Microbiological monitoring of the condition of dental biofilm (plaque) as a result of the application of chlorhexidine and xylitol in early childhood caries treatment

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Abstract

The high prevalence of early childhood caries (ECC) makes it necessary for treatment-and-preventive means, which influence the leading factor of the etiopathogenesis of the given disease – the pathogenic microflora – to be employed. In this study, the effectiveness of antibacterial methods that use xylitol and chlorhexidine is measured by means of comparing various data, as well as by comparing their influence on the structure of the microbiocenosis of dental biofilm.

Key words: early childhood caries, oral microflora, xylitol, chlorhexidine.

Despite an already substantial number of studies focusing on the etiopathogenesis, diagnostics, treatment and prevention of early childhood caries [1, 4, 15], this problem remains one of the most significant for a children's dentist, because of its widespread occurrence in many countries worldwide [1, 4, 15, 20].

Experience of the past decade shows that the leading role in causing caries, including early childhood caries, belongs to the Streptococcus microflora, and the *Streptococcus mutans* bacteria in particular [15]. However, the complex and varying composition of dental plaque proves that several other microorganisms are also able to cause caries, even though to a lesser extent: *Streptococcus sanguis*, *Streptococcus salivarius*, *Streptococcus milleri*, *Lactobacillus*, *Actinomyces viscosus* [6].

The most important biological aspects of *Streptococcus mutans* are its adhesive properties and the ability to produce lactic acid. Dental plaque is formed as a result of streptococci bacteria attaching themselves to the teeth, and this activity is intermediated by the synthesis of glucose polymers from sucrose found in food. The formation of glucans induces intercellular aggregation of *Str. mutans* and other bacteria present in plaque. The sticky glucan matrix of dental plaque prevents the diffusion of the large amount of acid formed by the microorganisms, prolonging its stay on the surface of the teeth, causing demineralization of the enamel, which leads to dental caries [6].

It has been proven that most often, the infection of a child by cariogenic microflora occurs primarily through the mother or other caregivers [15]. A decrease in the amount of *Streptococcus mutans* present in persons taking care of the child can reduce the risk of the child developing early childhood caries. Therefore, parents are advised to carry out regular oral hygiene procedures and to take thorough care of their teeth. Should a high level of presence of cariogenic microflora be discovered in their dental biofilm, they are advised to use medicinal products that lower its activity.



Fig.1



Fig.2

The age at which the infection of a child by *Streptococcus mutans* occurs is vital, since the earlier the infection occurs, the higher the risks and the intensity of the process of caries development [18, 19]. Earlier, researchers had thought that it was impossible for cariogenic microflora to infect the oral cavity of predentate children. However, recent clinical trials have shown that it is possible for *Streptococcus mutans* to form colonies in the grooves of the back of the tongue even before the infant has started to teethe [23, 26, 27].

Vertical transmission is not the only possible way in which cariogenic microflora can be transmitted from human to human. The genotypic analysis of *Streptococcus mutans* found in the mouth of 12-30 month-old children has shown that many children are infected by *Streptococcus mutans* of the same genotype [22, 25]. This proves that cariogenic microorganisms can be transmitted horizontally.

Dental afflictions in children are considered to be a clinical syndrome that can be labeled by several names. However, in recent times, the term "early childhood caries" has been prevalent in most medical publications.

Caries at an early age causes deciduous teeth to be afflicted almost as soon as they erupt. The first carious lesions are usually discovered on the vestibular surface of the upper incisors in the form of chalk-colored spots on the cervical margins. These spots usually change in color very quickly (2-3 months) to light yellow, with carious lesions taking their place soon after (Fig. 1).

The cariogenic process in the given case is characterized by swift progress, a widening size of the lesions and its ability to affect multiple teeth as soon as they erupt (barring the lower incisors). The resistance of the lower incisors to the process is explained by their position in the mouth, which makes them the teeth cleaned best by the tongue and saliva.

Often the incisors and canines are afflicted by circular caries, which can lead to the crowns breaking off. Sometimes, an unusual palatal caries localization on the cervical margins of the upper incisors and canines can occur.

