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ABSTRACT

Tetiana Timokhina
https://orcid.org/0000-0002-0220-0220
Department of Dental Therapy, Bogomolets National Medical University, Kyiv, Ukraine

Marina Kryvtsova
https://orcid.org/0000-0001-8454-2509
Department of Clinical Laboratory and Morphofunctional Diagnostics, Uzhhorod National University, Uzhhorod, Ukraine

Nina Proshchenko
https://orcid.org/0000-0001-7317-6590
Department of Prosthetic Dentistry, Bogomolets National Medical University, Kyiv, Ukraine

Mykola Spivak
https://orcid.org/0000-0002-4394-7275
D.K. Zabolotny Institute of Microbiology and Virology of National Academy of Sciences of Ukraine, Kyiv, Ukraine

CHARACTERISTICS OF THE ORAL MICROBIOTA IN CHILDREN WITH CONGENITAL CLEFT PALATE WITH CLEFT LIP

Introduction. The oral cavity microbiota plays a crucial role in maintaining oral health. However, its composition may change under the influence of exogenous factors or due to disruptions in the anatomical and consequently, functional features of various biotopes within the oral cavity. Under such conditions, the development of dysbiosis can lead to a chronic, persistent inflammatory process. These pathologies require further investigation to develop recommendations for correcting the identified imbalances.

Objective: To study the microbiota of oral cavity biotopes in children with cleft hard and soft palate with cleft lip.

Materials and Methods: In children with bilateral (n = 46) and unilateral (n = 44) cleft hard and soft palate with cleft lip was examined through dental assessment and microbiological analysis of cultured biological samples (smears from inflammatory sites), using differential diagnostic nutrient media. These media were used to cultivate conditionally pathogenic microbiota. For cultivating anaerobic microbiota, Shedler agar supplemented with 5% sheep blood ("Himedia", India) was used. Anaerobic conditions were created using an anaerostat with the AnaeroGen System ("Oxoid", United Kingdom).

Results: It was found that in children with unilateral and bilateral cleft hard and soft palate with cleft lip, the lowest values of the digital data of the hygiene indices was assessed as poor, the qualitative and quantitative composition of the oral microbiota was characterized by a predominance of conditionally pathogenic, aerobic, and facultative anaerobic microorganisms. The oral microbiota in these patients was classified as dysbiotic, with a dominance of facultative conditionally pathogenic microorganisms, which include opportunistic infection pathogens. A comparison of the oral microbiota in children with cleft hard and soft palate and cleft lip - both unilateral and bilateral - showed that the number of facultative microbiota representatives was higher in children with bilateral clefts. At the same time, the species composition of the dominant microbiota representatives showed little variation.

Conclusions: The oral microbiota in children with cleft hard and soft palate with unilateral and bilateral cleft lip was dominated by 2–5 associative complexes composed of gram-positive and gram-negative bacteria, microscopic fungi of the genus *Candida*, and periodontal pathogenic microorganisms, primarily from Socransky's orange complex**.** In cases of bilateral clefts of the hard and soft palate with cleft lip, the titer of facultative microbiota was higher. The structure of the microbial community included obligate anaerobes associated with periodontal pathogens. In the microbial coenosis of children with bilateral clefts of the lip and palate, anaerobic bacteria such as *Prevotella*, *Peptococcus*, *Peptostreptococcus*, and *Fusobacterium* were present against a background of high concentrations of conditionally pathogenic microorganisms.

Keywords: congenital maxillofacial anomalies, fissure of palate, oral hygiene, microbial coenosis, oral cavity biotopes, dental diseases, opportunistic microorganisms.

Corresponding author: *Tetiana Timokhina, Department of Dental Therapy, Bogomolets National Medical University, Kyiv, Ukraine*
e-mail: tanyatimokhina@gmail.com

РЕЗЮМЕ

Тетяна Тімохіна
https://orcid.org/0000-0002-0220-0220
Кафедра терапевтичної стоматології, Національний медичний університет імені О.О.Богомольця, Київ, Україна

Марина Кривцова
https://orcid.org/0000-0001-8454-2509
Кафедра клініко-лабораторної та морфофункціональної діагностики, ДВНЗ «Ужгородський національний університет», Ужгород, Україна

Ніна Прощенко
https://orcid.org/0000-0001-7317-6590
Кафедра ортопедичної стоматології, Національний медичний університет імені О.О.Богомольця, Київ, Україна

Микола Співак
https://orcid.org/0000-0002-4394-7275
Інститут мікробіології і вірусології імені Д. К. Заболотного НАН України, Київ, Україна

