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Evaluation of bacterial flora diversity in the teeth with pulp necrosis and purulent inflammation of the periapical tissues in adults using metagenomic analyses

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Background: Dental caries is one of the most common diseases in adults and children in the world. If not treated, it leads to irreversible inflammation of the dental pulp and purulent inflammation of the periapical tissues. At present, mainly classical culture methods are being used in laboratory diagnosis of infections. However, etiology of most of these complications remains unknown due to problems with isolation of strictly anaerobic bacteria as well as the fact that about 50% of oral microflora is uncultivable.
The aim of the study was to compare composition of bacterial microflora in the oral cavity and in inflammed dental root canals, using metagenomic sequencing techniques.
Material/methods: Samples were obtained from 4 patients with pulpitis and a periapical abscess. Material comprised of unstimulated saliva samples from the bottom of the oral cavity, dental plaque removed from the supragingival area of the affected tooth, and pus from the pulp chamber of the inflamed tooth.
From these samples the total DNA was isolated and subsequently used for preparation of amplicons of the V3-V4 regions of the marker gene 16S rRNA for metagenomic sequencing. Amplicons derived from saliva and the dental plaque were pooled into a single sample representing the bacterial flora of the oral cavity, and subsequently compared to the amplicons of the periapical purulent lesions. The results of high-throughput sequencing were subjected to the proper bioinformatic analysis in order to determine biodiversity in the tested samples and differences in the microbiome composition in the oral cavity and purulent periapical lesions.

Results: The results of this study indicate that the composition of saliva and dental plaque is similar in all examined individuals, whereas the composition of bacterial flora in the purulent lesions is unique in each patient. In samples of saliva and dental plaque bacteria of the genus *Capnocytophaga*, *Fusobacterium*, *Neisseria*, and *Leptotrichia* were most common. In the pus within the periapical lesions predominated bacteria classified in the following genera: *Prevotella*, *Fusobacterium*, *Pseudomonas*, *Megasphaera*, and *Veillonella.*

Conclusions:

1. High-throughput analyses allow fast and precise determination of the composition and diversity of microorganisms in different anatomical sites within the oral cavity.
2. The use of novel methods of metagenomic analyses in dentistry enables precise determination of unknown until now etiology of purulent processes in the periapical tissues, including uncultivable etiological agents.
3. Accessibility of the methods of metagenomic analysis will contribute to the development of endodontics in terms of exploration of the etiology of purulent complications and subsequently the use of new methods of treatment of the infected root canals.