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ORAL MICROBIOTA IN EARLY-CHILDHOOD CARIES PEDIATRIC PATIENTS — THE PILOT STUDY

© Elena A. Klimova, Natalia A. Sokolovich, Diana A. Kuzmina

Saint Petersburg State University, Faculty of Dentistry and Medical Technology. 21 Line V.O. 8a, Saint Petersburg, Russian Federation, 199106

Contact information:
Elena A. Klimova — Assistant of the Department of Dentistry. E-mail: doctor.klimova@yandex.ru ORCID: 0009-0007-1740-3294

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Abstract. Carious lesions continue to be an urgent problem of modern practical healthcare. On the territory of Russia, there is insufficient data from epidemiological studies among children under 3 years of age on the incidence of early childhood caries. In Western countries, patients with early childhood caries are treated without fail under general anesthesia with concomitant hospitalization, which is stressful for the child and family, as well as a costly method for healthcare. Modern metagenomics makes possible to study the fundamental aspects of the problem of the etiology of caries with the subsequent development of etiotropic therapy and means of prevention. Along with other microorganisms, the genus Streptococcus is the leader in the development of the carious process. The study of pathogens, as well as the function of specific genes in dental pathology, becomes possible with the help of metagenomic sequencing. In this paper, children with early childhood caries were studied by polymerase chain reaction followed by metagenomic sequencing. The spectrum of microorganisms in different children was studied and distributed among families. The study showed the need for further study of oral microbiota in children with early childhood caries to identify critically important pathogens.

Key words: *caries epidemiology; early childhood caries; metagenome; biofilm; caries*

МИКРОБИОТА ПОЛОСТИ РТА ПАЦИЕНТОВ ПРИ РАННЕМ ДЕТСКОМ КАРИЕСЕ — ПИЛОТНОЕ ИССЛЕДОВАНИЕ

© Елена Александровна Климова, Наталья Александровна Соколович, Диана Алексеевна Кузьмина

Санкт-Петербургский государственный университет, факультет стоматологии и медицинских технологий.
199106, г. Санкт-Петербург, 21-я линия В.О., 8а

Контактная информация:
Елена Александровна Климова — ассистент кафедры стоматологии. E-mail: doctor.klimova@yandex.ru
ORCID: 0009-0007-1740-3294

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Резюме. Кариозные поражения продолжают оставаться актуальной проблемой современного практического здравоохранения. По территории России недостаточно данных эпидемиологических исследований среди детей до 5 лет по заболеваемости кариесом раннего детского возраста. В западных странах пациентов с кариесом раннего детского возраста лечат в обязательном порядке в условиях общего обезболивания с сопутствующей госпитализацией, что является стрессом для ребенка и семьи, а также затратным методом для здравоохранения. Современная метагеномика дает возможность изучить фундаментальные аспекты проблемы этиологии кариеса с последующей разработкой этиотропной терапии и средств профилактики. Наряду с другими микроорганизмами по значению в развитии кариозного процесса лидирует род *Streptococcus*. Изучение патогенов, а также функции специфических генов при стоматологической патологии становится возможным при помощи метагеномного секвенирования. В настоящей работе исследован биологический материал с поверхности зубов детей с кариесом раннего детского возраста методом полимеразно-цепной реакции с последующим метагеномным секвенированием. Спектр микроорганизмов был изучен и распределен по семействам. Исследование показало необходимость дальнейшего изучения микробиоты у детей с ранним детским кариесом для выявления критически значимых патогенов и разработки специальных средств профилактики, персонализированных данному возрасту.

Ключевые слова: *эпидемиология кариеса; ранний детский кариес; метагеном; биопленки; кариес*

THE RELEVANCE OF THE PROBLEM

Early childhood caries (ECC) is a medical and social problem that seriously affects child health and the quality of life of children and their families. According to the World Health Organization, about 620 million children in the world suffer from this disease. ECC is classified as caries occurring in children younger than 72 months old, with the first signs of the disease manifesting between 18 and 24 months old.

In the scientific literature, early childhood caries (ECC) in children 3–5 years old is defined as one or more carious (C), cariesogenic missing (CM), or sealed (S) temporary anterior teeth or amount of CCMS surfaces more than 4 (at the age of 3 years), more than 5 (at the age of 4 years), or more than 6 (at the age of 5 years).

According to the literature, 7% of children under 3 years old in developed European countries have early childhood caries. In developing countries this indicator reaches 35%. The prevalence of dental caries in children under 3 years old in Moscow reaches 57%? according to L.P. Kiselnikova (2013),. There is no data from epidemiological studies among this age group in Russia.

In Western countries, patients with early childhood caries are treated under general anaesthesia with concomitant hospitalisation. This method is often psychologically stressful for the child and his family. It is also a very expensive method for the health care system. In addition, the statistics of caries recurrences in the next 2 years after ECC sanitation demonstrates a disappointing result. In our opinion, this disease requires a comprehensive study and development of special personalised methods of prevention before the onset of pathology, as well as after complete sanation.

