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Title: The mycobiome of the skin of patients with atopic dermatitis and healthy volunteers

Background: Atopic dermatitis (AD) is one of the most common inflammatory skin diseases, in which fungi are believed to act as triggering factors. Therefore, it is crucial to assess the composition of skin mycoflora for a better understanding of the etiopathogenesis of AD.

Objective: The aim of the study was to explore the skin mycobiome of AD patients and healthy individuals by using culture-dependent and metagenomics, culture-independent methodologies.

Methods: The study included 50 AD patients and 50 healthy individuals recruited between 2017 and 2019. Skin scrapings from elbows, necks and knees were collected with either a scalpel or OpSite dressings (Smith&Nephew Education; UK) in AD and healthy subjects, respectively. Culture-dependent species identification involved a battery of conventional phenotypic tests and PCR-based sequencing of the internal transcribed spacer (ITS) 1&2 regions (Jagielski et al., 2014, BMC Dermatol., 14:e3). Whereas, metagenomic sequencing, was performed directly on skin samples using ITS1-targeted primers, namely FungITS1 and FungITS2 (White et al., 1990, Mol. Ecol. 2:113-118).

Results: The overall positive culture rate was 44% for AD patients and 24% for healthy individuals. *Candida* spp. were recovered at the highest frequency (33.3%), followed by *Rhodotorula* spp. (21.6%) and *Malassezia* spp. (11.8%). Culture-based phenotypic and molecular (PCR-sequencing) methods used for species identification gave concordant results for 76.5% of the isolates cultured ($n=51$). Metagenomic sequencing, a total of 61 and 44 (considering the abundance above 1% within samples) various taxonomic fungal families were demonstrated on the skin of AD patients and healthy individuals, respectively. Among patients with AD, five families/orders, i.e. *Cladosporiaceae*, *Debaryomycetaceae*, *Malasseziaceae*, *Saccharomycetales* and *Botryosphaeriaceae* were represented at a higher frequency, when compared with healthy individuals. Contrarily, among the latter the family *Thermoascaceae* was overrepresented.

In conclusion, a higher positive culture rate, interpersonal diversity of the mycoflora, and a clear predominance of specific taxa were observed in AD patients when compared with healthy individuals.

Funding: This work was supported by the «luventus Plus» grant from the Polish Ministry of Science and Higher Education (0258/IP1/2016/74).