In vitro activity of amphotericin B and posaconazole against common agents of mucormycosis

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BACKGROUND

Mucormycosis are emerging and aggressive life-threatening infections, caused by siphonal fungi associated to the order Mucorales. An early and adequate antifungal therapy is the key to improve patient outcome. Generally recommended antifungal agents for mucormycosis are lipid formulations of amphotericin B (AMB) for systemic treatment and posaconazole (POS) as salvage therapy.

Aim of the study

We aimed to evaluate the activity AMB and POS for the various agents causing mucormycosis, to establish epidemiological cut-off values (ECOFF).

METHODS

A set of ninety three clinical isolates belonging to the order Mucorales was identified based on their morphology and by ITS direct sequencing analysis. A collection of 38 strains obtained from the CBS was used as reference for morphological classification. Species identification was performed by comparatice sequence analysis using GenBank database. Posaconazole and AMB were tested according to EUCAST guidelines (www.eucast.org) for determining the minimal inhibitory concentrations (MICs). Minimal fungicidal concentrations (MFCs) and MFC/MIC were determined as characteristic measures of antifungal activity, defining the drugs as either cidal or static.

RESULTS

Species identification – ITS as „pan-Mucorales“ barcode

ITS was found a good marker for species discrimination, with the exception of the sibling species *Lichtheimia* (*L*). *corymbifera* and *L. ramosa*. The most predominant species was *L. corymbifera* (n=41), followed by *Rhizopus* (*R*). *arrhizus* (n=29) and *R. microsporus* (n=23), as shown schematically in Figure 1.

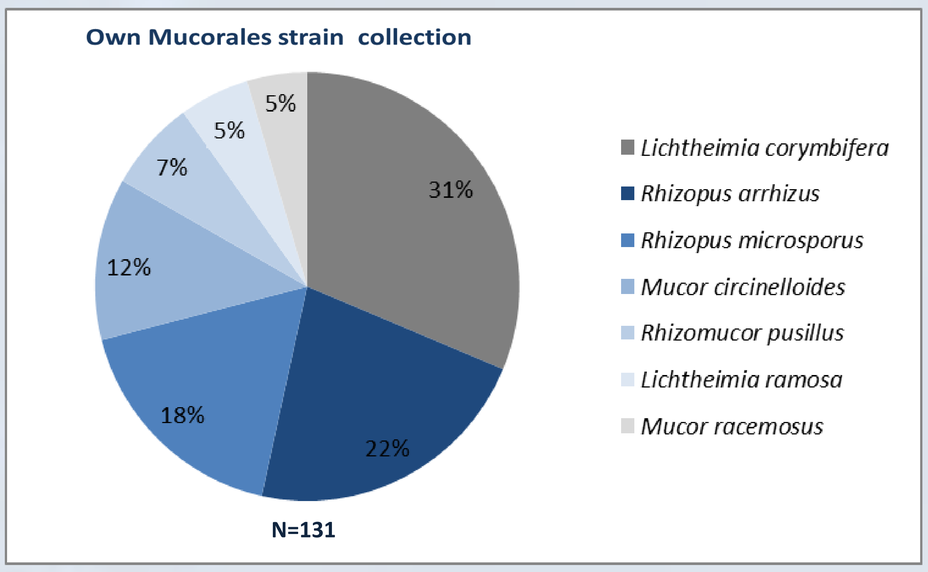


Fig 1. Species distribution schematic diagram of our Mucorales strain collection according to comparative ITS sequencing analysis with GenBank database.

Antifungal susceptibility testing - *in* vitro activity of AMB and POSA

1. AMB was the most potent drug against mucormycetes, but had limited activity against *R. arrhizus* (Fig.3C). AMB was found fungicidal for all mucormycetes (MFCs attained for all) , except for *L. ramosa,* with no MFC possible to be determined in any tested strain. In Figure 2 A, it is presented the MIC value for AMB (0.25µg/mL) and in Figure 2 B, the following lower AMB concentration (0.125µg/mL) for a *Mucor racemosus* strain.

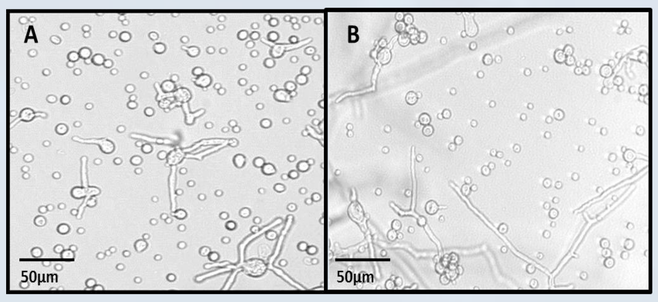


Fig. 2. Photomicrograph showing AMB activity obtained with a magnification of 400 x. The *Mucor racemosus* **M1** strain was tested for AMB MIC determination using EUCAST microbroth dilution method. **A)** the strain M1 is inoculated with AMB at a concentration of 0.25µg/mL in 100µL liquid RPMI (defined as MIC for M1); **B)** strain M1 inoculated with AMB at a concentration of 0.125µg/mL in liquid RPMI, with presence of mycelial growth.