ХАРАКТЕРИСТИКА МІКРОБІОТИ РОТОВОЇ ПОРОЖНИНИ У ДІТЕЙ З ВРОДЖЕНОЮ РОЗЩІЛИНОЮ ГУБИ ТА ПІДНЕБІННЯ

Вступ. Мікробіота ротової порожнини відіграє вирішальну роль у стоматологічному здоров'ї. Водночас, склад мікроорганізмів може змінюватись як під впливом екзогенних чинників, так і за умов порушення анатомічних, а, відповідно, й функціональних особливостей різних біотопів порожнини рота. За таких умов розвиток дисбактеріозу може призводити до хронічного персистуючого запального процесу. Такі патології потребують дослідження з метою подальшої розробки рекомендацій щодо корекції виявлених порушень.

Мета: дослідити мікробіоту біотопів ротової порожнини у дітей з розщілинами твердого та м'якого піднебіння з розщілинами губи.

Матеріали та методи: у осіб дитячого віку з двосторонньою (n=46) та односторонньою (n=44) розщілинами твердого та м'якого піднебіння з розщілинами губи проведено стоматологічне обстеження та дослідження посіву біоматералу (мазок із осередку запального процесу) з використання диференційно-діагностичних поживних середовищ. Для культивування умовно-патогенної мікробіоти використовували диференційно-діагностичні поживні середовища. Для культивування анаеробних представників мікробіоти використовували поживне середовище агар Шедлера +5 % овечої крові («Himedia», Індія). Для створення анаеробних умов був використаний анаеростат з системою створення анаеробних умов (AnaeroGen System – «Oxoid», Великобританія).

Результати: Встановлено, що у дітей з односторонніми та двосторонніми розщілинами твердого та м'якого піднебіння з розщілинами губи при найнижчих значеннях цифрових даних гігієнічних індексів показники були незадовільними, якісний та кількісний склад мікробіоти ротової порожнини характеризувався домінування умовно-патогенних, аеробних та факультаттивно анаеробних мікроорганізмів. Мікробіота ротової порожнини даних пацієнтів характеризувалась як дисбактеріоз із домінуванням факультативної умовно патогенної мікробіоти, до яких відносяться збудники опортуністичних інфекцій. Порівняння мікробіоти ротової порожнини у дітей із розщілинами твердого та м’якого піднебіння з розщілинами губи, односторонніми та двосторонніми показало, що кількість представників факультативної мікробіоти була вищою у дітей із двобічним ураженнями. Водночас, спектр видового складу домінуючих представників мікробіоти практично не відрізнявся.

Висновки: У мікробіоті ротової порожнини у дітей із розщілинами твердого та м’якого піднебіння з розщілинами губи, односторонніми та двосторонніми домінували 2-5 асоціативні комплекси, представлені грампозитивними, грамнегативними бактеріями та мікроскопічними грибами роду *Candida* у комплексі з пародонтопатогенними мікроорганізмами, здебільшого «помаранчевого комплексу Сокранського». Показано, що за двосторонніх розщілин титр представників факультативної мікробіоти був вищим, у структурі мікробного ценозу виявляли представники строгих: анаеробів, що відносяться до пародонтопатогенів. У мікробному ценозі із двосторонніми розщілинами твердого та м’якого піднебіння з розщілинами губи були присутні анаеробні бактерії *Prevotella, Peptococcus, Peptostreptococcus, Fusobacterium* на фоні високих титрів умовно патогенних мікроорганізмів.

Ключові слова: вроджені щелепно-лицеві аномалії, незрощення піднебіння, гігієна ротової порожнини, мікробні ценози, біотопи ротової порожнини, стоматологічні захворювання, опортуністичні мікроорганізми.

Автор, відповідальний за листування*: Тетяна Тімохіна, кафедра терапевтичної стоматології, Національний медичний університет імені О.О.Богомольця, Київ, Україна*
e-mail: tanyatimokhina@gmail.com

ABBREVIATIONS

CLP - Cleft Lip and Palate
CFU - Colony Forming Unit
OPI - Orthodontic Plaque Index
PI - O’Leary’s Plaque Index
GBI - Gingival Bleeding Index
PMA - Papillary-Marginal-Alveolar Index

INTRODUCTION

The infant’s oral cavity, initially sterile during intrauterine development, transforms into a diverse ecosystem colonized by numerous microorganisms within the first hours after birth. Alongside other bacteria, these microorganisms participate in the establishment of a “colonization cascade” that influences the subsequent development of local microbiota [1]. It has been suggested that infants acquire microorganisms during a specific phase of oral bacterial colonization, referred to as the “window of infectivity” [2].

