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PILOT STUDY FOR THE ANALYSIS OF THE ORAL MICROBIOTA

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ABSTRACT

The human oral microbiota, comprising a complex community of microorganisms, plays a crucial role in oral and systemic health. This study aims to create a biobank and database to characterise the diversity of oral microbiota using the sequencing of bacterial DNA. The primary objective is to assess the heterogeneity of oral microbiota among individuals by recording bacterial species. The secondary objective is to analyse the interplay between bacterial species and their potential involvement in systemic and musculoskeletal pathologies. Inclusion criteria involve patients aged 18 and above with negative bleeding on probing (BOP) test and absence of gingival inflammation. Clinical assessments and oral swabs will be collected, followed by DNA extraction and library preparation for sequencing. The bioinformatics analysis will identify and classify bacterial species using the 16S rRNA gene and reference databases. We believe the study will shed light on the oral microbiota's biodiversity, facilitating a better understanding of its impact on health and disease through advanced sequencing and data analysis techniques. The establishment of a biobank will provide a valuable resource for future investigations in oral microbiota research.

KEYWORDS: *microbiota, microorganisms, bacteria, DNA, oral cavity*

INTRODUCTION

Human oral microbiota is the ecological community of commensal, symbiotic, and pathogenic microorganisms in the oral cavity (1). The oral microbiome is an ensemble of more than 1,000 different microorganism genomes in the oral cavity (2, 3).

It is widely accepted that oral microorganisms are responsible for various diseases, mainly by a synergistic or cooperative manner, and the interspecies interactions within the oral community play a crucial role in determining whether oral microbiota elicit diseases or not (4-6). The oral microbiota is also associated with several systemic diseases, namely cardiovascular disease, pneumonia, heart disease, metabolic syndrome, rheumatoid arthritis, pancreatic cancer, colorectal cancer, esophageal cancer, and stroke (1, 4).

For this study, we have designed a study protocol with the aim of creating a biobank to record the different microorganisms of the oral cavity using modern metagenomics. The primary goal of the study is to create a database with

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the names of the bacterial species to evaluate the oral microbiota heterogeneity between different individuals. The secondary objective of the study will be to evaluate and highlight the presence of a network between the different bacterial species present in these patients and to analyze their dynamics in the development of systemic and musculoskeletal pathologies.

MATERIALS AND METHODS

The inclusion criteria of patients enrolled in this study are as follows: age over 18, negative BOP (bleeding on probing), absence of clinical signs of gingival inflammation (enlarged gingival profiles due to edema or fibrosis, chromatic transition towards a red and/or bluish red hue, increased gingival exudate), absence of plaque and tartar deposits, patients undergoing regular IOP, signature of informed consent for the analysis of the oral microbiome and data processing.

Patients who do not agree to participate in the study will be excluded from this study.

Clinical protocol

A specialist dental check-up will be performed according to the normal clinical practice of the complex operating unit of dentistry at Verona University.

Following an explanation of the purpose of the study and the signing of informed consent, patients will receive, in addition to the dental specialist visit and routine diagnostic radiographs (intraoral radiographs, orthopantomography of the dental arches and CT scan of the dental arches), an oral swab (Swab Collection and DNA Preservation System, Norgen Biotek Corp.) of the oral cavity to sample the microbiota (7).

Sequencing

All bacterial DNA will be purified from the sampling swabs (Microbiome DNA Isolation Kit - Norgen Biotek). We will prepare sequencing libraries (QIAseq 16S/ITS - Qiagen kit) of the 9 hypervariable regions of the gene for the 16S subunit of bacterial ribosomal RNA. Using an Illumina NGS sequencing platform (MiSeqDX), and following bioinformatics and statistical analysis, it will be possible to identify the individual bacterial species in the initial sample (8).

The swabs will be performed directly at the control visit. After being collected, the samples will be analysed in the biology and genetics section of the Department of Neuroscience, Biomedicine, and Movement at the University of Verona.

RESULTS AND DISCUSSION

Bioinformatic analysis

The bioinformatics analysis involves the V3-V4 hypervariable regions of the 16S rRNA gene. We will conduct our analysis working with the Amplicon Sequence Variants (ASVs), inferring DNA sequences within a sample. Each of these sequences belongs to a possible different bacterial species in the human oral cavity.

A taxonomic classification will be assigned to each ASV according to the known reference sequences available in public databases, reaching, if possible, the 7 taxonomic levels (kingdom, phylum, class, order, family, genus, species). The Divisive Amplicon Denoising Algorithm (DADA2) and the pre-trained classifier provided by the Human Oral Microbiome Database (HOMD) will be used to identify microorganisms in the oral cavity (9).

CONCLUSIONS

This study will provide a first glimpse of the extent of the oral microbiota's biodiversity, which has been limited until today. With the creation of a biobank, we could identify a greater number of microorganisms, which would provide effective statistical power for understanding the biological mechanisms underlying the state of health and disease of the oral cavity using modern sequencing and data analysis techniques.

Statement of Ethics

Ethics approval was given by the Verona University Ethics Committee (Prog.3032-CESC).

Conflict of interest

The authors declare that they have no conflict of interest.

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