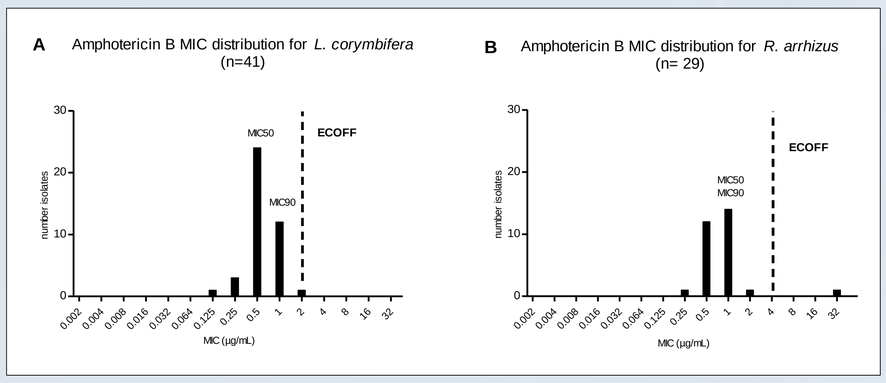


Fig. 3. AMB MIC distribution for **A)** *L. corymbifera* and **B)** *R. arrhizus* populations. MIC50, MIC90 and ECOFF values are presented for both strain collections.

2. POS showed limited activity against mucormycetes (e.g., *Mucor spp*. and *R. arrhizus* - Fig.5B). POS was found fungistatic for all mucormycetes (as shown in Fig. 4). In all tested species, either no MFCs were obtained (e.g., *Mucor spp.*), or when achieved, MFC/MIC > 4 (e.g., *R. arrhizus*, *L. corymbifera*, *R. microsporus*) were observed.

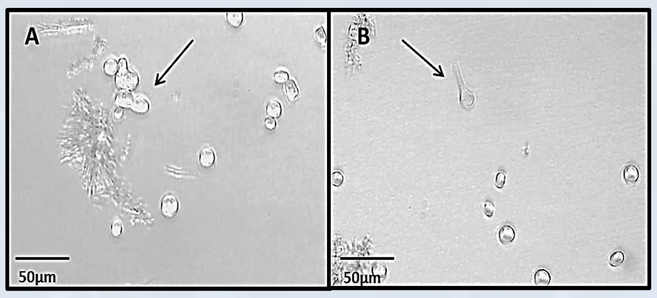


Fig. 4. Photomicrograph showing POS activity with a magnification of 400 x. The *L. ramosa* **LR1** strain was tested for POS MIC determination using EUCAST microbroth dilution method. **A)** strain **LR1** inoculated with POS at a concentration of 16µg/mL in 100µL liquid RPMI; **B)** strain **LR1** inoculated with POS at a concentration of 8 µg/mL – no mycelia growth, but germlins are observed in both concentrations shown with arrows. MIC was 1µg/mL.

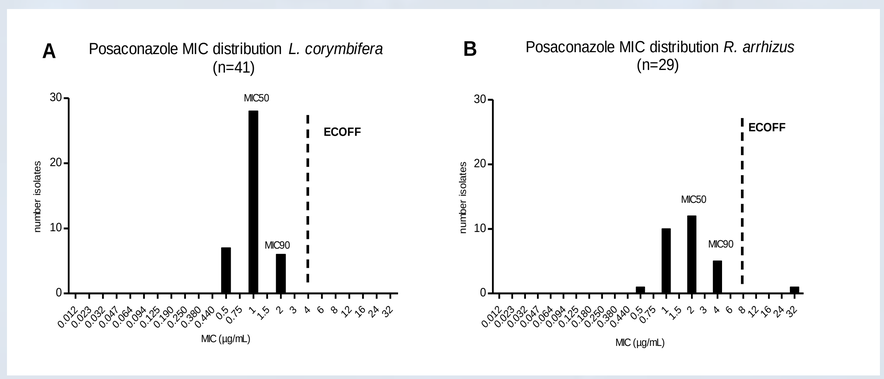
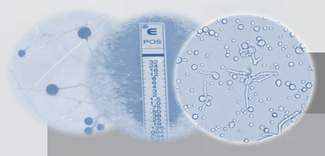


Fig. 5. POS MIC distribution using EUCAST method for **A)** *L. corymbifera* and **B)** *R. arrhizus*. Measures of antifungal activity, MIC50, MIC90 and ECOFF are presented in both strain collections.



CONCLUSION

ITS sequencing was suitable for the identification of mucormycetes, with some limitations for the discrimination of sibling species; in general, AMB was confirmed to be the most active agent against mucormycosis, but had limited activity against *R. arrhizus*. While AMB was clearly fungicidal, POS was found fungistatic with restricted activity against most species.