A significant amount of plaque is usually present in the oral cavity of children afflicted by ECC. The plaque is yellow in color and is hard to remove. It can also cause catarrhal gingivitis, hyperemia and alveolar osteitis (Fig. 2).

A lasting positive effect when treating ECC can only be attained as a result of a combination of different treatment-and-preventive techniques, aimed at all parts of the ethiopathogenesis, including the cariogenic microflora [5].

Most often, chlorhexidine, iodides, and varying combinations of the two form the basis for treatments discussed in foreign medical research [15, 20, 28].

It is also known that xylitol possesses a particular antibacterial quality with regards to the most cariogenic microorganisms – the *Streptococcus mutans.* A bacteria cell obtains energy from the zymolysis of sugars. Cariogenic microorganisms consume xylitol as part of their vital activity, however, they do not have any means of processing it, leading to an excess of it in the cell. As a result, the bacterial cell either dies, or excretes the excess xylitol back into the oral cavity. The latter case is also known as an "idle cycle", whereby even though the bacteria do not die, they use their energy in vain, leading to a slowing down of growth and spread of the bacteria [8, 21].

Also, xylitol lowers the adhesive qualities of cariogenic microorganisms in relation to the dental surfaces, preventing the formation of plaque and improving the health of the oral cavity [2, 14, 24].

AIMS OF THE STUDY

A comparison of the effectiveness of different antimicrobial treatments belonging to different pharmacological groups – antiseptics (chlorhexidine) and sugar substitutes (xylitol) – and their effect on the microbiocenosis of dental plaque.

MATERIALS AND METHODS

30 patients aged 12-36 months, all suffering from ECC (18 boys and 12 girls), were placed under our supervision. Clinical examination of their oral cavities was carried out by recording various observed data for each individual on separate cards, part of the data being the values for the Decayed, Missing, Filled teeth (DMFT) and Decayed, Missing, Filled surfaces (DMFS) indices. Dental hygiene was evaluated using the dental plaque assessment in young children (IDPA) index (Kuzmina E.M., 2000). The pres-

ence/absence of catarrhal gingivitis was determined visually.

None of the children were complaining about any kind of pain, and they were all prescribed the following preventive treatment plan:

1. Improve oral hygiene
2. Normalize the food intake (in order to get rid of the carbohydrate factor)
3. Antimicrobial therapy to be carried out at home
4. Local remineralization therapy with products containing calcium phosphate
5. Local application of fluorides once a month (with the help of a dentist)
6. Sealing of the fissures in the deciduous molars
7. Preventive endogenous fluoride treatment (if the child lives in an area with drinking water with a low fluoride concentration
8. Regular visits to the pediatrician in order to check for possible somatic dysfunctions and hypercalcemia
9. Health center supervision

The patients were randomly divided into two groups of 15. The patients in the first group were subjected to a one-month local antimicrobial and remineralization therapy using the R.O.C.S. Medical Minerals adhesive gel containing calcium glycerophosphate, magnesium chloride and xylitol (10%). The applications of the R.O.C.S. Medical Minerals remineralizing gel were to be carried out 4-5 times daily, after a prior brushing of the teeth.

The patients in the second group were prescribed daily applications of a 0.1% chlorhexidine solution for 7-10 days, for 1-2 minutes every day as part of an antimicrobial therapy. For remineralization purposes, the "Belagel Ca/P" gel was to be used daily for the duration of one month. The patients were to have the gel applied 4-5 times daily, after a prior brushing of the teeth as well.

All the above procedures were to be carried out at home, with the help and supervision of the patients' parents, who had also received detailed instructions about the use of the gels.

Bacteriological and molecular-biological studies of each patient's oral cavity using a multiplex PCR (polymerase chain reaction) developed at the Scientific Research Institute for Medicine and Dentistry were performed.

As part of the bacteriological study, material samples of dental plaque were collected from the vestibular surface of the upper incisor (prior to the use of any chemical and hygienic compounds).