THE FUNDAMENTAL RATIONALE OF THE RESEARCH

Metagenomics provides an opportunity to study the fundamental aspects of the problem of caries etiology with the subsequent development of etiotropic therapy and means of prevention. Along with other microorganisms, the Streptococcus leads in terms of importance in the development of the carious process. The study of pathogens, as well as the function of specific genes in dental pathology becomes possible with the help of metagenomic sequencing.

Oral diseases, including periodontal diseases and dental caries, occupy leading positions in terms of prevalence among human infectious diseases [2]. Caries continues to be an urgent problem in medicine in all regions. Its destructive effect leads to significant expenses in the health care system [3].

The human oral cavity is a moist, warm environment which can be easily colonised by micro-organisms. The presence of both hard and soft tissues, each with a complex microanatomy, favours the creation of many different niches and supports the existence of a diverse microbiota. It is now known that the most common oral diseases, the dental caries and its complications, are likely to be caused by multispecies communities rather than by single, isolated pathogens [1]. Different microbial species coexist and form a polymicrobial biofilm, the plaque in the oral cavity [2, 6].

Metagenomics using next generation sequencing (NGS) technology reproduces bacterial composition and genomic profiles of bacteria to study the relationships between microbial diversity, genetic variations and oral diseases. According to Richard J. Lamont, the study of the genome of oral microorganisms is at an early stage of development [6]. The roles of pathogenic species and the function of specific genes in the development of dental disease have been discovered by metagenomic analysis. Annotations of oral microbial genomes support the hypothesis of association of specific genes or metabolic pathways with oral health and specific diseases [6]. Scientists have also proposed a model of three-level interactions occurring in the microbiome and determining the state of dental health or disease [2].

Streptococcus are the microorganisms with the most important relationship to pathological processes. These microbes are often the first surface colonisers and numerically dominant in the human oral cavity. A large number of interactions between *Streptococcus* and other bacteria have been documented and suggest their critical importance in the development of multispecies microbial communities and for the transition from dental health to disease states.

Streptococcus are Gram-positive facultative anaerobic cocci. Improvements in microscopy techniques and biofilm models allow a detailed view of the spatial distribution of Streptococcus in oral biofilm. There has been an increase in the use of methods to analyse specific genes that modulate interspecies interactions. A number of studies have also found that Streptococcus produce a spectrum of extracellular factors that facilitate their integration into multi-species communities and enable them to form "social networks" with their "neighbouring" species. These "community integration factors" include adhesins and receptors that promote aggregation and small signalling molecules.

Bishop et al. (2009) identified 4 groups of oral *Streptococcus* (Figure 1) [1]. Two species of Streptococcus group *Mutans*, named *S. mutans* and *S. sobrinus*, were found to be associated with human dental caries. Their acid-producing properties and the potential of being able to exist in an acidic environment are directly related to the cariesogenic potential of these bacteria. In order to survive in the acidic conditions of the human oral cavity with hundreds of competing bacteria, both species have evolved many adaptation mechanisms [4].

The cariesogenic potential of *S. mutans* may be due to multiple virulence factors. These factors are: 1) the ability to metabolise carbohydrates with concomitant release of lactic acid (acid generation); 2) the tolerance to and ability to survive in an acidic environment; 3) the ability to facilitate hydroxyapatite binding and promote intercellular adhesion; 4) the formation of multi-bacterial grouped structures in dental plaque (biofilm formation); and 5) the successful elimination of other bacterial strains through the production of bacteriocins. The biofilm enters a state of progressive cariesogenic potential when these virulence factors of *S. mutans* and other micro-organisms are expressed phenotypically and work together [9].

Molecular studies have shown that *S. mutans* is not always present in caries. Other acid-producing bacteria may also play a role in the pathogenesis of dental caries in some individuals are *Lactobacillus* spp., *Bifidobacterium dentium, S. sobrinus, S. salivarius/S. vestibularis* and *S. parasanguinis* [7–9].

If *S. mutans* is involved, the additional presence of the bifidobacterium *Scardovia wiggsiae* correlates closely with dental caries [5].

The quantitative prevalence of primary colonisers *S. gordonii* and *S. sanguinus* in the oral cavity contributes to limiting the growth of *S. mutans* [7, 8]. The availability of oxygen to *S. gordonii* and *S. sanguinus* and their production of hydrogen peroxide allow these microorganisms to compete effectively with other streptococcal species, including *S. mutans* [7–9]. In contrast, *S. mutans* can antagonise the growth of other oral *Streptococcus* by the formation and release of bacteriocins [7, 8]. Interspecies antagonism, similar to other environmental factors in the oral cavity, helps to determine the outcome of the competitive struggle between *Streptococcus* which is primary colonising the oral cavity and *S. mutans*. Ultimately, the patient's dental health status is shaped or diseases being diagnosed, including dental caries.

MATERIALS AND METHODS

Six children 20–36 months old with no concomitant general pathology, with an unremarkable allergological anamnesis and who had not previously used antibacterial therapy were under observation for 3 months. The children underwent a complete dental examination and the need for routine sanation under general anaesthesia was identified. The patients were divided into two groups according to gender.

