

**SPECIES OF STREPTOCOCCUS CAUSING CARIES IN CHILDREN AND THEIR
LABORATORY DIAGNOSIS**

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Abstract. This article highlights the role of bacteria belonging to the genus *Streptococcus*, particularly *Streptococcus mutans* and *Streptococcus sobrinus*, as the primary etiological factors of dental caries in children. Nowadays, dental caries is thought to be an ecological imbalance in the oral biofilm that causes the hard tissues of the tooth to dissolve. Dental decay has historically been believed to be caused by two species of the *Streptococci* group: *Streptococcus mutans* (SM) and *Streptococcus sanguinis* (SS). The pathogenesis of caries development, biofilm formation mechanisms, and demineralization processes are explained on a scientific basis. Laboratory diagnostic methods—microscopic, bacteriological, biochemical, immunological, and molecular (PCR) methods—are also extensively covered. The importance of assessing the risk of caries and prescribing preventive measures through modern diagnostics is demonstrated. Early detection of this disease in children plays an important role not only in maintaining dental health but also in strengthening overall health. Microbiological control, individual hygiene, and proper nutrition are considered interconnected factors in the prevention of caries.

Keywords. Caries, *Streptococcus mutans*, *Streptococcus sobrinus*, biofilm, plaque, demineralization, laboratory diagnostics, PCR, pediatric dentistry, karyogenic bacteria.

Introductio. Dental caries is one of the most common chronic infectious diseases worldwide, occurring particularly frequently among children. According to the World Health Organization, 60-90% of school-age children have caries. Caries is a multifactorial disease associated with biofilm, and microorganisms, dietary habits, the protective properties of saliva, and the structural state of tooth enamel play an important role in its development. (1, 4). Microbiological studies conducted in recent years indicate the *Streptococcus mutans* complex as the primary etiological factor of caries. These bacteria produce organic acids by forming a biofilm on the tooth surface and fermenting carbohydrates, resulting in the demineralization of tooth enamel. Caries is significant not only for damaging local dental tissues but also for negatively affecting the child's nutrition, speech, general development, and quality of life. Early-onset caries is clinically significant as a source of pain, insomnia, decreased appetite, and chronic inflammation. (5,6) The high prevalence of caries in children makes this disease one of the most pressing problems in dentistry. In particular, if milk tooth caries is not detected in time, it can



negatively affect the development of permanent teeth. Therefore, it is important to study the microbiological foundations, etiological factors, and modern diagnostic methods of caries. (4,7). Dental caries is a prevalent, complex, and multidimensional disease that is influenced by the host's genetic, environmental, socioeconomic, and microbiological characteristics. The teeth get demineralized when a high-sugar diet and the presence of bacteria that create acid in the dental biofilm disrupt the equilibrium in the interface. The dental biofilm is composed of organized bacterial populations at a solid/liquid contact. It has been demonstrated that early colonization with the cariogenic SM in newborns and young children may increase the severity of the lesions and predispose them to future caries, even though caries can affect persons of all ages. Furthermore, caries affects 60–90% of children worldwide, with low-income countries suffering the brunt of the disease, despite other countries experiencing a decrease in its frequency (8,9,10). The current view states that a major factor in the development of dental caries is the ecological change in the microbial composition towards cariogenic species brought on by sugar. In the cariogenic environment, some conditional infections flourish because they can more successfully compete with commensal microbes for the tooth surface, leading to illness. Only 20% of the supragingival bacteria in the oral biofilm are oral streptococci, despite making up 80% of the first colonizers during early biofilm development. The body benefits from the commensal microorganisms in the dental biofilm because they keep pathogenic bacteria from colonizing and causing illness. In the cariogenic environment, some conditional infections flourish because they can more successfully compete with commensal microbes for the tooth surface, leading to illness. Carious lesion formation and dissemination are associated with some Streptococci. There is disagreement on the connection between the growth in SM and the occurrence of caries, despite the fact that SM is essential to the development of caries (11,12,13).

The main purpose of the presented manuscript is to provide species of streptococcus causing caries in children and their laboratory diagnosis, based on the results of authoritative scientific works, regarding the relevance of improving their prevention and treatment.