The material was collected in the mornings, when the teeth were yet to be brushed, with the help of a sterile paper endodontic pin of standard size (30), which was then placed in a semiliquid Stuart transport medium. Prior to being transported, the samples were kept at the temperature of 2-4°C, with the transport procedure itself taking up to one hour. Further bacteriological research was then carried out in accordance with all clinical anaerobic microbiology standards and rules.

Next, the streak plate technique was used to sow the bacteria onto mediums designed for the cultivation of Streptococcus and other oral cavity bacteria in aerobic and anaerobic conditions.

Colonies of obligate and facultative anaerobes were obtained in anaerobic conditions with the help of a 5% chocolate blood agar (CBA), prepared from the Difco brand Brain-Heart Infusion by adding ferriheme (5 μg/ml) and menadione (0.1 μg/ml), with the compulsory placement of the medium inside anaerobic culture apparatuses with anoxic gas mixtures consisting of 80% nitrogen, 10% hydrogen and 10% carbon dioxide. Reduction of oxygen residue was carried out using a palladium catalyst.

The results of the quantitative study were recorded in colony-forming units (CFU) per millileter (CFU/mL).

Isolated colonies of bacteria in aerobic conditions were obtained by combining:

- 5% chocolate blood agar made from Brain-Heart Infusion,
- selective and differential mitis-salivarius agar,
- Himedia brand M1353 chromogenic medium,
- Himedia brand spore differentiation chromogenic medium.

After identification of the different obtained isolated colonies and their quantification, pure cultures of bacteria were obtained in either the Brain-Heart Agar, or the transport medium. The cultivation in anaerobic condition was carried out for up to 7 days, and in aerobic conditions – for up to 3 days.

The type of bacteria obtained was determined by morphological, cultural and biochemical analysis.

The biochemical identification of the pure cultures of anaerobic bacteria, streptococcus organisms, and gram-negative bacteria was performed using API (France) and Roche (Germany) brand testing systems.

The results were then interpreted with the help of bacterial presence norms.

A polymerase chain reaction was induced with the help of a "MultiDent" testing set ("GenLab Ltd.", Russia), in order to detect the five main types of periodontal bacteria: Actinobacillus actinomycetemcommitans, Porphyromonas gingivalis, Prevotella intermedia, Bacteriodes forsythus, and Treponema denticola.

Sample taking for the PCR diagnostics for periodontitis was performed in accordance with the recommendations of Tsarev V.N. et al. (2002) and was used to identify the cultures of enzyme-forming bacteriodes and actinobacilli.

DNA was extracted from the samples by preparing it using several clinical reagents manufactured by "GenLab Ltd." (Russia), including:

- "Extragen" – ion exchanger granule suspension mixture (1 flask, 10 mL)
- "Enzymix" proteolytic compound (1 freeze-dried test tube)
- "Enzymix" solvent

  (1 test-tube, 100 µl)

The further extraction and detection of periodontal bacteria was performed as required by the ФС-2006/043-У medical technology developed in the department of microbiology, virusology and immunology at the Moscow State University of Medicine and Dentistry.

STUDY RESULTS AND DISCUSSION

Clinical examination of the oral cavities of the patients in the 1st group established a high level of intensity of caries of deciduous teeth: the DMFt index value was measured at 5.9±1.4, while the DMFs index value at 12.9±3.1. The oral hygiene level was assessed to be "poor": the average value of the IDPA plaque index (Kuzmina E.M., 2000) was measured at 0.75±0.09. 73.3% of patients had symptoms of catarrhal gingivitis, and 53.3% suffered from symptoms of hyperemia and alveolar osteitis.

The evaluation of the microbial flora of the dental plaque of the patients in the first group at the start of the study showed a high level of microbial presence of both resident and aggressive types of cariogenic and periodontal pathogenic bacteria (Table 1).