Congenital cleft lip and palate (CLP) significantly alters the oral microbiome, resulting in a microbiological profile that increases the risk of carious, periodontal, and hypoplastic lesions, as well as systemic diseases in affected young children and adolescents. The oral microflora of CLP patients has been shown to harbor potentially pathogenic fungi and bacteria, including *Candida, Staphylococcus aureus, Lactobacilli, and Streptococcus mutans* species. These changes may lead to oral disease and postoperative complications, potentially necessitating additional surgical intervention [3].

Furthermore, malocclusion and the use of orthodontic appliances can alter the local oral environment, causing an imbalance in the oral microbiome [4]. Compromised innate immunity increases the risk of oral infections, including candidiasis, herpetic gingivostomatitis, aphthous ulcers, and severe periodontal disease [5, 6]. Surgical procedures are typically performed within the first few months after birth to repair CLP. A higher prevalence of Gram-negative microorganisms is observed before surgery, while an increased presence of Gram-positive microorganisms is noted afterward. This shift is associated with the surgical closure of the nasopharyngeal space. Additionally, children with CLP have a markedly elevated risk of early colonization by pathogenic microorganisms [7].

Anatomical changes in CLP inevitably affect the composition of oral microbiota, leading to increased colonization by potentially pathogenic fungi and bacteria, including *Candida species, Staphylococcus aureus, Lactobacilli,* and *Streptococcus mutans*. These changes often promote the formation of dysbiotic biofilms, as supported by numerous studies [4, 8]. The cariogenic and periodontal microbiota in young children and adolescents with CLP contains highly pathogenic microorganisms, which contribute to more severe disease progression and complications during postoperative recovery. Biofilms on tooth surfaces produce acids that demineralize enamel, thereby facilitating the invasion of cariogenic bacteria into the enamel, dentin, and even the pulp [9]. In CLP patients, the oronasal connection differs from the postoperative condition, resulting in a unique oral microbiome. Microbiota analysis of saliva samples identified a total of 23 microbial types across 38 classes, 111 orders, 184 families, 327 genera, and 612 species. The predominant genera in patients with and without CLP were *Streptococcus* (29.61% and 30.27%), *Neisseria* (18.62% and 5.99%), and *Prevotella* (8.82% and 8.93%). Notably, the microbial community in CLP patients exhibited a more even distribution, as indicated by the Pielou index, whereas species distribution was uneven in non-CLP patients. These findings indicate that oral microbial communities in CLP patients have unique ecological characteristics and structural differences. Cariogenic bacteria and periodontal pathogens may serve as biomarkers for CLP and correlate with a higher prevalence of dental caries and periodontal diseases [10]. A meta-analysis of 18 studies assessing periodontal parameters (plaque index, gingival index, bleeding index, periodontal probing depth, clinical attachment level) revealed a correlation between CLP and a higher risk of poor oral health [11]. Patients with cleft lip and palate who wore fixed braces exhibited poor oral health and elevated microbial counts, underscoring the necessity for enhanced oral hygiene practices in this population [12].

Given the challenges of maintaining oral hygiene postoperatively in children with cleft lip and palate, this study aimed to investigate the oral microbiota in children with these developmental anomalies.

Objective: to investigate the microbiota of various oral biotopes in children with cleft hard and soft palate with cleft lip.

MATERIALS AND METHODS

The study included 102 children aged 8 to 18 years who are receiving comprehensive rehabilitation at the Consultative and Diagnostic Polyclinic and in the Department of Reconstructive and Plastic Microsurgery of the National Children's Specialized Hospital "OKHMATDYT" (Kyiv, Ukraine). The participants were assigned to four groups: 46 patients with congenital cleft hard and soft palate with bilateral cleft lip; 44 patients with congenital cleft hard and soft palate with unilateral cleft lip; and a comparison group including 12 patients with combined incomplete nonunion. The control group (n=25) included healthy adolescents without congenital developmental anomalies.

The clinical examination involved a comprehensive evaluation of children's oral mucosa and periodontal tissues: gingivitis indices (PMA), plaque index (PI), gingival bleeding intensity (GBI). The microbiological analysis involved plating oral swabs on differential and diagnostic culture media.