SPECIES OF STREPTOCOCCUS AND THEIR ROLE IN CARIA. The oral cavity is considered a complex micro-ecosystem, containing hundreds of species of microorganisms. Bacteria belonging to the genus Streptococcus occupy a special place in this microflora and are of great importance in the development of caries. Among streptococci, Streptococcus mutans and Streptococcus sobrinus are considered the primary etiological agents of caries, as they possess the ability to produce strong acid and biofilm. (2,5) At the same time, species such as Streptococcus sanguinis, Streptococcus gordonii, and Streptococcus mitis belong to the normal oral microflora, but they can also indirectly affect the caries process under certain conditions. These species are among the microorganisms that initially colonize the tooth surface and subsequently create conditions for other bacteria. When the ecological balance in the oral cavity is disrupted, karyogenic species gain the upper hand. (8,9) The leading role of Streptococcus mutans and Streptococcus sobrinus in caries is explained by their high acidogenic and aciduric properties. They actively break down carbohydrates, form a large amount of organic acids, and remain viable in an acidic environment. This leads to the gradual erosion of tooth enamel. (5,10)

MUTANT BIOLOGY AND PATHOGENICITY OF STREPTOCOCCUS. Streptococcus mutans is a Gram-positive, facultative anaerobic coccus and is the primary etiological factor in caries. The pathogenicity of this microorganism is determined by several important characteristics. First of all, it produces the enzyme glucosyltransferase, which converts sucrose into viscous glucans, thereby firmly adhering to the tooth surface. (10,11). In addition, it has the ability to form a strong biofilm and plays a leading role in the formation of dental plaque. S. mutans produces lactic acid by fermenting carbohydrates, which leads to the destruction of the enamel. At the same time, the bacterium possesses aciduric properties that allow it to survive in



low pH environments, giving it an advantage over other microorganisms. (11,12). This bacterium easily binds to the acquired pellicula of the tooth using adhesins on the cell surface. Subsequently, the synthesis of extracellular polysaccharides intensifies, ensuring the interaction of bacteria and the formation of a dense biofilm. Metabolic activity is high within this biofilm, and acids act on the tooth surface for a long time. (13,14). *Streptococcus sobrinus* also possesses karyogenic properties, and in some patients, the risk of caries increases when found in combination with *S. mutans*. Some studies have shown that the coexistence of these two types is associated with the more rapid development of caries. (2,15)

PATHOGENESIS OF CARIES. The development of caries is a complex and step-by-step process, in which bacteria attach to the gum layer on the tooth surface during the first stage. At the next stage, a biofilm is formed using glucans, and the bacteria are firmly attached to each other. (6,13) During fermentation, carbohydrates are broken down, and organic acids, primarily lactic acid, are formed. Under the influence of these acids, mineral substances in the tooth enamel, namely calcium and phosphate ions, are removed, and the demineralization process begins. This process is typically activated when the critical pH level is below 5.5. If the process continues, cavitation occurs in the tooth and a caries site is formed. (5,16); The time factor is also important in pathogenesis. That is, the more frequently carbohydrate foods are consumed, the more the pH level on the tooth surface decreases, and there is insufficient time for remineralization. As a result, the enamel recovery process slows down, and demineralization begins to prevail. (4,17) Caries first begins at the stage of white spots. During this period, a clouded white spot appears clinically on the surface of the enamel. At this stage, the process can be reversed with proper preventive measures. If therapeutic and preventive measures are not taken, a cavity may form in the enamel, with subsequent damage to the dentin and pulp. (16,18)

FACTORS OF CARIES DEVELOPMENT IN CHILDREN. The development of caries in children occurs under the influence of a number of endogenous and exogenous factors. Endogenous factors include decreased salivary secretion, insufficient mineralization of dental enamel, and incomplete formation of the immune system. Due to the fact that the enamel of milk teeth is thinner and less mineralized than permanent teeth, caries develops faster in them. (7,18) Exogenous factors are more related to lifestyle and are explained by conditions such as excessive consumption of sugary products, non-compliance with oral hygiene, and frequent meals. The transmission of karyogenic bacteria from mother to child, i.e., vertical transmission, is also one of the important factors. The microflora in the mother's oral cavity can be important in the formation of the child's early microbiota. (3,19,22) Furthermore, habits such as drinking sweet drinks at night, feeding for a long time with sweet mixtures in a bottle, and rubbing sugar into the nipple accelerate the development of early-onset caries. Such conditions cause the development of rapid and numerous caries foci, especially in frontal canines. (20) Social factors are also of great importance. If the parents' dental literacy is low, the child develops poor oral hygiene. Inadequate preventive examinations, the lack of fluoride products, and an unhealthy diet increase the risk of caries. (1,4)

ORAL BIOPHILM AND THE SIGNIFICANCE OF PLACE. Biofilm plays a central role in the development of dental caries. The biofilm is a complex biological structure adhering to the tooth surface, composed of microorganisms, their metabolic products, and polysaccharides. Dental plaque is the clinical manifestation of this biofilm. (6,13). Inside the biofilm, bacteria are protected to a certain extent from the external environment. They interact metabolically, utilize nutrients efficiently, and are more resistant to antimicrobial agents. Therefore, a simple mouthwash cannot completely remove the biofilm; it must be removed mechanically using a toothbrush and additional hygiene products. (9,14) Under conditions of biofilm compaction and an abundance of carbohydrates, the number of karyogenic bacteria increases. This causes the pH



to remain low for a long time, and the demineralization process intensifies. Thus, the biofilm is one of the central links in the pathogenesis of caries. (10.17)

