

Comparison of the Sensititre YeastOne with CLSI M38-A2 microdilution methods for MIC determination of amphotericin B and azoles against *Aspergilli*

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Introduction

The 2017 ESCMID-ECMM-ERS guideline recommended performing antifungal susceptibility testing on clinically relevant *Aspergillus* isolates. Recently, the Sensititre YeastOne method has been increasingly adopted in both clinical and research settings for susceptibility testing of *Aspergillus* species. However, available studies comparing the MICs resulting from the YeastOne and CLSI methods were mostly conducted before 2008, and azole-resistant *A. fumigatus* isolates with environmental resistance mechanisms had not been evaluated yet. Hence, this study compared the YeastOne and reference CLSI M38-A2 broth microdilution methods for antifungal susceptibility testing of *Aspergillus* species to investigate whether the Sensititre YeastOne method is suitable for routine use in clinical laboratories.

Materials and Methods

The minimum inhibitory concentrations (MICs) of antifungal agents against 100 isolates, comprising 31 *Aspergillus fumigatus* (5 TR₃₄/L98H isolates), 23 *Aspergillus flavus*, 13 *Aspergillus terreus*, and 10 *Aspergillus niger* clinical isolates, and 23 *A. fumigatus* environmental isolates (19 TR₃₄/L98H isolates), were determined using both Sensititre YeastOne (Trek Diagnostic Systems, Ltd.) and the reference CLSI M38-A2 broth microdilution methods.

Results and Discussion

The MIC results are shown in Table 1. The overall agreement (within two twofold dilutions) between the two methods was best for voriconazole (100%), followed by posaconazole (95%), itraconazole (92%), and amphotericin B (90%). For all isolates, the voriconazole GM MICs determined by the two methods were nearly identical (0.732 vs. 0.758 mg/L), whereas the itraconazole and posaconazole GM MICs from YeastOne were about one twofold dilution lower than those from the M38-A2 method (itraconazole 0.346 vs. 0.688 mg/L; posaconazole 0.095 vs. 0.214 mg/L). In contrast, the amphotericin B GM MIC was 3.3-fold higher in YeastOne than in M38-A2 (1.959 vs 0.602 mg/L).

Table 1. The MIC results for *Aspergillus* spp. by both methods.

Strains, no.	MIC (mg/L)				% Agreement with the following twofold dilution	
	Sensititre YeastOne		CLSI M38-A2		within ± 1	within ± 2
	GM	MIC ₅₀ /MIC ₉₀	GM	MIC ₅₀ /MIC ₉₀		
Amphotericin B						
All, 100	1.959	2/4	0.602	0.5/1	41 (41)	90 (90)
<i>A. fumigatus</i> , all, 54	1.828	2/2	0.526	0.5/1	14 (25.9)	50 (92.6)
<i>A. flavus</i> , 23	2.470	2/4	1.095	1/2	18 (78.3)	23 (100)
<i>A. terreus</i> , 13	1.896	2/2	0.766	1/1	9 (69.2)	13 (100)
<i>A. niger</i> , 10	1.741	2/2	0.230	0.25/0.5	0 (0)	4 (40)
Itraconazole						
All, 100	0.346	0.12/>16	0.688	0.25/>8	77 (77)	92 (92)
<i>A. fumigatus</i> , all, 54	0.906	0.12/>16	1.644	0.5/>8	41 (75.9)	50 (92.6)
<i>A. flavus</i> , 23	0.120	0.12/0.12	0.175	0.12/0.5	20 (87.0)	23 (100)
<i>A. terreus</i> , 13	0.067	0.06/0.12	0.114	0.12/0.12	12 (92.3)	13 (100)
<i>A. niger</i> , 10	0.186	0.25/0.25	1.510	0.5/>8	4 (40)	6 (60)
Posaconazole						
All, 100	0.095	0.06/0.5	0.214	0.12/1	66 (66)	95 (95)
<i>A. fumigatus</i> , all, 54	0.110	0.06/0.5	0.321	0.25/2	27 (50)	50 (92.6)
<i>A. flavus</i> , 23	0.110	0.12/0.12	0.146	0.12/0.25	23 (100)	23 (100)
<i>A. terreus</i> , 13	0.048	0.06/0.06	0.083	0.06/0.12	10 (76.9)	13 (100)
<i>A. niger</i> , 10	0.074	0.06/0.12	0.199	0.12/0.5	6 (60)	9 (90)
Voriconazole						
All, 100	0.732	0.5/4	0.758	0.5/4	100 (100)	100 (100)
<i>A. fumigatus</i> , all, 54	0.913	0.5/4	0.999	0.5/4	54 (100)	54 (100)
<i>A. flavus</i> , 23	0.599	0.5/1	0.547	0.5/1	23 (100)	23 (100)
<i>A. terreus</i> , 13	0.474	0.5/0.5	0.474	0.5/0.5	13 (100)	13 (100)
<i>A. niger</i> , 10	0.616	0.5/1	0.660	0.5/1	10 (100)	10 (100)

Of 24 TR₃₄/L98H *A. fumigatus* isolates, itraconazole and voriconazole MICs by YeastOne correlated well with those by M38-A2 in terms of the GM MICs, the MIC ranges, the high-level agreement (within one dilution) (100% for itraconazole and voriconazole), and categorial agreement (100% for itraconazole and 91.7% for voriconazole). The very major error rate (16.7%) noted for posaconazole might be explained by the observation that posaconazole YeastOne MICs were generally one dilution lower than the corresponding M38-A2 MICs.

The M38-A2 method found that four *A. niger* strains exhibited trailing growth with microscopically aberrant small, rounded, compact hyphal forms in wells with itraconazole concentrations ranging from 0.5 to 8 mg/L, and the itraconazole MICs were read as >8 mg/L (Figure 1), whereas the corresponding itraconazole MICs determined by YeastOne were all ≤ 0.5 mg/L without trailing growth. The clinical significance of trailing growth causing in vitro itraconazole resistance is not clear due to the rarity of related reports, which warrants further investigations.

Conclusions

YeastOne might be a suitable alternative method for the susceptibility testing of itraconazole, posaconazole, and voriconazoles against common *Aspergillus* spp. and for detecting azole-resistant TR₃₄/L98H *A. fumigatus*. Nevertheless, YeastOne tended to yield higher amphotericin B MICs and failed to detect *A. niger* isolates which exhibited trailing growth with itraconazole in the M38-A2 method. Hence, MIC interpretations in both cases should be undertaken with caution.

Figure 1. Determination of MICs of itraconazole against four *Aspergillus niger* strains by CLSI M38-A2. In wells with itraconazole concentrations ranging from 0.5 to 8 mg/L, (A) trailing growth, and (B) microscopically aberrant growth (small, rounded, compact hyphal forms, C01-014) are illustrated.