In the study, we used a swab from the palate and gums, collected with a sterile applicator in a transport tube (AMIES). The material was seeded onto nutrient media according to the Gold sector culture method, using nutrient media (Himedia): Sabouraud Dextrose Agar for the cultivation of microscopic fungi; blood agar (MPA + 5% blood) for bacteria of the *Streptococcus and Neisseria genus*; streptococci isolated using Streptococcus Selective Agar (Himedia) and Mitis salivarius agar (Himedia); Endo and Levin media (Farmaktiv, Ukraine) for bacteria of the Enterobacteriaceae genus, while yolk-salt agar with mannitol for bacteria of the *Staphylococcus genus*; enterococci isolation was carried out on Bile Esculin Azide Agar, and *Pseudomonas aeruginosa* was cultured on Pseudomonas Isolation Agar (HiMedia). Bacteria and microscopic fungi were identified using morphological, tinctorial, and biochemical characteristics, implementing the identification systems ENTERO-test, STREPTO-test, and STAPHYLO-test produced by Erba Lachema (Czech Republic) [13].

Data were analyzed with the statistical package IBM SPSS Statistics Base (version 22) and EZR. All results were considered statistically significant at a value of $p<0.05$. Quantitative data are presented as mean (M) ± standard deviation (SD), unless otherwise stated. The normality of the data distribution was checked using the Shapiro-Wilk test.

RESULTS

Children with cleft hard and soft palate with cleft lip usually experience some complications, which make them more predisposed to oral health deterioration compared to those without congenital maxillofacial anomalies. Our data revealed that children from both groups were susceptible to inflammatory diseases affecting the oral mucosa, lips, and gums [14, 15]. A higher incidence was noted in patients with cleft hard and soft palate with bilateral clefts, who exhibited varying degrees of gingivitis, generalized lesions, swollen and sore gums, bleeding gums, particularly pronounced in the frontal and lateral regions of the upper jaw – areas that often coinciding with substantial dental plaque accumulation. Patients with cleft hard and soft palate with unilateral cleft lip had a less severe clinical picture due to the involvement of only one side. However, most patients exhibited considerable inflammation in the marginal gingiva, suggesting moderate gingivitis. Our findings indicated that at the lowest hygiene index values, specifically O'Leary's Plaque Index (PI) the oral hygiene of these patients was assessed as poor during ongoing monitoring (Table 1, Fig.1).

Table 1 – Index assessment of oral hygiene and periodontal tissues in children with cleft hard and soft palate with unilateral cleft lip and cleft hard and soft palate with bilateral cleft lip

Index	Cleft hard and soft palate with unilateral cleft lip (n=44)	Cleft hard and soft palate with bilateral cleft lip (n=46)	p
O'Leary's Plaque Index (PI), %	60.59±6.52	76.25±4.48	<0,001
Gingival Bleeding Index (GBI), %	36.21±4.56	45.46±4.95	<0,001
Papillary-Marginal-Alveolar Index (PMA), %	51.60±4.11	55.33±3.35	<0,001

Notes: p - the probability indicator difference between groups

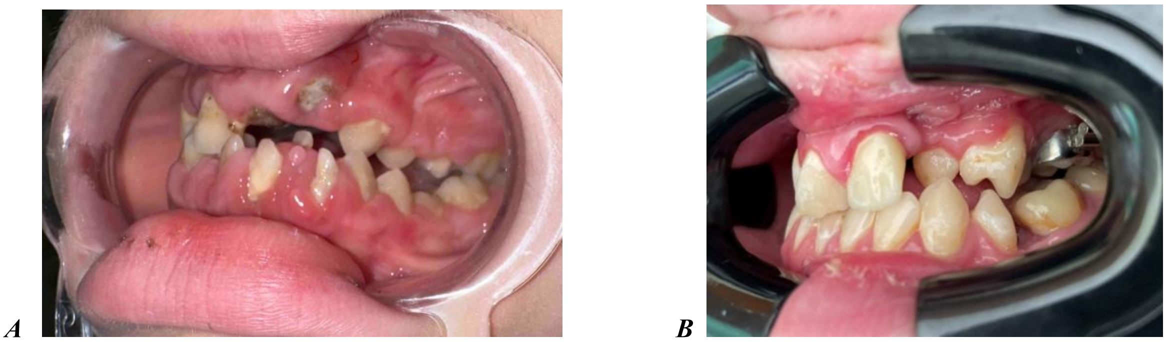

Figure 1. A) Cleft hard and soft palate with unilateral cleft lip; B) Cleft hard and soft palate with bilateral cleft lip

Gum inflammation is triggered by various mechanisms, including local damaging factors associated with congenital pathologies, multi-stage surgical interventions, orthodontic appliances, and impaired self-cleaning that contribute to a significant bacterial impact on the oral organs and tissues.

Research indicates that the microbiota of the "palate" and "gum" biotopes in adolescents with cleft lip and palate exhibit oral dysbiosis, characterized by a predominance of facultative, conditionally pathogenic microbiota and the absence of autochthonous microbiota representatives.

