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Methodological Pilot Study for Dental Plaque and Calculus Microbial Characterization.

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Objectives A pilot study was conducted to present a modified methodology for microbial characterization at different taxonomic levels of human dental plaque and calculus.

Methods A no-smoker, healthy volunteer, with a good oral hygiene, no drugs taken in the last 3 months, and abstained from eating and drinking in the 2 hours before test, was selected. Dental plaque was collected with sterile paper cones from the buccal surfaces of incisors and molars. Tartar deposits were removed from the lingual and buccal surfaces of the lower incisors with a currette. Plaque and calculus samples were individually stored in sterile eppendorfs and sent for genomic analyses. Total microbial DNA extraction from dental plaque was performed with DNeasy PowerSoil Pro kit (Qiagen) directly from the sterile cone. Total microbial DNA extraction from one part of dental calculus was performed with the same kit with a modified protocol. The remaining calculus samples were incubated overnight with EDTA and proteinase K to dissolve the inorganic fraction. DNA was amplified with universal bacterial primers, then sequenced with an Illumina MiSeq platform. Data were statistically analyzed ($p<0.05$).

Results A total of 97.335 read counts were performed. Dominant phyla in plaque samples (53% Firmicutes, 29% Actinobacteria, 13% Proteobacteria and 5% others) and in calculus (54% Actinobacteria, 22% Firmicutes and 18% Proteobacteria and 6% others) were characterized. Dominant families in plaque were 35% Streptococcaceae, 13% Actinomycetaceae and 12% Micrococcaceae while in calculus were 21% Actinomycetaceae, 20% Micrococcaceae, 17% Streptococcaceae and 11% Corynebacteriaceae. Streptococcus (35%), Rothia (12%) and Actinomyces (11%) were identified in plaque, while Actinomyces (20%), Rothia (19%), Streptococcus (17%) and Corynebacterium (11%) in calculus.

Conclusions A modified characterization protocol of dental plaque and calculus was presented as to better define oral microbiota. Further studies with a larger number of patients are currently ongoing.