TITLE: IDENTIFICATION OF FILAMENTOUS FUNGI IN A PHARMACEUTICAL INDUSTRY USING MATRIX-ASSISTED LASER DESORPTION IONIZATION – TIME OF FLIGHT MASS SPECTROMETRY (MALDI-TOF MS)

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ABSTRACT:
Microbial contaminants represent a risk to the production processes of immunobiological products in pharmaceutical industries. The Matrix-Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry (MALDI-TOF MS) technology has been applied as a fast and easy-to-perform method for identification of isolates through mass and ionic charge analysis of the microbial proteome. The aim of this study was to evaluate the performance of the MALDI-TOF MS methodology (VITEK® MS RUO – bioMérieux) in the identification of filamentous fungi in a pharmaceutical industry. Forty-nine filamentous fungi strains isolated between 2019 and 2021 from: environmental monitoring samples (n=30), sterility test of culture medium, intermediate and/or final products (n=5), bioburden analysis of solutions (n=5), analysis of water samples (n=4), asseptic simulation (n=2), culture medium and cell line used in production (n=2) and cell line used in quality control testing (n=1). Only results with datacount between 100-200 were considered satisfactory. The 49 strains were reactivated in Potato Dextrose Agar and transferred to tubes with Tryptic Soy Broth. For cell lysis and extraction, samples were washed with sterile deionized water and treated with ethanol, 70% formic acid and acetonitrile. The supernatant from each sample was added to the slide wells and alpha-cyano-4-hydroxy-cinnamic acid matrix was added. After matrix crystallization, the slides were introduced into VITEK® MS RUO. The extraction results were considered satisfactory for 40 (81.6%) out of 49 strains, while the datacount ranged between 18 and 98 for nine (18.4%) strains. Among the 40 strains whose extraction was satisfactory, 34 (85.0%) were not identified. The remaining six (15.0%) strains were identified at species level as: *Aspergillus sydowii* (n=3), *Aspergillus unguis* (n=1), *Penicillium chrysogenum* (n=1), *Scopulariopsis brevicaulis* (n=1). In conclusion, an improvement of the extraction method is necessary to achieve an acceptable datacount for all strains. Moreover, the implementation of other methodologies, mainly based on acid nucleic sequencing, are necessary for the identification of strains that are not included in the database of Saramis. Afterwards, the superspectra of these strains can be created and inserted in the database, so that these filamentous fungi species will be identified if they are isolated in the pharmaceutical industry again.

Keywords: filamentous fungi, MALDI-TOF MS, pharmaceutical industry, immunobiological

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