A study of the microbiota of the palate and gums in children with congenital cleft hard and soft palate with unilateral cleft lip revealed that the examined biotopes primarily harbor associations of microorganisms, mainly facultative oral microbiota, including opportunistic pathogens. Disruptions in these microbial coenoses, characterized by the dominance of facultative and allochthonous species, indicate an imbalance resulting from anatomical defects and surgical procedures (Table 2, Fig. 2, 3).

The predominant microorganisms in the microbial coenoses were bacteria of the *genus Staphylococcus, Enterobacteralis, microscopic fungi* of the *genus Candida*, etc. The microbiota of the studied biotopes typically consisted of a microbial association of 2 to 5 species.

Table 2 – Prevalence of facultative oral microbiota in children with congenital cleft hard and soft palate with unilateral cleft lip, n=44

Species/genus of the microorganism	Frequency of detection in the microbiota structure, abs. quantity/%	Average titer of detection of microorganisms in the biotope, CFU
Staphylococcus aureus	24 / 54.5	10^{5}
Bacteria of the genus *Enterobacteralis*	24 /54.5	10^5
Microscopic fungi of the genus *Candida*	26 / 59.0	10^5
Bacteria of the genus *Peptostreptococcus*	8 /33.3	10^5
Streptococcus pneumoniae	8 / 33.3	10^4
Bacteria of the genus *Prevotella*	8 / 33.3	10^5
Bacteria of the genus *Peptococcus*	6 / 13.6	10^5
Fusobacterium nucleatum	4 / 9.0	10^2
Veilonella parvula	4 / 9.0	10^4
Streptococcus pyogens	4 / 9.0	10^5
Porphyromonas gingivalis	2 / 4.5	10^5
Actinomises spp.	2 / 4.5	10^5

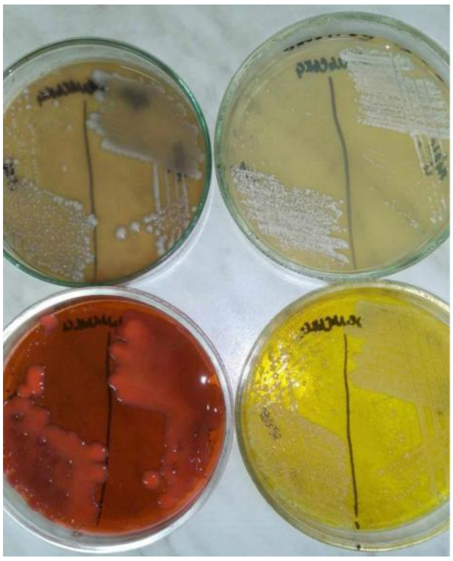

Fig. 2 Results of the microbiological analysis of the oral microbiota in a patient with congenital cleft hard and soft palate with unilateral cleft lip *(S.aureus, Klebsiella spp., Candida albicans, Petostreptococcus spp.)*

Fig. 3 Results of the microbiological analysis of the oral microbiota in a patient with congenital cleft hard and soft palate with unilateral cleft lip *(Staphylococcus epidermidis, Enterobacter aerogenes, Candida albicans)*

Within *Enterobacteralis*, common species included *E.coli, H. alvei, K. pneumoniae, Citrobacter freundii, and Enterobacter spp*. The microbiota also contained anaerobic microorganisms associated with periodontal disease, such as *Prevotella and Peptostreptococcus bacteria*. *C.albicans* was the most frequently observed fungus, with *C. pseudotropicalis* found only occasionally (2 cases). Overall, 63.6% of patients with unilateral nonunion exhibited periodontal infections linked to anaerobic bacteria (*Prevotella intermedia, Peptostreptococcus micros, Veilonella parvula, Fusobacterium nucleatum, Actinomises spp., Porphyromonas gingivalis*).

The comparison of oral microbiota in children with unilateral and bilateral cleft hard and soft palate with cleft lip revealed a higher prevalence of facultative microbiota in those with cleft hard and soft palate with bilateral cleft lip, estimated at 105-107 CFU/ml. The spectrum of the most prevalent microbiota species showed minimal differences between the groups (Table 3, Fig. 4). Bacteria from the genus *Streptococcus* were less frequently found in cases of cleft hard and soft palate with bilateral cleft lip to unilateral clefts. 69.6% of individuals with periodontal pathogenic microorganisms (*Prevotella intermedia, Peptostreptococcus micros, Veilonella parvula, and Fusobacterium nucleatum*) had a slightly higher incidence in unilateral clefts. However, the microbial titers of these pathogens were significantly greater in bilateral clefts. Bacteria from the genera *Staphylococcus, Enterococcus*, and anaerobic periodontal pathogens were found in 34.8% of patients with bilateral cleft lip and palate. Patients with this microbiota profile had the most pronounced signs of inflammatory periodontal changes. Obligate anaerobes, identified as pathogens in inflammatory periodontal diseases, were more commonly detected in cases of bilateral cleft lip and palate (Table 4).

