**Objectives:**
The ID-Fungi plate (IDFP) is a specialized medium designed for MALDI-TOF/MS-based identification of filamentous fungi. It features a thin film layer on the agar surface, facilitating easy transfer of material onto the target, without agar contamination. In an effort to contribute to improved patient management, this study aimed to i) evaluate identification performance of MALDI-TOF/MS for clinical Mucorales isolates using IDFP in comparison to conventional MALDI-TOF/MS pretest settings and ii) determine the Minimum Inhibitory Concentration (MIC) distributions of amphotericin B, posaconazole, and isavuconazole for the tested strains.

**Materials & Methods:**

Clinical Mucorales isolates obtained in Mycology Laboratory of Hacettepe University Faculty of Medicine Hospitals from 1999 to 2023 were revived from stock cultures and their purities were confirmed. Genus/species level identifications were performed by internal transcribed spacer (ITS) sequencing as the reference method. For MALDI-TOF/MS, three approaches were evaluated: direct transfer from Sabouraud Dextrose Agar (SDA), mycelial extraction from Sabouraud Dextrose Broth (SDB), and utility of IDFP. Antifungal susceptibility was tested using the EUCAST reference broth microdilution method (EUCAST E.DEF. 9.4).

**Results:**

A total of 61 isolates were included. The clinical specimens from which the strains were isolated were lower respiratory tract (n=27), biopsy (n=10), urine (n=8), upper respiratory tract (n=5), wound/pus (n=5), and other (n=6).

Identifications according to the ITS sequencing results were as follows: *Rhizopus arrhizus* 54.1% (n=33), *Mucor circinelloides* 14.8% (n=9), *Lichtheimia corymbifera* 9.8% (n=6), *Rhizomucor pusillus* 9.8% (n=6), *Rhizopus arrhizus/delemar* 4.9% (n=3), *Lichtheimia ramosa* 3.3% (n=2), *Rhizopus stolonifer* 1.6% (n=1), and *Mucor plumbeus* 1.6% (n=1).

Genus-level identifications were identical for all methods. Species-level identifications (scores ≥1.70) were 54.1% for transfer from SDA, 59.0% for extraction from SDB and 68.9% for transfer from IDFP. When scores were not taken into consideration, the compliance increased to 93.4%, 95.1% and 93.4%, respectively. Identification results were presented in Table 1.

MIC values of the tested antifungal drugs were summarized in Table 2. One *R.arrhizus* and one *R.stolonifer* isolate did not show sufficient grow when tested for antifungal susceptibility.

**Conclusions:**

MALDI-TOF/MS was efficient for rapid and accurate identification of Mucorales genera using all three methods. Interestingly, species level identification was compatible with sequencing results in most isolates, even if the scores were low. Direct transfer from SDA was adequate to identify most isolates rapidly and without additional cost. Extraction from SDB improved identification scores. Consistent with previous studies and importantly, the IDFP method yielded higher identification scores. Sample transfer to target was easier and faster when using IDFP. Since Mucorales strains grow rapidly and aerial hyphae are easier to harvest, the impact of IDFP on Mucorales identification might have remained limited as compared to that of other moulds having colonies firmly adhering to the agar surface. For *R.arrhizus* which included adequate number of isolates for analysis, the most active drug in vitro was amphotericin B, followed by posaconazole, and isavuconazole, in rank order. The number of isolates were limited for other genera and species and expanded data are needed to assess potential differences in the *in vitro* antifungal activities.