Table 1. Composition of cariogenic and resident microflora of dental plaque before and after the application of the R.O.C.S. Medical Minerals gel containing xylitol (n=15)

Bacteria genus and species	Before the treatment		After the treatment		P
	M+m lg CFU/mL	I	M+m lg CFU/mL	I	
Streptococcus mutans	5.45±0.25	73.3	4.45±0.20*	40.0*	<0.05
Streptococcus sanguis	7.15±0.26	100.0	6.52±0.21*	100.0	<0.05
Streptococcus salivarius	4.67±0.18	60.0	4.38±0.24	53.3	>0.05
Enterococcus spp.	5.92±0.19	60.0	5.53±0.14	86.7**	<0.05
Peptostreptococcus anaerobius	5.14±0.14	46.7	5.29±0.28	46.7	>0.05
Actinomyces spp.	4.67±0.23	60.0	5.00	33.3*	<0.05
Veillonella parvula	4.20±0.09	33.3	4.00±0.12	46.7**	<0.05

*value significantly lower than before the treatment

**value significantly higher than before the treatment

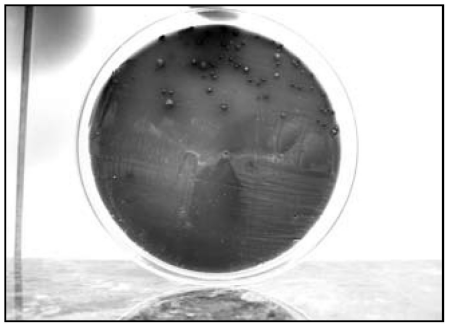


Fig. 3. Colonies of alpha viridans cariogenic streptococci *S. mutans* (large) and *S. sanguis* (small) cultivated from children's dental plaque in a 5% chocolate blood agar

The main cariogenic type – *Streptococcus mutans* – was discovered in 73.3% of the patients, with the present amount (expressed in powers of 10) measured at 5.45±0.25 (about 10^5 CFU/mL). The present amount of *Streptococcus sanguis* also turned out to be significant (7.15±0.26, or about 10^7 CFU/mL), while being found in all (100%) of the patients (Fig. 3). The *Actinomyces spp*. bacteria, which also have strong adhesive properties and produce significant amounts of acid, were discovered in 60% of patients, but in more moderate amounts of 4.67±0.23 (about 10^5 CFU/mL).

Moreover, the presence of the *Veillonella parvula* bacteria in the dental plaque was significantly lower than by cariogenic types, and was recorded to be only 4.20±0.09, and was found in 33.3% of the patients. *Veillonella parvula* are known to be different from most metabolic cariogenic types, since they utilize and neutralize acids, making them a leading microbial factor in caries resistance [11, 16].

Other resident bacteria types also play a certain role in slowing down the spread of cariogenic streptococci colonies, but this role is not as vital. Presence of other resident types of bacteria in patients of

the 1st group was found to vary between 10^4 and 10^5 CFU/mL, with the frequency of their presence ranging from 46.7% to 60.0%.

As a whole, the microbiological conditions of patients in the first group are no doubt typical for carious processes.

Especially noteworthy was the evaluation of the presence of periodontal pathogen types of anaerobic bacteria (Table 2).

Most patients were found to carry 2-3 types of periodontal pathogens at a time, measured to range from 10^5 to 10^6 CFU/mL.

The frequency of presence of the main periodontal pathogen – *Porphyromonas gingivalis* (40.0%) – was most important, as well as that of the virulent anaerobic *Prevotella melaninogenica* type (60.0%). Other periodontal and virulent types – *Fusobacterium spp.* and *Klebsiella spp.* were discovered in 20.0% and 13.3% of the patients respectively. Relatively often (20.0% of the patients), the *Candida albicans* bacteria, a diploid fungus, were discovered to be present in significant concentrations (4.77±0.37).

As a result of the clinical examinations of the oral cavity of patients in group 2, it was established that patients in this group also scored relatively high in the DMFt (6.5±1.5) and DMFs (14.2±3.3) indices. Oral hygiene of the patients was scored according to the IDPA plaque index (Kuzmina E.M., 2000), with the values being measured to be 0.78±0.1. Catarrhal gingivitis was diagnosed in 66.7% of the patients, and hyperemia and alveolar osteitis in 46.7% of the patients.

The analysis of the starting frequency of the presence of cariogenic types of bacteria and its presence of dental plaque in patients of the 2nd group, who were treated with a 0.1% solution of chlorhexidine, had shown the following (Table 3).

