Development of main spectral profiles database for MALDI-identification of common aspergillosis causative agents from the colonies obtained in liquid medium


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Background

MALDI-TOF-mass-spectrometry of the cell extract has been successfully used to identify clinical isolates of micromycetes, including Aspergillus spp. Modern approaches of main spectral profiles (MSPs) are intended mainly for working with cultures in 'pellet' that are grown in a liquid medium on a rotator. Working with 'pellet' is associated with some inconveniences, in this regard many microbiologists prefer to work with membranous cultures obtained on liquid medium without rotation.

Purpose

The purpose of the study is to compose the MSP-database of Aspergillus spp. – the most common causative agents of invasive aspergillosis – to identify their cultures grown in liquid nutrient medium without mechanical agitation.

Materials and methods

Study was performed using the Laser ToF LT2Plus MALDI-TOF-mass-spectrometer (SAI, UK) and BactoSCREEN software (Lytch, Russia). In order to create the MSP-database strains of A. fumigatus, A. flavus, A. niger-complex (A. niger & A. awamori) and A. nidulans identified by the target DNA-sequencing of ITS and β-tubulin loci according to the CLSI MM18 standard (2nd Ed.) were obtained from the Russian collection of pathogenic fungi. Only the most detailed mass-spectra with low noise levels were selected. The MSP-database was designed according to the instructions of the software manufacturer. 166 Aspergillus spp. of 5 species were processed for a positive identification control, 199 fungal cultures of 37 species and 117 bacterial cultures of 85 species were included in the trial for negative control (specificity control). All of the strains used in the study were isolated from human biomaterials. The processing of mycelial fungi cultures was carried out as described previously [Riabinin I.A. et al., AAA-2014]. Bacterial and yeast cultures were prepared by direct deposition and acid etching on MALDI-target. As a trial's resume the effectiveness parameters were calculated for the created MSP-database relative to the main commercial MSP-database of software manufacturer.

Results

The «AMPSL» (Aspergillosis Main Pathogens Spectral Library) database was created, which included high-quality MSP of 47 Aspergillus spp. cultures. Compared to the manufacturer’s main MSP-base, AMPSL was more sensitive (100% vs 76.8%), although slightly less specific (98.2% vs 100%). False positive identification using the AMPSL-database was detected for individual isolates of Penicillium sp., P. digitatum, Purpureocillium lilacinum and Scopulariopsis bireusicaulis. These micromycetes are closely related to Aspergillus spp. and have very similar composition of MALDI-mass-spectra, which explains their inaccurate identification. However, the conventional microbiological methods (stereomicroscopy of colonies, microscopy of a preparation from a culture) such problem strains are easy to determine correctly. In general, the created «AMPSL» base showed greater diagnostic efficiency compared to the standard MSP-base of mass-spectrometer (98.5% vs 96.7%). A distinctive feature of the AMPSL utilization is the more successful identification of A. flavus and A. terreus isolates.

Conclusion

The study allowed us to create the MSP-database suitable for the cultural diagnosis of aspergillosis using MALDI-TOF-mass-spectrometry. In the future, it is advisable to expand the range of species of the database, taking into account the more rare but actual causative agents of aspergillosis (e.g. A. sydowi, A. ochraceus, A. candidus, A. calidoustus and others).