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Comparison of Antifungal Susceptibility Testing Methods for Filamentous Organisms: Microbroth Dilution vs. Agar Gradients Strips vs. Agar Dilution Panels

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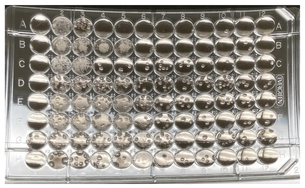
INTRODUCTION

Clinical Laboratory Standards Institute (CLSI) broth microdilution (BMD) is a reference method for mold antifungal susceptibility testing (AFST) but is time consuming to perform and requires considerable expertise with interpretation. Alternatively, gradient strips (AGS) and agar dilution panels (ADP) methods are relatively easy to perform and interpret. Here, we compared AGS and ADP from LIOFILCHEM® s.r.l with the BMD method for AFST of 100 mold isolates from hyaline hyphomycetes Mucorales and dematiaceous groups against amphotericin B (AB), voriconazole (VZ), itraconazole (IT), posaconazole (PZ), isavuconazole (IS) and micafungin (MF).

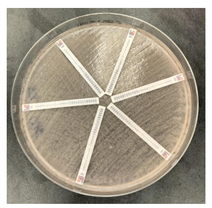
METHODS

Setup and read using CLSI guidelines



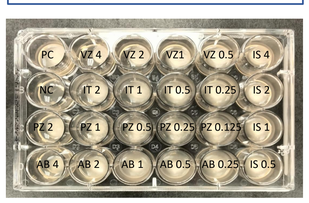
Picture 1: Standard antifungal microbroth-dilution panel. Column 1 is negative control, column 2 is positive control, and column 3-12 have dilutions of the antifungal, starting with 0.03 ug/mL (column 3) and ending with 16 ug/mL (column 12). For filamentous fungi, the first well without growth is the MIC.

Setup on RPMI media and incubated at 30C until confluent growth observed (1-5 days). MIC read where inhibition ellipse intersects the MIC scale



Picture 2: 48h of *F. oxysporum* inoculated on LFC RPMI medium with AGS (voriconazole, itraconazole, posaconazole, isavvuconazole, amphotericin B and micafungin). MIC =100% inhibition for AB and 80% inhibition for azoles and micafungin.

25 ul of suspension was inoculated in each well except negative control (NC). Incubated at 30C for 1-5 days. Read when there is sufficient growth in the positive control (PC)



Picture 3: 48 h of *F. oxysporum* inoculated on AD panel comprising antifungals of various concentrations. MIC is read in the well at which there is a marked reduction in appearance of growth compared to PC.

RESULTS

	BMD	AGS (No/ Insufficient growth)	AD	AGS vs BMD: % Essential agreement	ADP vs BMD: % Essential agreement	AGS vs ADP: % Essential agreement
AMPHOTERICIN B						
Dematiaceous	27	19 (8)	27	53 (10/19)	**100 (27/27)**	63 (12/19)
Hyaline hyphomycetes	48	44 (4)	48	68 (30/44)	**100 (48/48)**	70 (31/44)
Mucorales	19	19	19	26 (5/19)	**95 (18/19)**	26 (5/19)
Total	94	82 (12)	94	55 (45/82)	**99 (93/94)**	59 (48/82)
VORICONAZOLE						
Dematiaceous	30	23 (7)	30	78 (18/23)	**90 (27/30)**	65 (15/23)
Hyaline hyphomycetes	48	45 (3)	48	80 (36/45)	**94 (45/48)**	82 (37/45)
Mucorales	20	20	20	35 (7/20)	**95 (19/20)**	85 (17/20)
Total	98	88 (10)	98	69 (61/88)	**93 (91/98)**	78 (69/88)
ITRACONAZOLE						
Dematiaceous	27	22 (5)	27	77 (17/22)	85 (23/27)	86 (19/22)
Hyaline hyphomycetes	43	41 (2)	43	83 (34/41)	65 (28/43)	68 (28/41)
Mucorales	18	18	18	44 (8/18)	78 (14/18)	67 (12/18)
Total	88	81 (7)	88	73 (59/81)	74 (65/88)	73 (59/81)
POSACONAZOLE						
Dematiaceous	27	23 (4)	27	74 (17/23)	81 (22/27)	83 (19/23)
Hyaline hyphomycetes	46	44 (2)	46	84 (37/44)	57 (26/46)	82 (36/44)
Mucorales	19	18 (1)	19	72 (13/18)	84 (16/19)	44 (8/18)
Total	92	85 (7)	92	79 (67/85)	70 (64/92)	74 (63/85)
ISAVUCONAZOLE						
Dematiaceous	25	21 (4)	25	48 (10/21)	84 (21/25)	48 (10/21)
Hyaline hyphomycetes	50	46 (4)	50	80 (37/46)	**90 (45/50)**	85 (39/46)
Mucorales	18	18	18	83 (15/18)	83 (15/18)	78 (14/18)
Total	93	85 (8)	93	73 (62/85)	87 (81/93)	74 (63/85)
MICAFUNGIN						
Dematiaceous	23	20 (3)		40 (8/20)		
Hyaline hyphomycetes	46	44 (2)		61 (27/44)		
Mucorales	16	16		**100 (16/16)**		
Total	85	80 (5)		64 (51/80)		

RESULTS SUMMARY

- 100 medically important filamentous fungi were isolated from a variety of patient specimens: 30 dematiaceous fungi (D), 50 hyaline hyphomycetes (H), and 20 Mucorales (M)
- Comparison of CLSI broth microdilution method (BMD) with agar dilution method (AD) showed 99% essential agreement (EA) in testing 94 filamentous fungi for amphotericin B (AMB), and 93% EA in testing 98 filamentous fungi for voriconazole (VOR).
- For gradient strips (AGS), up to 12% of the tested plates showed no growth (NG) or insufficient lawn of growth (INS) of filamentous fungi on RPMI plate media, mostly seen in Dematiaceous group. Compared to BMD, 100% Essential agreement was seen in Mucorales tested for micafungin (MICA) with all isolates showing resistance.
- Comparison of gradient strips vs agar dilution panels didn't show acceptable concordance, with majority of the MIC results from gradient strips being lower than those from agar dilution panels.

CONCLUSIONS

- Agar dilution panels could be an alternative methods for testing Amphotericin B and Voriconazole for filamentous fungi.
- Insufficient or no growth of organism on culture plates may limit the utility of gradient strips.
- Broth microdilution (BMD) remains the gold standard for filamentous organism antifungal testing.

REFERENCES

CLSI. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi*, 3rd ed. CLSI standard M38. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.

ACKNOWLEGEMENTS

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