The main cariogenic type – *Streptococcus mutans* – was discovered to be present in 2/3 of the patients (66.7%), while the present amount, expressed as a power of 10, was found to be 5.50±0.32 (between 10^5 and 10^6 CFU/mL). The present amount of another cariogenic type, the *Streptococcus sanguis*, was even higher: 6.97±0.21 (or about 10^7 CFU/mL), notably being found in all (100%) of the patients. Finally, *Antinomyces spp.*, which possess an adhesive quality and produce significant amount of acids, were discovered to be present in 60.0% of the patients, although in a moderate amount: 5.14±0.24 (about 10^5 CFU/mL).

As such, the frequency of the presence of the main cariogenic types of bacteria slightly differed from that of the 1st group, but a high amount was nevertheless present in both groups.

As for the antagonistic microflora, the infection of dental plaque by *Veillonella parvula* in the given group was much less pronounced, as was the case in the 1st group, than by the cariogenic types and was measured to be 4.50±0.24, with the

Table 2. Composition of virulent microflora of dental plaque before and after the application of the R.O.C.S. Medical Minerals gel containing xylitol (n=15)

Bacteria genus and species	Before the treatment		After the treatment		P
	M+m lg CFU/mL	I	M+m lg CFU/mL	I	
A. actinomycetemcomitans	Not present	0	Not present	0	-
Porphyromonas gingivalis	5.45±0.29	40.0	5.78±0.25	40.0	>0.05
Prevotella melaninogenica	4.44±0.23	60.0	5.40±0.11**	33.3*	<0.05
Fusobacterium nucleatum	5.33±0.12	20.0	4.50±0.14*	13.3*	<0.05
Staphylococcus aureus	Not present	0	4.00	6.7	>0.05
Klebsiella pneumoniae	4.50±0.14	13.3	4.20±0.09	33.3**	<0.05
Candida albicans	4.77±0.37	20.0	Not present	0*	<0.05

Table 3. Composition of cariogenic and resident microflora of dental plaque before and after the local application of the 0.1% chlorhexidine solution (n=15)

Bacteria genus and species	Before the treatment		After the treatment		P
	M+m lg CFU/mL	I	M+m lg CFU/mL	I	
Streptococcus mutans	5.50±0.32	66.7	4.90±0.26*	66.7	<0.05
Streptococcus sanguis	6.97±0.21	100.0	6.33±0.29*	100.0	<0.05
Streptococcus salivarius	5.50±0.11	40.0	5.52±0.24	53.3	>0.05
Enterococcus spp.	6.33±0.09	46.7	6.08±0.15	26.7*	<0.05
Peptostreptococcus anaerobius	4.96±0.25	46.7	4.60±0.42	33.3*	<0.05
Actinomyces spp.	5.14±0.24	60.0	5.83±0.08**	40*	<0.05
Veillonella parvula	4.50±0.26	26.7	4.60±0.23	33.3	>0.05

*value significantly lower than before the treatment

**value significantly higher than before the treatment

particular type of bacteria being found in about a quarter of the patients (26.7%).

Other resident types of bacteria, which play a significant role in slowing down colonization by aggressive streptococci, were found to be present in the same number of patients as in the 1st group.

The present amount in the second group by the given type was measured to be between 10^5 and 10^6 CFU/mL, with the frequency being recorded to be 40-46.7%. These values correspond with medical literature which states that in healthy persons, resident bacteria is present in 50-60% of people, in amounts of 10^4 to 10^6 CFU/mL. This type of bacteria plays an important role in forming the normal microbial flora of the oral cavity [3, 12].

The microbiological conditions in the oral cavity obviously play an important role in determining the development and spread of caries. A sudden change in the balance of the microbial flora, indicated by a sharp increase in the amount of acid-producing streptococci bacteria, and in 46.7% of cases – by the interaction of *Streptococcus mutans* with actinomycetes as a result of the absence of a sufficient amount of antagonistic flora, Veillonella in particular, promotes the enamel demineralization and cariogenic processes.