Table 3 – Prevalence of facultative oral microbiota in children with cleft hard and soft palate with bilateral cleft lip, n=46

Species/genus of the microorganism	Frequency of detection in the microbiota structure, abs. quantity/%	Average titer of detection of microorganisms in the biotope, CFU
Staphylococcus aureus	22 / 47.8 %	10^5
S.haemolyticus	4 / 8.7	10^3
S.epidermidis (with hemolytic properties)	4 / 8.7	10^3
Bacteria of the genus *Enterobacteralis*	34 /73.9	10^5
Microscopic fungi of the genus *Candida*	32 / 69.6	10^5
Streptococcus pneumoniae	8 / 17.4	10^3
Bacteria of the genus *Peptostreptococcus*	12 / 26.0	10^5
Bacteria of the genus *Peptococcus*	10 / 21.7	10^5
Bacteria of the genus *Prevotella*	14 / 30.4	10^6
Veilonella parvula	4 / 8.7	10^3
Fusobacterium nucleatum	2 / 4.3	10^3

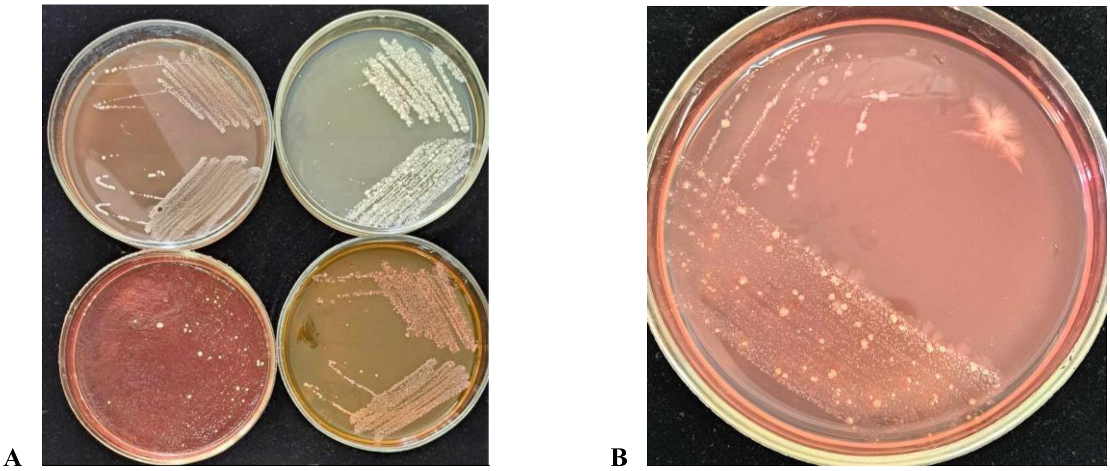

Fig. 4. Results of the microbiological analysis of the oral microbiota in a patient with cleft hard and soft palate with bilateral cleft lip (A-*Candida albicans, Citrobacter freundii*; B-*Peptococcus spp., Veilonella parvula*)

Table 4 – Number of species of microbial associates - representatives of facultative microbiota – in children with cleft hard and soft palate with unilateral cleft lip or cleft hard and soft palate with bilateral cleft lip

Number of microbiota representatives	Cleft hard and soft palate with unilateral cleft lip, abs. number of patients/%, n=44	Cleft hard and soft palate with bilateral cleft lip, abs. number of patients/%, n=46
Two-species association	10 / 22.7	14 / 30.4
Three-species association	16 / 36.4	12 / 26.4
Four-species association	12 / 27.3	18 / 39.3
Five-species association	6 / 13.6	2 / 4.3

We also studied the microbiota in patients with a “combined type of cleft” (n = 12). The microbiocenoses of the examined biotopes exhibited qualitative and quantitative differences in microbiota profile compared to healthy individuals. We observed an increased concentration of conditionally pathogenic aerobic and anaerobic microorganisms. There was no statistically significant difference between the groups of individuals with unilateral or bilateral clefts.

Thus, anatomical changes resulting from unilateral or bilateral nonunion and recurrent interventions influence the composition of oral microbiota. This often leads to colonization by conditionally pathogenic and periodontal pathogenic microorganisms. An increased level of these microorganisms serves as an etiological factor for chronic inflammatory processes in the mucous membrane and periodontal tissues.