LABORATORY DIAGNOSIS METHODS. Laboratory diagnostics are crucial for identifying streptococci that cause caries in children. The diagnostic process begins with the collection of clinical material. Usually, dental plaque, saliva, or material from a caries site is examined. These materials are delivered to the laboratory in specialized transport media, such as Amies or Stuart media. Proper sampling and rapid delivery increase the reliability of the test results. (9.21) The Gram staining method is used for microscopic examination, and it is determined that the Gram-positive cocci are arranged in a chain. However, this method does not accurately distinguish the bacterial species. Therefore, microscopy is more often used as a preliminary guiding method. (9) In bacteriological studies, selective media such as Mitis Salivarius Agar and MSB Agar are used. In these environments, *Streptococcus mutans* colonies grow in small, bluish, granular, and sticky forms. The number of bacteria is estimated in CFU/ml. A high number of colonies indicates an increased risk of caries. (11.21) Biochemical tests are used to determine the enzymatic properties of bacteria, such as the fermentation of mannitol and sorbitol, and a negative catalase test are important diagnostic features. These tests help differentiate streptococci from other Gram-positive cocci. (8,9) Bacterial antigens can be detected by immunological methods, including ELISA and latex agglutination tests. Although these methods are fast, their sensitivity and specificity may be lower in some cases than molecular methods. (12.21)

MODERN MOLECULAR DIAGNOSTICS (PCR). Among modern diagnostic methods, PCR technology is the most accurate and sensitive. Using this method, the *gtfB* gene and the 16S rRNA gene specific to *Streptococcus mutans* are identified. The advantage of the PCR method is that it can detect even a very small number of bacteria and gives a result in a short time. (12.15) The Real-Time PCR method allows for the determination of bacterial count, which is important for assessing the risk of caries development. Especially in stages where clinical signs are not yet clearly manifested, the increase in karyogenic microflora can be detected in advance using the PCR method. (15.21) Molecular methods are also widely used in scientific research. With their help, the composition of the oral microbiota, the microbiological profile of caries, and individual risk factors are analyzed in depth. Therefore, PCR is of great importance not only for diagnosis but also for forecasting and developing a preventive plan. (7.12)

DIFFERENTIAL DIAGNOSIS. It is important to distinguish caries from other diseases of the hard dental tissues. Specifically, enamel hypoplasia, fluorosis, erosion, and non-carious lesions in the clinical cervical region can have a caries-like appearance. However, in these cases, the bacterial factor is not dominant. (16.18) Differential diagnosis takes into account clinical examination, medical history, localization, symmetry of the lesion, appearance of the surface, and laboratory data. For example, fluorosis is often symmetrical, while enamel hypoplasia is associated with disorders during tooth formation. In caries, biofilm, softening, and bacterial activity predominate. (4,18);

PREVENTION AND PREVENTION. A comprehensive approach is essential for the prevention of caries. First of all, it is recommended to reduce sugar consumption and brush your teeth at least twice a day. Using fluoride toothpastes helps to strengthen the enamel. Fluorine increases the acid resistance of enamel and enhances remineralization. (1,17) Furthermore, rinsing the mouth with chlorhexidine solutions, the use of probiotics, and regular dental check-ups play an important role in the prevention of caries. Sealing of fissures, professional hygiene, and individual caries risk assessment are also effective preventive measures. (3.20) Prevention in children cannot be effective without the participation of parents. Therefore, explanatory work



should be conducted with parents regarding proper nutrition, the avoidance of sweet drinks at night, the care of a child's teeth from an early age, and regular dental supervision. (4,19).

CONCLUSION. Dental caries in children is primarily an infectious process associated with the *Streptococcus mutans* complex. These bacteria form a biofilm, produce acid, and lead to the demineralization of tooth enamel. Modern laboratory diagnostic methods, especially PCR technology, are important for early detection of the disease and the establishment of effective preventive measures. (5, 12, 15)

The development of caries occurs due to the interaction of microbiological, biochemical, and hygienic factors. Therefore, in its prevention, not only treatment but also early diagnosis, individual risk assessment, and comprehensive preventive measures are of great importance. Especially in pediatric dentistry, the microbiological approach is an important factor in ensuring the formation of healthy permanent teeth in the future. (1,3,7)

The current in vivo investigation, which was conducted to identify and correlate SM and SS in children with CA and CF, can be used to draw the following conclusions: The relatively simple PCR method is an extremely accurate and sensitive identifying instrument. It was therefore used to examine, recognize, and measure microorganisms in kids with CF and CA. While children without caries showed higher amounts of SS, children with caries had significant levels of SM. Caries scores and SM are significantly correlated. The SM and Caries scores increased statistically substantially by 4.74 units at $p < 0.001$ with a 34% variance in SM levels, with 34% acting as the regression mode, according to the results of the simple linear regression analysis.

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