We had also analyzed the samples obtained from patients of the 2nd group for presence of periodontal pathogenic types of anaerobic bacteria (Table 4).

As was the case in the 1st group, the analyzed patients were found to contain 2-3 types of periodontal pathogens, in the amount of 10^4-10^5 CFU/mL.

Bacteria of the virulent anaerobic type *Prevotella melaninogenica* were the most widespread (33.3%). Bacteria of the main periodontal pathogen type, *Porphyromonas gingivalis,* were found to be slightly less widespread (present in 20% of the patients). Other periodontal pathogen and virulent types of bacteria – *Fusobacterium spp.*, *Klebisella spp.*, were discovered in a less significant number of patients: 6.7% and 13.3% respectively. More significant was the amount and frequency of presence of *Candida* spores: 5.50±0.12.

Clinical examination of the oral cavity of patients in the 1st group a month after the start of the prescribed treatment has shown the absence of the spread of carious lesions and areas afflicted by demineralization; pigmentation of chalk-colored spots was starting to return to normal. Among other positive developments was the disappearance of catarrhal gingivitis symptoms and a satisfactory value for the IDPA plaque index (Kuzmina E.M., 2000) for all patients – 0.38±0.07. Also, the spread of carious defects had been stopped, dental tissue afflicted by caries had hardened, meaning the caries had taken on a more compensated form.

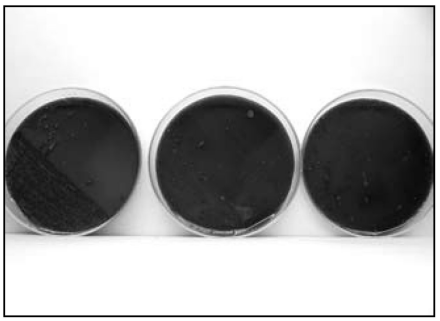


Fig.4 The decreasing amount of *Streptococcus mutans* present in a patient in the process of treatment with the R.O.C.S. Medical Minerals gel with xylitol: 1st dish – before (10^6 CFU/mL); 2nd dish – immediately after the end of the one-month treatment (10^4 CFU/mL); 3rd dish – 1 week after the end on the one-month treatment (10^3 CFU/mL).

Data obtained from repeated analysis of the microbial flora of dental plaque in patients of the 1st group after the use of the R.O.C.S. Medical Minerals gel with xylitol is shown in Tables 1 and 2.

The amount of cariogenic *Streptococcus mutans* and *Streptococcus sanguis* bacteria was found to have decreased drastically: from 5.45 to 4.45 CFU/mL and from 7.15 to 6.52 CFU/mL respectively, while the amount of *Actinomyces spp.* underwent practically no changes, however, a significant decrease in its frequency was nevertheless noted – down from 60% to 33.3%, even though 33.3% is still a relatively high frequency (Fig. 4).

At the same time, an increase in the population of *Veillonella parvula* was rec-

Table 4. Composition of virulent microflora of dental plaque before and after the local application of the 0.1% chlorhexidine solution (n=15)

Bacteria genus and species	Before the treatment		After the treatment		P
	M+m lg CFU/mL	I	M+m lg CFU/mL	I	
A. actinomycetemcomitans	Not present	0	Not present	0	-
Porphyromonas gingivalis	6.73±0.05	20.0	6.48*	6.7*	<0.05
Prevotella melaninogenica	5.12±0.19	33.3	4.65±0.21*	40.0	<0.05
Fusobacterium nucleatum	7.00	6.7	5.00±0.14	33.3**	<0.05
Staphylococcus aureus	Not present	0	Not present	0	-
Klebsiella pneumoniae	4.50±0.14	13.3	4.50±0.43	13.3	>0.05
Candida albicans	5.50±0.12	26.7	5.83±0.08**	40.0**	<0.05

