mutans* number was also found after the use of Colgate Plax Cool Mint® mouthwash, which, as mentioned above, contains 225 ppm of fluoride.

In Japanese primary school students aged 7, 10 and 12 years, presence of *S. mutans* and *L. acidophilus* bacteria was evaluated using Dentocult SM Strip and Dentocult LB Dip Slick tests. An amount of the tested bacteria was again evaluated after over 2 years of using mouthwash containing 500 ppm of fluoride. Significant decrease in *S. mutans* and *L. acidophilus* number was noted [14]. In own studies decrease in *S. mutans* and *Lactobacillus* spp. titer was proved. It should be presumed that in the case of own studies, longer period of using the evaluated mouthwash could have an influence on significantly higher reduction of microorganisms colonies number.

In the study of the Polish researchers, titer of *S. mutans* and L. acidophilus in saliva was assessed using also SM and LB CRT Bacteria tests. After the use of Dentofresh Junior mouthwash containing sodium fluoride, decrease in the titer of *S. mutans* from 32.7×10^5 to 18.40×10^5 CFU/ml in saliva, and L. acidophilus – from 42.9×10^5 to 34.7×10^5 CFU/ml in saliva was noted [15].

In recent years more and more popular as mouthwashes have been products containing essential oils. They have a positive impact on the condition of teeth and periodontium. Significant influence of mouthwashes including essential oils and chlorhexidine on reduction of dental plaque, compared to an influence of the evaluated in present study Colgate Plax Cool Mint® mouthwash was indicated. Effectiveness of Colgate mouthwash was considerably lower than other mouthwashes. The mouth rinse containing essential oils contributed to reduction of dental plaque bacterial microflora by 62.4%, whereas the mouthwash with sodium fluoride – by 3.8% [16]. Found in own studies significant influence of Colgate Plax Cool Mint® mouthwash containing sodium fluoride and cetylpyridine chloride on reduction of *S. mutans* bacteria titer in saliva could result from synergistic action of both these active ingredients. In the previously cited studies, greater influence of the mouthwash containing 0.2% sodium fluoride on *S. mutans* number compared to a mouthwash with essential oils was determined, however the difference was statistically insignificant [8]. Positive influence of sodium fluoride on reduction of *S. mutans* titer in saliva (from 1.259×10^5 CFU/ml to 0.214×10^5 CFU/ml) in children aged 6-12 years, who used the mouthwash including 0.2% fluoride for 15 days was proved [17].

The results of own studies and mentioned above results of other authors indicate various level of antimicrobial efficacy of mouthwashes against microorganisms present in dental plaque, which is one of the main causal factors contributing to oral diseases, including caries and periodontal diseases. Effectiveness of the antimicrobial preventive actions of mouthwashes is based on inhibiting the development of dental plaque by reducing an amount of its microorganisms. The evaluated Colgate Plax Cool Mint® mouthwash shows proper antibacterial and antifungal efficacy. It should be widely propagated and used in everyday oral hygiene as prophylaxis.

CONCLUSION

Colgate Plax Cool Mint®, the most frequently used by the youth in their daily oral hygiene, shows effectiveness in reduction of *Lactobacillus* spp. and *S. mutans* bacteria, as well as *C. albicans* yeasts present in the saliva, what allows for inhibition of formation and development of plaque, which proves the usefulness of this mouthwash in prophylaxis of oral diseases, including caries and periodontal diseases.

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AUTHORS' STATEMENT

The authors declare that they have no competing interests.

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