Metagenomic analysis as a support to clinical diagnosis of onychomycosis

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Introduction. Onychomycosis can be caused by yeasts and by dermatophytic and nondermatophytic moulds. These fungi lead to the gradual destruction of the nail plate. Antifungal therapies, both oral and topical, take a long time with very often disappointing results due to the lack both of accurate diagnosis and tests for sensitivity to antifungal drugs before therapy. Thus dermatologists increasingly require the analysis for the pathogens responsible of onychomycosis, including the sensitivity to antifungal drugs, after a too brief interruption of therapy; as a consequence fungal culture, essential to establish the susceptibility to the drug, give frequently false-negative results. In order to overcome the above mentioned problems, the aim of the present work was to compare two different methods: the cultural methods and the sequence analysis method based on the metagenomic approach.

Results. 23 samples (69,69%) were from 20 patients with previous antifungal treatments. With Metagenomics, in samples both from treated and not treated patients high percentages of pathogenic fungi was detected (probably from nails where the pathogen was still alive) and low percentages (1,06%- 2,52%, probably resulting from nail fragments healed from the infection or not colonized by the fungus) (Fig.1). The culture method has led to the isolation and identification of pathogens in the 39,39% of the samples, while with the metagenomic method pathogens were detected (high percentage) in the 57,57% of the samples (Fig.2). In two samples a mixed infection was detected with both methods: one *Trichophyton rubrum* plus *Candida parapsilosis* and the other *Candida parapsilosis* plus *Debaryomyces hansenii*. In the 45,45% of the specimens a few moulds were isolated and therefore considered contaminants. The metagenomic data confirmed their presence in a percentage too low to be regarded as pathogens.

Methods. 33 nail samples from 29 patients with clinical diagnosis of suspected onychomycosis in feet and/or hands were investigated. Fungal cultivation was performed using Sabouraud dextrose agar (Oxoid) amended with antibiotics, with and without cycloheximide, to detect yeasts, dermatophyte and nondermatophytic moulds. Total DNA was obtained from nail powder. To get fungal amplicons, the ribosomal ITS1 region was targeted, by using primers BITS and B58S3 (Bokulich and Mills, 2013) linked to Illumina adapters. Sequencing libraries were finally constructed through the link of indexes (Nextera XT Index Kit, Illumina, San Diego, CA), quantified, normalized and pooled. Libraries were subjected to paired-end sequencing (2 x 250 bp, nano format). Data analysis was performed using the pipeline Qiime and high-quality reads were clustered into operational taxonomic units (OTUs) at 97%. Relative abundances of microbial taxa in each sample were calculated and compared.

Conclusions. Comparing the results obtained with the 2 methods it can be seen that fungal cultures had only 18.18% of false negatives. Metagenomic methods led to important results: 1) the recognition of higher amount of positive cases; 2) the detection of low percentage of pathogens probably dead for the previous treatment or, in the case of long-lasting infections (Fig.3), for having collected fragments in a part of the nail where the fungus was not still alive; 3) the confirmation that the developed moulds were only contaminants. The two methods provide the dermatologist different information: a) if the dystrophic nails have a fungal origin or not; b) if it is a mycoses, the cultural isolation allows to report the sensitivity to the antifungal agents; c) if the mycosis is resolved by a previous therapy, metagenomics highlights it

Fig. 1 - Metagenomics: detection % of fungal pathogens in treated and untreated patients

Fig.2 - % of positive cases and fungal pathogens species detected with the two methods

Fig. 3 – Long-lasting onychomycosis

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