*value significantly lower than before the treatment

**value significantly higher than before the treatment

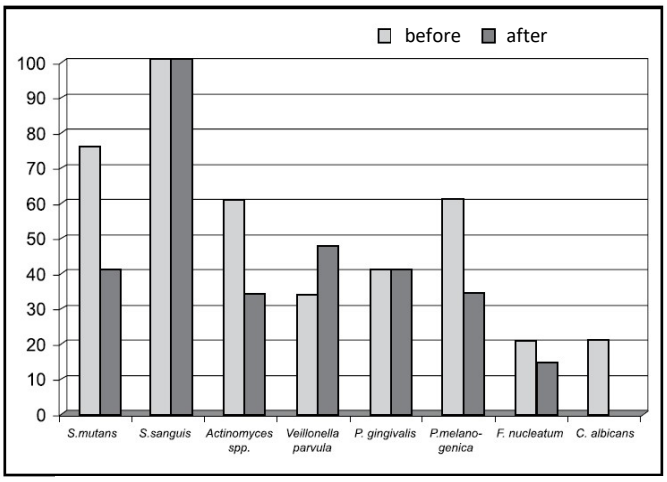


Fig.5. Changes in the frequency of the presence of bacteria in dental plaque before and after treatment with R.O.C.S. Medical Minerals gel with xylitol (10%)

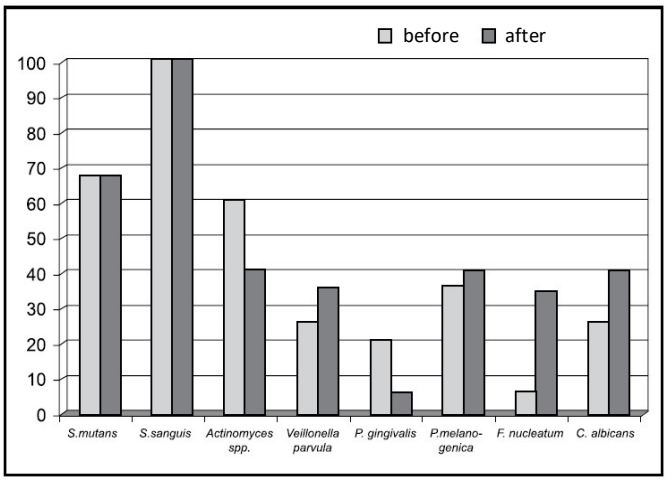


Fig.6. Changes in the frequency of the presence of bacteria in dental plaque before and after treatment with 0.1% chlorhexidine solution

orded in 46.7% of the patients, which can be seen as a positive result that serves to increase a patient's resistance to caries [12, 16].

As such, the obtained data serves to show that the R.O.C.S. Medical Minerals gel with xylitol has a positive impact on the ratio of resident to cariogenic types of bacteria, which, in particular, is expressed in the decrease of the presence frequency of *Streptococcus mutans* from 73.3% to 40%, as well as the decrease of the Veillonella population (the main antagonist of the cariogenic flora).

Another fact worth noting is the lowering of the frequency of one of the main virulent anaerobic bacteria types – *Prevotella melaninogenica* – by the end of the R.O.C.S. Medical Minerals gel treatment (although without lowering the present amount), as well as a significant decrease in the frequency of another anaerobic type of bacteria – *Fusobacterium nucleatum.* The amount of *Porphyromonas gingivalis* and the frequency of the given periodontal pathogen (40.0%) did not change.

Another important positive development in the composition of the microbiocenosis of dental plaque was undoubtedly the disappearance of the *Candida* spores.

The obtained data lets us conclude that the components of the R.O.C.S. Medical Minerals gel have a positive impact on the composition of the microbiocenosis of dental plaque, normalizing the species composition and the amount of cariogenic bacteria, while stabilizing the resident types. Moreover, the use of the gel facilitated the disappearance of *Candida* spores from the dental plaque, as well as the decrease in the amount and frequency of certain periodontal types of anaerobic bacterial pathogens (Table 5); nevertheless, their total elimination from dental plaque could not be achieved [9, 10, 17].

Clinical examination of the oral cavity in patients from the 2nd group a month after the prescribed treatment have shown: an absence of the spread of carious lesions and areas of demineralization; the disappearance of the symptoms of catarrhal gingivitis in 80% of the patients (in relation to the total number suffering from the symptoms at the start); a "satisfactory" level of oral hygiene in 73.3% of the patients, with the average value of the IDPA plaque index (Kuzmina E.M., 2000) measured to be 0.45±0.09.

