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In Vitro Post-Antifungal Effect of Amphotericin B, Fluconazole, and Micafungin against *Candida albicans*, *Candida glabrata*, and *Candida parapsilosis*

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Introduction

- Invasive candidiasis is an important healthcare-associated fungal infection mostly caused by *Candida albicans*, *C. glabrata*, and *C. parapsilosis*.
- Post-antifungal effect (PAFE) is the amount of time fungal growth is inhibited after a brief exposure to an antifungal agent.
- Understanding PAFE is clinically useful when evaluating antifungal therapeutic regimens.
- Amphotericin B, fluconazole, and micafungin are representative of systemic antifungal agents used to treat candidiasis.
- In this study, the PAFE of amphotericin B, fluconazole, and micafungin were evaluated against *C. albicans, C. glabrata,* and *C. parapsilosis* isolates.

Materials and Methods

- *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, and one clinical isolate of *Candida glabrata* were tested.
- Antifungal susceptibility testing was performed using the Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution method.
  - Amphotericin B (Sigma-Aldrich), fluconazole (USP), and micafungin (Sigma-Aldrich) were tested.
  - Each isolate was tested in triplicate to establish baseline MIC values (bMIC).
  - Modal MIC values were used to determine drug concentrations for PAFE testing.
- For PAFE determinations, antifungal concentrations of 1X, 4X, and 16X the baseline MIC were used.
- A starting inoculum of 1-5x10^5 CFU/mL from a fresh culture was added to RPMI with the respective antifungal at the desired concentration, either 1X, 4X, or 16X the bMIC.
  - A growth control that was not exposed to an antifungal agent was used to determine the standard 1$\log_{10}$ increase.
- After a 1 h exposure to the antifungal agent, the cells were washed three times with RPMI and reconstituted with pre-warmed RPMI to a final volume of 10mL.
- Colony counts were performed at T0 (pre-exposure) after the 1 h drug exposure and after the cell wash (T1).
  - Test cultures were re-incubated following final cell wash.
  - Colony counts were performed at T2, T4, T8, T12, T24, and T48 hours.
- PAFE was calculated as the difference in time required for isolates to grow 1$\log_{10}$ after the final cell wash compared to the untreated growth control.
  - PAFE = T – C, where T is time required for isolate to increase 1$\log_{10}$ (in CFU) after drug removal and C is time required for untreated growth control to increase 1$\log_{10}$ after undergoing the same process performed on the test culture.
- The reduction in starting inocula, in log-kill, was also calculated over the 48 h study period.

Results

- PAFE results and bMICs for amphotericin B, fluconazole, and micafungin are shown in Table 1.
- Amphotericin B exhibited prolonged PAFE (>40 h) against all *Candida* species, regardless of the concentration tested (Figure 1)
  - *C. glabrata* displayed a PAFE of 40.1 h at 1X bMIC and >46.1 h at 4X and 16X bMIC.
  - PAFE values were >45.7 h at all concentrations tested against *C. albicans.*
  - The amphotericin B PAFE against *C. parapsilosis* was >41.5 h at all concentrations tested. Notably, amphotericin B was the only antifungal displaying prolonged PAFE against *C. parapsilosis* (Figure 1).
    - At 16X bMIC, no cells were recovered following 1 h of amphotericin B exposure against *C. albicans*, *C. parapsilosis*, and *C. glabrata* (Table 2).
    - In addition, no viable cells were recovered at any amphotericin B concentration tested against *C. glabrata* or at 4X the bMIC against *C. albicans* (Table 2).
    - A reduction of 1.91 and 2.71 $\log_{10}$ in the viable cell counts were observed for amphotericin B at 1X and 4X the bMIC against *C. parapsilosis*, respectively (Table 2).
- Short PAFE times were observed for fluconazole against *C. albicans* (range, 0.6-2.5 h) regardless of the concentration tested (Figure 2, Table 1).
  - No PAFE was observed for fluconazole at the concentrations tested against *C. parapsilosis*.
  - No decrease in the viable cell counts (log-kill) was observed after 1 h exposure to fluconazole for *C. albicans* or *C. parapsilosis* (Table 2).
  - *C. glabrata* showed elevated fluconazole bMIC values; PAFE was not tested against this isolate.
- Micafungin exhibited prolonged PAFE against *C. albicans* and *C. glabrata*, but no PAFE was observed against *C. parapsilosis* irrespective of the concentrations tested (Figure 3, Table 1).
  - PAFE was >41.1 h against *C. albicans* at all concentrations tested and the $\log_{10}$ reduction in the viable cell counts varied from 0.11 at 1X the bMIC to 0.95 at 16X the bMIC.
  - *C. glabrata* displayed PAFE values of 37.4 h at 1X bMIC and >46.4 h at 4X and 16X the micafungin bMIC.
  - *C. glabrata* exposure to micafungin caused a reduction in the viable cell counts in a concentration-dependent manner (Table 2; Figure 3).
  - No decrease in the viable cell counts was observed after 1 h exposure to micafungin against *C. parapsilosis,* regardless of the concentrations tested (Table 2).

Figure 1. Amphotericin B post-antifungal effect (PAFE) against *C. albicans*, *C. parapsilosis*, *and C. glabrata* at 1X, 4X, and 16X the baseline MIC values.

