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Review Article

Lab-Test® 4: Dental caries and bacteriological analysis

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ABSTRACT

Dental caries is one of the most common infectious multifactorial diseases worldwide, characterized by the progressive demineralization of the tooth, following the action of bacterial acid metabolism. The main factors predisposing the onset of the carious process are: 1) the presence of bacterial species able to lower the pH until critical values of 5.5, 2) the absence of adequate oral hygiene, 3) an inefficient immune response anti-caries, 4) the type of alimentary diet and 5) the structure of the teeth. Among the 200 bacterial species isolated from dental plaque the most pathogenic for dental caries are: *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus acidophilus*, *Actinomices viscusus* and *Bifidobacterium dentium*. Our laboratory (LAB® s.r.l., Codigoro, Ferrara, Italy) has developed a test for absolute and relative quantification of the most common oral cariogenic bacteria. The test uses specific primers and probes for the amplification of bacteria genome sequences in Polymerase Chain Reaction Real Time. The results provide a profile of patient infection, helpful for improving the diagnosis and planning of preventive treatment to reduce the bacterial load.

Key Words: Bacteria, dental caries, real-time polymerase chain reaction

Dental caries is one of the most common infectious multifactorial diseases worldwide, characterized by the progressive demineralization of the tooth, following the action of bacterial acid metabolism.[1]

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Sucrose is the most cariogenic carbohydrate present in the diet, because, in addition to being fermented, it is also the substrate for the synthesis of extracellular polysaccharide (EPS),[5] which favors the bacterial adhesion to the tooth surfaces modifying the matrix biofilm.[6]

Among the 200bacterial species isolated from dental plaque the most pathogenic for dental caries are: *Streptococcus mutans* (serotypes C, E and F), *Streptococcus sobrinus* (serotype C and G),[7] *Lactobacillus acidophilus*,[8] *Actinomices viscusus* and *Bifidobacterium dentium*.[9,10]

These bacteria are defined acid-tolerant because they are able to survive in strong acid medium. They join to the tooth surface, metabolizing carbohydrates and producing organic acids that cause drastic lowering of the pH, resulting in the demineralization of tooth enamel.[11]

An uncontrolled increase in bacterial load leads to infiltration of the dentin and infection of the soft tissue of the pulp, causing excruciating pain, necrosis of the dental pulp, tooth loss and systemic infections.

Streptococcus mutans is a gram-positive bacterium that adheres to tooth enamel by the antigen

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I/II (Ag I/II) and builds a network of polysaccharides which favors the adhesion and proliferation of other microorganisms.[12]

S. mutans has the ability to survive, to grow and to maintain its metabolism in acid conditions.[3] This is due to a protein called membrane-bound F-ATPase, that drives protons out of the cells, preventing the decrease of intracellular pH.[13] This characteristic makes *S. mutans* very cariogenic and it is for this reason that specific treatments are reqired.

Lactobacillus acidophilus is not able to adhere directly to the tooth enamel. Together with *S. mutans* it is the leading producer of lactic acid responsible for the demineralization of tooth enamel.[8]

Another important risk factor for dental caries is the infection by *Streptococcus sobrinus*. In fact, a co-infection of *S. sobrinum* and *S. mutans* causes an increased incidence of the disease.[14]

In recent years, in the oral cavity, *Bifidobacterium dentium* has also been isolated, the only pathogenic bacterium of the group of Bifidobacteria (gastrointestinal microorganisms).[10]

Bifidobacterium dentium infection can be acquired from probiotic food and is responsible for 8% of all caries. It survives in the environment of the mouth and interacts with the bacterial microflora.[10]

Dental caries is a multifactorial pathology. For this reason have been adopted a number of strategies in the prevention of the disease, that include:

1. Administration of topical and systemic fluoride, which acts both on the surface of the teeth and bacterial flora, making a primary prevention;
2. Good oral hygiene that includes use of fluoride toothpaste, flossing and specific mouthwash. The mechanical removal of plaque deposits associated with the pharmacological actions of a toothpaste, decreases the bacterial load and facilitates the disintegration of the colonies;[15-17]
3. Balanced diet, low in sugar;[18]
4. Application of sealants that reduce colonization niches.[19]

In recent decades, studies have focused on the obtaining of anti-caries vaccines directed toward *S. mutans*. These vaccines contain antibodies against the bacteria surface receptors Ag I/II which prevent adhesion of the microorganism to the tooth enamel.[20]

Another type of vaccine has been synthesized against glucosyl transferase enzyme secreted by *S. mutans*. This enzyme catalyzes the synthesis of extracellular glucans from sucrose introduced with the diet, forming a matrix responsible for bacteria accumulation.[21]

A recent *in vivo* study in mice has used modified cholera toxin, in order to stimulate the production of antibodies. This toxin is a potent mucosal immunogen capable of amplifying the antibody response to any different antigens administered in combination. In this case, it revealed a good antibody response in the saliva after nasal administration of Ag I/II of *S. mutans*.[22]

The administration of vaccines consisting of glucosyl transferase associated with aluminum phosphate, resulted in a modest production of salivary antibodies and a slow recolonization of the surface of the teeth by *S. mutans*.[23]

An increase in the concentration of anti-*S.mutans* in the human saliva was also obtained after administration of oral or nasal glucosyl transferase conveyed with liposomes.[24]

Best results were obtained with the passive immunoprophylaxis of anti-Ag I/II topical application. Volunteers subjected to this treatment showed a drastic reduction in bacterial load of *S. mutans* for over two years.[25]

This form of passive immunization seems to have the concrete possibility of practical application, especially in patients with xerostomia, at high risk of dental caries.[26] However, some studies have demonstrated cross-reactivity between surface antigens of *Streptococcus mutans* and the human heart tissue.[27]

Currently, several studies have shown that the seeds of cloves (Syzygium aromaticum), the bitter fruits of cola (Garcinia Kola) and the leaves of tobacco (Nicotiana species) can be used in the composition of toothpastes, because they are able to reduce the cariogenic action of *S. mutans*.[28]

However, to date, there is no effective vaccine against dental caries; therefore the best treatment for this disease is the early detection and identification of pathogenic bacterial species and their elimination with specific antibiotics.

Our laboratory (LAB® s.r.l., Codigoro, Ferrara, Italy) has developed a test for absolute and relative quantification of the most common oral cariogenic bacteria (*Streptococcus mutans, Streptococcus sobrinus, Lactobacillus acidophilus, Actinomices*

viscusus, Bifidobacterium dentium). The test uses specific primers and probes for the amplification of bacteria genome sequences in PCR Real Time.

The results provide a profile of the patient infection, helpful for improving the diagnosis and planning of preventive treatment to reduce the bacterial load.

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