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ePoster Viewing

Highlights from molecular mycology

A NEW MULTIPLEX REAL TIME PCR FOR DETECTION AND IDENTIFICATION OF ZYGOMYCETES AND ASPERGILLUS SPP. IN HUMAN BIOSAMPLES

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Objectives

The success of treatment of fungal infection of the lungs depends to a large extent on early etiological diagnostics, which is often problematic when conventional methods of analysis are used. The aim of the study was to develop a PCR for simultaneous detection and identification of *Zygomycetes* and *Aspergillus spp.* in human biological samples.

Methods

A multiplex real time PCR with High Resolution Melt analysis (mHRM-RT-PCR) was developed for detection and identification of *Zygomycetes* and *Aspergillus spp.* using fungi isolates from Russian collection of pathogenic fungi of Kashkin Research Institute of Medical Mycology (Saint-Petersburg). Identification of the fungi isolates was carried out using morphological criteria and by sequencing of ITS and D1/D2 regions of rRNA gene and β -tubulin gene fragment of micromycetes. The mHRM-RT-PCR was tested on clinical samples (sputum, bronchoalveolar lavage, blood, autopsy material) obtained from patients with mycotic infection of the lungs. Samples from the patients were also analyzed using direct DNA sequencing, a TaqMan PCR for detection of *Aspergillus spp.*, microbiological (microscopy and culture), and Platelia-Aspergillus EIA (BioRad).

Results

The mHRM-RT-PCR was developed using 64 fungal strains: 22 strains of *Zygomycetes*, 22 strains of *Aspergillus* and 20 strains of other clinically relevant fungi. Specific reproducible amplification of proper DNA fragments was achieved for aspergillus- and zygomycetes-specific primer pairs separately and in the multiplex PCR setting. The mHRM-RT-PCR allows to identify the representatives of *Aspergillus* and *Absidia* to the genus level, *Rhizomucor pusillus*, *Rhizopus microsporus*, *Mucor circinelloides*, *Cunninghamella echinulata*, *Syncephalastrum racemosum*, *Lichtheimia corymbifera* to the species level and *Rhizopus arrhizus*/ *Rhizopus stolonifer* and *Mucor racemosus*/ *Mucor plumbeus* to the pair of species level. We are currently evaluating our mHRM-RT-PCR on clinical material from patients with mycotic infection of the lungs.

Conclusions

We have developed a multiplex RT-PCR for detection and identification of *Zygomycetes* and *Aspergillus spp.* in human biological samples. The preliminary results of the evaluation of the mHRM-RT-PCR on clinical samples indicate that it may be a very useful tool for revealing of etiologic agent of mycotic infection of the lungs, particularly in the case of a mixed infection by *Aspergillus* and *Zygomycetes spp.*