The analysis of the microbial flora of dental plaque collected from the patients of the 2nd group one month after the prescribed treatment has shown only limited positive changes in the ratio of cariogenic

Table 5. Comparative changes in the frequency of presence of different types of bacteria in dental plaque after treatments of 0.1% chlorhexidine solution and R.O.C.S. Medical Minerals gel with xylitol (10%)

Bacteria genus and species	0.1% Chlorhexidine	R.O.C.S. medical minerals with xylitol
Streptococcus mutans	No change	45.4% decrease
Actinomyces spp.	33.3% decrease	44.5% decrease
Veillonella parvula	24.7% increase	40.2% increase
Porphyromonas gingivalis	66.5% decrease	No change
Prevotella melaninogenica	20.0% increase	44.5% decrease
Fusobacterium nucleatum	480.0% increase	33.5% decrease
Candida albicans	49.8% increase	Eliminated by the treatment

and normal bacterial flora: the average amount of *Streptococcus mutans* present has steadily been going down, although not in large amounts (4.90±0.26), while the frequency did not show any tendency to decrease at all and remained 66.7%, a significant amount. Another cariogenic type of bacteria, the *Streptococcus sanguis* also showed a tendency to decrease in amount in the dental plaque, but remained present in all of the patients (100%). The quantitative data for actinomycetes colonization has not changed significantly, but a decrease in its frequency (from 60% to 40%) was nevertheless observed (Table 3).

No changes were recorded in the presence of other resident types of bacteria, including *Veillonella*, while the amount of enterococci and their frequency did go down significantly (from 46.7% to 26.7%).

The obtained results indicate that chlorhexidine only had a weak effect on the resident/cariogenic microflora amount ratio in our treatment, as only a weak tendency to lower the amount of cariogenic streptococci and actinomycetes was noted, and the frequency of the main types of streptococci practically did not change at all (Fig. 6).

At the same time, the amount and frequency of resident-type bacteria – the enterococci – had decreased. No recovery in the population levels of the bacteria antagonistic to the acid-producing microflora – the *Veillonella* – was noted.

As such, it can be concluded that the given data indicates a caries resistance mechanism different to that which occurs as a result of the application of the R.O.C.S. Medical Minerals gel with xylitol. The effects of the latter, in our view, are more physiological with respect to the regulation of the resident/cariogenic microflora ratio for the patient's oral cavity.

The effects of the local application of the chlorhexidine solution on the virulent microflora were as follows: lowering of the frequency of the *Porphyromonas gingivalis* bacteria by three times; a decrease in the average amount of *Prevotella melaninogenica* (Table 4); an increase in the amount of *Fusobacterium nucleatum* in dental plaque (from 6.7% to 33.3%).

It was also worth noting that the frequency of *Candida albicans* spores increased from 26.7% to 40.0% (Table 5). At the same time, a significant increase in the amount of the spores present in the dental plaque was noted (up to 5.83±0.08 CFU/mL). These observations indicate the development of microbial dysbiosis in the dental plaque as a result of chlorhexidine use.

CONCLUSIONS

- The use of a combination of treatment-and-preventive techniques, which should be aimed at all etiopathogenic factors of caries development in early age children, allows for the stabilization of the process in a relatively short timeframe.
- The inclusion of xylitol and chlorhexidine as part of an antimicrobial therapy into early childhood caries treatment plans leads to a decrease in the frequency and amount of cariogenic and periodontal bacteria in dental plaque.
- The application of a 0.1% chlorhexidine solution has less of a physiological effect on the microbial composition of dental plaque, when compared to the R.O.C.S. Medical Minerals remineralizing gel containing xylitol (10%).
- The combination of remineralizing and antimicrobial qualities of the R.O.C.S. Medical Minerals gel allows for a decrease in the timeframe of suggested treatments, which significantly decreases the difficulty of carrying it out on children of an early age.

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