TITLE: EVALUATION OF DNA PRE-EXTRACTION PROTOCOLS OF SPUTUM TO ASSESS THE MICROBIOME OF THE AIRWAYS FROM CYSTIC FIBROSIS PATIENTS.

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ABSTRACT: Cystic fibrosis (CF) is a recessive multisystem genetic disease in which, pulmonary manifestations are the principal cause of high morbidity and mortality. The relationship between the structure and the composition of the microbiome of the CF patient represents an important factor in his states of health. For microbiome analysis, the quality of DNA extracted has a pivotal impact in the sample representativeness. The aim of this study was to evaluate different protocols of DNA extractions in order to obtain the best method to be used in the microbiome analysis of sputum from Cystic Fibrosis (CF) patients. The extraction kit QIAamp DNA Mini Kit (QIAgen, Valencia CA) was used with three different pre-treatments: The first procedure (1A) followed the manufacturer protocol recommendation. The second procedure (1B) included a proteinase K treatment for 60 min at 56°C followed by bead-beating with zirconia/silica bead in a FastPrep 24 5G system (Qbiogene, CA), for 10 seconds at 6.0 m/sec (repeated 4 times). The third procedure (1C) was based in the sputum digestion with equal volume of N-acetyl-cysteine (100mg/mL). The last procedure (1D) used a TE buffer (10mM Tris-HCl [pH 8.0], 1mM EDTA) with the sputum and bead-beating as described above. The concentration of DNA was measured at Qubit 3.0 Fluorometer (Thermofisher) and the DNA integrity was verified with the 4200 Tape Station (Agilent). The amplicon library was prepared following the Illumina 16S Metagenomic Sequencing Library Preparation protocol and the high-throughput sequencing performed with MiSeq 600V3 kit. An average of 190,000 reads were obtained per

sample. The total genus-level of taxonomic categories identified (TCI) was approximately 215 per sample. The total of species-level TCI was: 238 for 1A; 320 for 1B; 179 for 1C and 241 for 1D. We selected a *Pseudomonas aeruginosa* and a *Rothia mucilaginosa* to evaluate the effectiveness of DNA extraction from gram-negative and gram-positive bacteria. The detection of *P. aeruginosa* and *R. mucilaginosa*, respectively, for each protocol extraction was: 1A, 22.08% and 0.85%; 1B 29.10% and 4.46%; 1C, 31.15% and 0.68% and 1D, 26.92% and 8.56%. In conclusion, the protocol 1B presented the best performance considering the total of species-level TCI. Noteworthy, both protocols 1B and 1D, which use the bead-beating strategy, increased the yield of Gram-positive bacterial DNA extraction.

Keywords: Microbiome, DNA extraction, Cystic Fibrosis

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