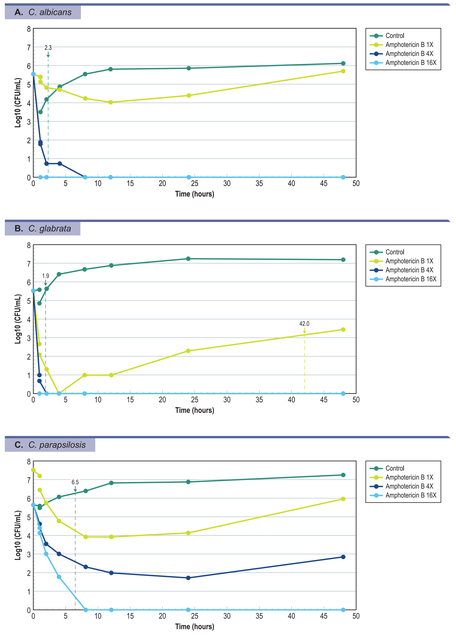


Figure 2. Fluconazole post-antifungal effect (PAFE) against *C. albicans* and *C. parapsilosis* at 1X, 4X, and 16X the baseline MIC values

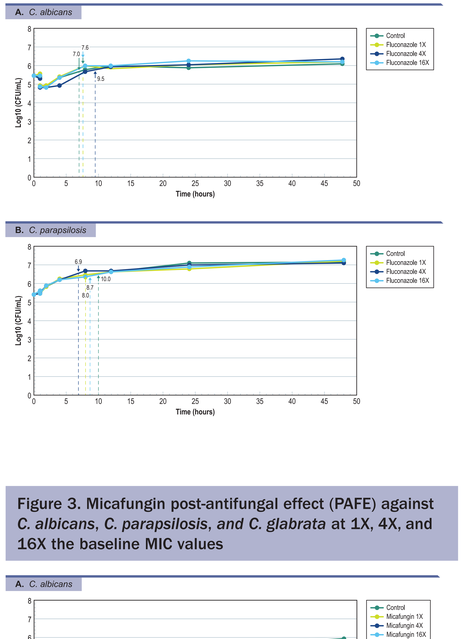


Table 1. Post-Antifungal Effect (PAFE) for amphotericin B, fluconazole, and micafungin against *C. albicans*, *C. parapsilosis*, and *C. glabrata*

Antifungal / Microorganism tested	bMIC (mg/L)	PAFE (hours) at the following multiple of bMIC		
		1X	4X	16X
Amphotericin B				
C. albicans	1	>45.7	>45.7	>45.7
C. parapsilosis	1	>41.5	>41.5	>41.5
C. glabrata	1	40.1	>46.1	>46.1
Fluconazole				
C. albicans	0.25	0.6	2.5	0.6
C. parapsilosis	1	≤0.0	≤0.0	≤0.0
C. glabrata	128	NT	NT	NT
Micafungin				
C. albicans	0.015	>41.1	>41.1	>41.1
C. parapsilosis	1	≤0.0	≤0.0	≤0.0
C. glabrata	0.03	37.4	>46.4	>46.4

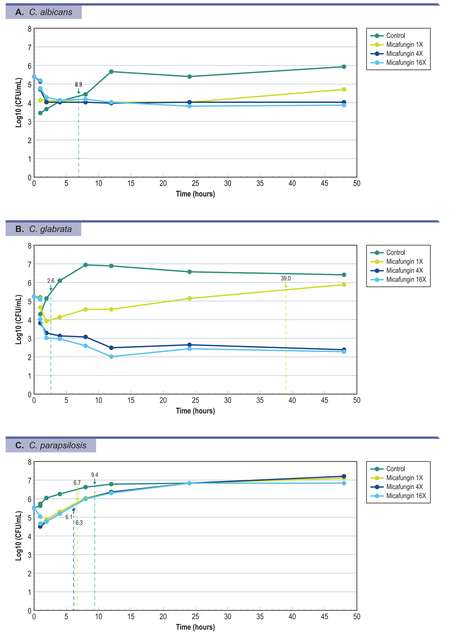
Abbreviations: bMIC, baseline MIC; NT, not tested.

Table 2. Reduction in starting inocula (PAFE, in log-kill) of *C. albicans*, *C. parapsilosis*, and *C. glabrata* after 1-hour exposure to amphotericin B, fluconazole, and micafungin

Antifungal / Microorganism tested	bMIC (mg/L)	Maximum log reduction in colony counts over 48-hour period		
		1X	4X	16X
Amphotericin B				
C. albicans	1	1.07	ND	ND
C. parapsilosis	1	1.91	2.71	ND
C. glabrata	1	ND	ND	ND
Fluconazole				
C. albicans	0.25	–0.02	–0.02	0.02
C. parapsilosis	1	–0.33	–0.39	–0.43
C. glabrata	128	NT	NT	NT
Micafungin				
C. albicans	0.015	0.11	0.70	0.95
C. parapsilosis	1	–0.26	–0.29	–0.13
C. glabrata	0.03	0.70	1.49	2.04

Abbreviations: ND, not determined, or below the limit of detection (10^2 CFU/mL); NT, not tested.
Negative values indicate that there was no reduction in colony counts compared with the starting inoculum.

Figure 3. Micafungin post-antifungal effect (PAFE) against *C. albicans*, *C. parapsilosis*, *and C. glabrata* at 1X, 4X, and 16X the baseline MIC values



Conclusions

- Differences in PAFE against *Candida* spp. are useful when considering the dosing of antifungal agents.
- Amphotericin B had the most sustained activity following drug removal among the antifungal agents tested against this set of *Candida* isolates.
- Micafungin displayed prolonged PAFE against *C. albicans* and *C. glabrata,* but no PAFE was observed against *C. parapsilosis*.
- Fluconazole had no activity against *C. glabrata* and limited PAFE against *C. parapsilosis* and *C. albicans* isolates.

Acknowledgements

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