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Oxford Nanopore Sequencing for Preliminary Microbiome Analysis Amongst Active/Latent Tuberculosis Patients vs Healthy Individuals in Sri Lanka

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The human microbiome has long been considered to play a vital role in the immune response against infections including Tuberculosis (TB). However, even with an annual disease burden of 8000 cases, the gut and oral microbiome of Sri Lankan TB patients are yet to be studied. Moreover, currently prominent sequencing technologies used for generating microbiome data namely; Illumina and Ion Torrent require sophisticated instrumentation in contrast to the more feasible Oxford Nanopore sequencing technology (ONT). Thus, this study focused on observing the microbial diversity in oral and gut microbiome among TB patients and healthy individuals in Sri Lanka via 16S rRNA gene analysis using ONT. Microbial DNA was extracted from sputum and stool samples of TB infected individuals (n=5) who were tested positive for TB interferon-gamma release assay (IGRA) and IGRA (-) healthy individuals (n=5) using Qiagen QIAmp microbiome kit which were then sequenced using ONT via 16S barcoding followed by bioinformatics analysis using EPI2ME software. A total of 115 genera were identified from sputum samples of both study groups with Gemella, Granulicatella, Streptococcus, Veillonella being the most dominant while 150 genera were identified in stool samples of both cohorts where genera Blautia, Faecalibacterium, Streptococcus were the most abundant. No compositional changes were observed between microbiomes of TB infected and healthy individuals albeit the infected cohort having lower read counts. However, optimisation of ONT workflow to increase the read counts is continuing and a larger sample size is needed to obtain more comprehensive microbiome profiles for both the cohorts studied.

Keywords: *tuberculosis, microbiome, Oxford Nanopore sequencing, 16S rRNA gene analysis*