DISCUSSION

The microbiota of patients with CLP has been extensively studied, revealing that young children and adolescents with CLP may exhibit elevated levels of *Streptococcus mutans, Candida spp*., and *Lactobacillus spp*. in their saliva [16]. The colonization rate of *Candida* species in patients with CLP (63.3%) was significantly higher than that in healthy individuals (18.3%). *Candida* colonization was most prevalent in CLP patients who had undergone at least three surgeries (78.2%) and in those with bilateral clefts (77.7%) [17]. Moreover, abnormal oral microbiomes arising from the development and surgical correction of CLP can significantly affect oral health of CL/P patients, as well as influence the bacterial composition and condition of other bodily structures. Our research also found elevated concentrations of microscopic *Candida spp*. in both unilateral and bilateral clefts.

Our research revealed the persistence of anaerobic bacteria from Socransky’s orange complex, particularly *Prevotella* species, which were the most prevalent in dental plaque biofilms on teeth adjacent to the cleft site in CLP patients compared to those without a cleft. Nevertheless, studies indicate that *Streptococcus* and *Lactobacillus* are the next most common constituents of oral microbiome in CLP patients [16]. *Aggregatibacter actinomycetemcomitans* is another significant pathogenic bacterium in dental biofilms associated with CLP. The concentrations of *A. actinomycetemcomitans, P. gingivalis*, and *T. forsythia* were significantly elevated in the subgingival biofilm of CLP patients, emphasizing the importance of early screening of adolescents with CLP and permanent dentition to prevent periodontal disease [18]. *Porphyromonas gingivalis* in young children and adolescents signals potential immunological changes, as the microorganism locally infects periodontal tissues while escaping host defenses. It employs virulence factors that disrupt innate immunological and inflammatory responses. Regarding periodontal microbiota, bacteria such as *Campylobacter spp., Fusobacterium spp., Fusobacterium nucleatum, Prevotella intermedia/nigrescens, Parvimonas micra,* and *Porphyromonas gingivalis* were identified in CLP patients [20]. *Porphyromonas gingivalis* levels were elevated in both supragingival and subgingival plaque biofilms on teeth adjacent to CLP. However, Perdikogianni et al. (2009) found no significant difference in P. gingivalis colonization in subgingival plaque samples from the cleft area versus control sites, suggesting a specific localization of oral bacteria associated with CLP [20]. Given that age correlates positively with the progression of periodontal disease and individuals with CLP have an increased risk of plaque accumulation and gingivitis, clinicians should prioritize preventive dental care for these patients from an early age [21].

Our findings align with those of Escobar-Arregocés et al. (2024), who aimed to characterize the oral microbiota and explore the correlation between dental caries and periodontal health in patients aged 0–18 years with non-syndromic CLP. The cariogenic microbiota in young children and adolescents with CLP resembled that of their counterparts without CLP, although there was a higher prevalence of *Streptococcus mutans* and *Lactobacillus spp*. The periodontal pathogenic microbiota was linked to the presence of *Campylobacter spp., Fusobacterium spp., Fusobacterium nucleatum, Prevotella intermedia/nigrescens, Parvimonas micra, and Porphyromonas gingivalis*, microorganisms recognized for their significant pathogenic potential. The microbiota’s heterogeneity before and after surgery included *Staphylococcus aureus, Streptococcus beta-hemolyticus, Klebsiella pneumoniae, Klebsiella oxytoca, Moraxella catarrhalis,* and multiple *Candida species*, such as *Candida albicans, Candida krusei,* and *Candida tropicalis*. The meta-analysis indicated that patients with CLP were 2.03 times more susceptible to caries compared to young children and adolescents without CLP. The authors concluded that the microbial diversity within the oral cavity is substantial and may vary based on the type of cleft and surgical procedures performed [22].

Zhang et al. (2022) investigated the impact of alveolar bone grafting on the oral microbiota in children with non-syndromic CLP (n=28) [23]. Patients provided unstimulated saliva and plaque samples before surgery, 2 days, 1 month, and 3 months after surgery. Using the Illumina MiSeq sequencing of the V3-V4 hypervariable regions of the 16S rRNA gene, it was observed that microbial diversity in both saliva and plaque significantly decreased 2 days after surgery, then gradually increased at 1 and 3 months. The salivary and plaque microbiota profile at 2 days after surgery was markedly different from that at other intervals. However, by 1 and 3 months, the microbiota began to revert toward the preoperative profile. Alveolar bone grafting changed the salivary microbiota of children with CLP within 3 months after surgery [23]. Based on the clinical examination of the surgical sites 3 months after surgery, patients (n=28) were divided into a non-inflammatory group (n=15) and an inflammatory group (n=13). The diversity of the operative taxa in the inflammatory and non-inflammatory samples and the microbial variations in the oral cavity were significantly different between the two groups before and after surgery. Analysis of the relative taxa abundance before surgery revealed 26 units with a relative abundance above 0.01%, reflecting a significant difference between the groups. According to the principal component analysis of the preoperative samples, the taxa associated with inflammation included *Tannerella sp., Porphyromonas sp., Gemella sp., Moraxella sp., Prevotella nigrescens, and Prevotella intermedia*. These results indicate that preoperative oral microbiota profiles may be associated with the risk of postoperative inflammation [24]. Our studies have observed the persistence of facultative and periodontal pathogenic microbiota in samples taken in the remote postoperative period.

