Comparison of three read-out methods for Sensititre YeastOne Alamar blue assay for determining Minimum Inhibitory Concentrations

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Introduction

Madurella mycetomatis (*M.mycetomatis*) is the major causative agent of eumycetoma. Eumycetoma is a neglected, inflammatory tropical infection characterized by painless lesions, grains and draining sinuses. Currently, susceptibility testing for *M. mycetomatis* is not routinely performed. This is possibly due to the need to use hyphal suspension as a starting material, the slow growth rate, labour-intensiveness and the need to use expensive dyes for endpoint visualization. Furthermore, pyomelanin is produced by several of the isolates, which might hamper the use of other viability dyes in *in vitro* susceptibility testing. Commercial systems such as the Sensititre YeastOne YO10 are available but are currently not used in clinical practice with regards to eumycetoma.

Objectives

- Determine if the Sensititre YeastOne YO10 Alamar blue assay can be used to determine the *in vitro* susceptibility of *M. mycetomatis* towards several antifungal agents.
- Assess if pyomelanin formation interferes with the various endpoint read-out systems, by comparing isolates secreting pyomelanin with isolates which do not secrete pyomelanin

Materials & Methods

- Eight pyomelanin producing (P1, MM25, MM30, MM44, MM45, MM49, MM55, MM68) and two non-pyomelanin producing (MM14 and MM50) *M. mycetomatis* isolates were used in this study.
- In vitro* susceptibilities of 10 clinical isolates of *M. mycetomatis* were determined with the Sensititre YeastOne Alamar blue susceptibility assay following manufacturer's instructions. The Minimum Inhibitory concentrations, (MICs) were determined by visual reading, SWIN reading and spectrophotometry.

Results

- M. mycetomatis* is most susceptible towards the azole class of antifungal agents
- Different MICs were obtained with different read-outs system
- Better agreement between visual and SWIN endpoints compared to visual and spectrophotometer and/or SWIN

Figure 1: Sensititre YeastOne panel (non-melanized pink and melanized, brown). MICs distribution for isolates of *M. mycetomatis* with three different read-out methods, black bars:visual, gray bars: SWIN and white bars: spectrophotometric

Table 1: Comparison of the different endpoint read-out methods for Sensititre YeastONE assay for isolates of *M.mycetomatis*

Antifungal agent	Agreement (%)					
	Visual vs SWIN		Visual vs Spectrophotometric		SWIN vs Spectrophotometric	
	Identical MICs	MICs within 1 dilution	Identical MICs	MICs within 1 dilution	Identical MICs	MICs within 1 dilution
Itraconazole	80	100	50	70	50	80
Vorinacozole	80	100	40	80	30	70
Posaconazole	90	100	40	80	50	80
Fluconazole	30	70	0	30	20	50
Amphotericin B	70	100	50	80	30	80
5-Flucytosine	100	100	90	90	90	90
Caspofungin	100	100	80	90	80	90
Micafungin	100	100	90	90	90	90
Anidulafungin	100	100	90	100	90	100

Conclusion

- The present data suggest the Sensititre YeastOne YO10 is suitable for the susceptibility testing of *M. mycetomatis* and could vital in resource-limited setting
- M. mycetomatis* isolates are susceptible to azoles and amphotericin B but not echinocandins and 5-flucytosine