27th ECCMID Vienna, Austria 22 – 25 April 2017
The congress of ESCMID

Session: P044 Diagnostic mycology I

Category: 6b. Diagnostic mycology (incl molecular)

23 April 2017, 13:30 - 14:30
P0977

Impact of DNA extraction protocol on the fungal microbiota identified from respiratory samples through high throughput DNA sequencing

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Background: The respiratory tract contains a vast community of fungi that remain largely unknown, especially due to the limitations of culture-based methods. Metagenomic studies are of great interest in this area. For fungi, standardized methods regarding the DNA extraction protocols or the PCR amplification targets still remain to be determined. Here, we investigated the use of 2 different DNA extraction methods and two different amplification targets on the fungal communities identified in human sputum.

Material/methods: Six sputa from 6 patients hospitalized in Creteil Hospital were subjected to genomic DNA extraction using two methods, both associated with a mechanic lysis: the automatic extraction with DSP DNA Midi kit on QIAsymphony® (QSE) (Qiagen, France) and the manual Powersoil® Mobio (PSM) kit (MoBio, USA). Two of these sputa, with different yeasts in culture, were spiked with conidia of *Aspergillus fumigatus* and *A. niger* (10^5 conidia/ml) to add filamentous fungi in known concentrations. After their extraction, the ITS1 and ITS2 region were used for high-throughput DNA sequencing analysis. Amplicon libraries were prepared using MiSeq Reagent kit V3 (Illumina, France). Data generated were analysed with PyroMIC© (IDDN FR.001.400018.000.S.P.2014.000.31230) and Genoscreen pipelines (Lille, France), and compared in terms of diversity and relative abundance with the culture results (Sabouraud medium and blood agar).

Results: The QSE method, combined with prior mechanic lysis, led to a lower DNA purity, but presented the highest DNA yield (1.5 to 16-fold) and required less time than the PSM extraction. For each spiked sputum, both extraction methods allowed the detection of similar yield of *Aspergillus* DNA by 28S and mitochondrial RT PCR. As for metagenomic analysis, both methods allowed identifying the major taxa, but their relative abundance varied significantly according to the extraction protocol. The relative abundance between *Candida* and *Aspergillus* sp. and among the different *Candida* sp. was closer from that observed in culture with QSE while PSM created distortion with a significant increase of *Candida glabrata* and *Aspergillus* sp. reads. By contrast, no significant differences in terms of diversity or abundance of major taxa were found according to the amplification target ITS1 or ITS2. However, more minor taxa were observed with ITS2 than ITS1. Whatever the extraction method used, the metagenomic analysis allowed us to identified 4 or 5 species not found in culture, one of them (*Saccharomyces cerevisiae*) being a major taxon (relative abundance > 1%).

Conclusions: For fungi, our data highlight the importance of carefully choosing the method of DNA extraction, which impacts the yield of DNA extracted and its purity. These points, little described in the literature for respiratory specimens, could secondarily impact the success of the NGS amplification and influence the fungal communities identified, especially in terms of relative abundance of the major and minor taxa.