Alveolar bone grafting (ABG) is usually performed in adolescence, leaving the cleft in the oral cavity for a long time. The research [25] also highlights that the microbial composition of dental plaque in patients with alveolar clefts significantly differed from that in healthy age-matched patients in terms of *Neisseria, Haemophilus, Fusobacterium, Rhodococcus, Aggregatibacter, Gemella and Porphyromonas, with Capnocytophaga, Rhodococcus, and Actinomyces* being particularly abundant. There were also phenotypic differences in anaerobic, gram-negative, gram-positive bacteria, and oxidative stress responses between patients with larger alveolar clefts and healthy individuals.

Current understanding emphasizes that immune system functioning and changes in the oral microbiome are central to the development of oral diseases [26]. Therefore, clinical teams should focus on reducing the risk of diseases affecting the hard tissues of the teeth, periodontal tissues, and oral mucosa by closely monitoring oral hygiene, microbiota profiles, and adhering to established clinical guidelines. This includes outpatient therapeutic preparation for surgical interventions, orthodontic treatments, and postoperative rehabilitation to optimize patient outcomes.

CONCLUSIONS

1. The oral microbiota in children with cleft hard and soft palate with cleft lip was characterized by dysbiosis, marked by the predominance of facultative, conditionally pathogenic microbiota. The microbial coenoses included opportunistic pathogens within the oral biotopes.
2. The oral microbiota was primarily composed of 2 to 5 associative complexes, including Gram-positive and Gram-negative bacteria, fungi of the genus Candida, and periodontal pathogenic microorganisms, predominantly belonging to Socransky’s orange complex.
3. The titer of facultative microbiota was higher in patients with bilateral cleft hard and soft palate with cleft lip, and their microbial coenoses contained obligate anaerobes associated with periodontal diseases. Anaerobic bacteria such as *Prevotella, Peptococcus, Peptostreptococcus,* and *Fusobacterium* were detected in cases of bilateral cleft hard and soft palate with cleft lip, with higher concentrations of conditionally pathogenic microorganisms.

PROSPECTS FOR FUTURE RESEARCH

Identified disturbances in the oral microbiota composition highlight the relevance of developing methodological approaches for microbiome correction in the oral cavity, particularly under conditions of congenital anomalies such as cleft lip and palate.

AUTHOR CONTRIBUTIONS

Tetiana Timokhina – conceptualization and design, methodology, data collection, statistical processing and data analysis, manuscript writing, final approval of the article; **Marina Kryvtsova** – conceptualization, writing (review and editing); **Nina Proshchenko** - final approval of the article; **Mykola Spivak** - critical review.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ARTIFICIAL INTELLIGENCE DISCLOSURE

The authors confirm that no artificial intelligence-based technologies were utilized in the writing or editing of the manuscript.

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INFORMATION ABOUT THE AUTHORS

Tetiana Timokhina

C.M.S., Associate Professor of the Department of Dental Therapy, Bogomolets National Medical University, 13, T. Shevchenko blvd. Kyiv, Ukraine, 01601

e-mail: tanyatimokhina@gmail.com

Marina Kryvtsova

D.B.S., Professor, Head of Department of Clinical Laboratory and Morphofunctional Diagnostics, Uzhhorod National University, 3 Uzhhorod Narodna Square, Transcarpation region, Uzhhorod, Ukraine, 88000

e-mail: maryna.krivcova@uzhnu.edu.ua

Nina Proshchenko

D.M.S., Associate Professor of the Department of Prosthetic Dentistry, General Director of University clinic of the Bogomolets National Medical University, 13, T. Shevchenko blvd. Kyiv, Ukraine, 01601

e-mail: khrolnina@gmail.com

Mykola Spivak

D.B.S., Professor, Director of D.K. Zabolotny Institute of Microbiology and Virology of National Academy of Sciences of Ukraine, 154, Akademika Zabolotnogo St., Kyiv, Ukraine, 03143

e-mail: spivak@imv.org.ua

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