Group 1 — 3 female patients on mixed feeding.

Group 2 — 3 male patients on mixed feeding.

The selection criteria was presence of nocturnal breastfeeding and sugar in the diet, when patients were involved in the groups. Patients were subjected to bacteriological and molecular biological tests by polymerase chain reaction (PCR) with real-time fluorescence detection of amplification results before sanitation.

Material used for bacteriological examination was collected from the surfaces of the first temporary molars of the mandible and incisors of the maxilla without special hygienic treatment. The material was taken in the morning, before the brushing procedure, using a sterile paper endodontic pin of standard size (No. 30) and disposable sterile polypropylene probes with synthetic pile, which were then placed in the solution "DNA Express" for subsequent transport. The system was kept at 2–4 °C until transport. Then samples were transferred to the laboratory in a refrigerated state for 1 hour. Bacteriological examination was performed according to the generally accepted rules of clinical anaerobic microbiology.

The samples were then subjected to high-throughput metagenomic sequencing at the SPbSU Resource Centre using an Ion Torrent PGM

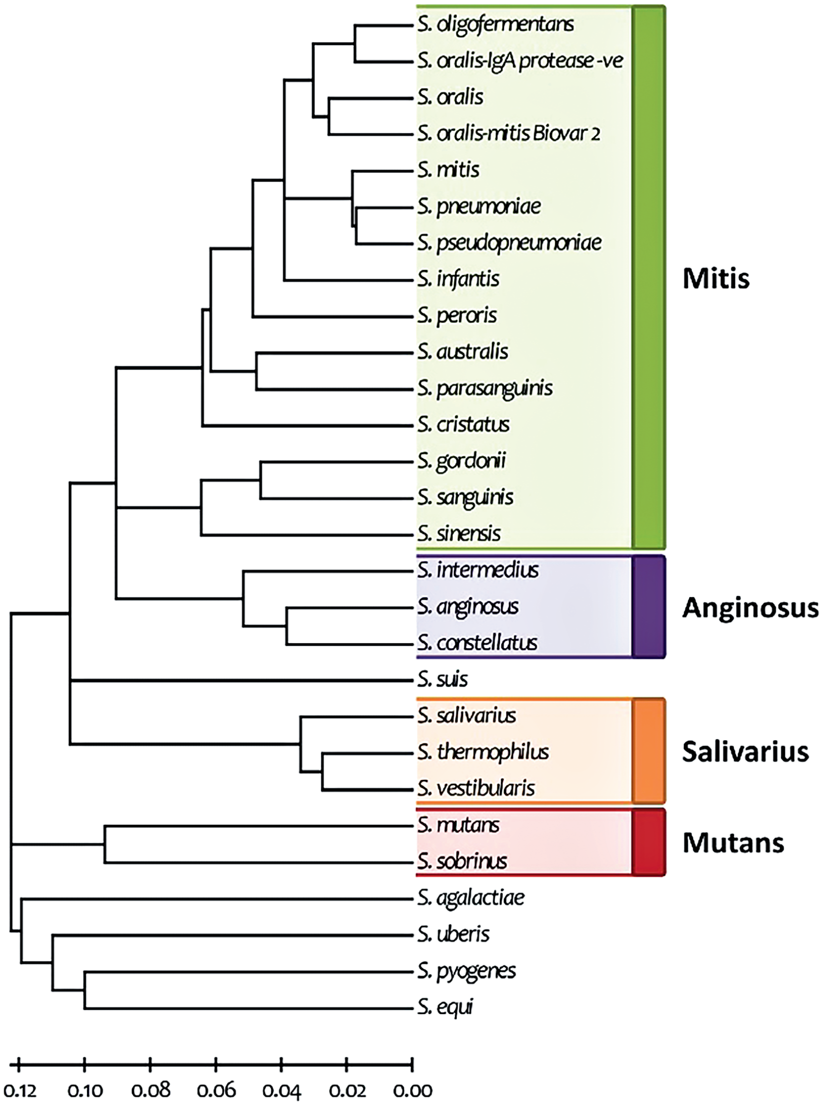

Fig. 1. Phylogenetic tree of the genus Streptococcus (according to Bishop et al., 2009)
Рис. 1. Филогенетическое древо рода *Streptococcus* (по Bishop et al., 2009)

sequencer (Life Technologies) for 16S RNA marker genes using "universal" primers to identify the microbial community.

CONCLUSIONS

Plaque samples obtained from the teeth of 1st group showed a higher number of opportunistic and cariesogenic microflora in contrast to samples from group 2. Due to the high cost of the study, these results need quantitative support and future confirmation or refutation. In our opinion, they are worthy of discussion in the professional community on the basis of the significance of this pathology in terms of the proportion of ECC among dental diseases of children.

The next step is to study the changes in the microbiota during 6–18 months after sanitation to draw preliminary conclusions on the materials used. The other step is to change the composition of child's microbiota under conditions of complete sanitation. This publication also aims to encourage paediatric dentists to actively participate in research on ECC.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Competing interests. The authors declare that they have no competing interests.

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Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

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