

Activity of amphotericin B, caspofungin and anidulafungin on planktonic and biofilm *Candida* spp. by microcalorimetry

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Objectives: *Candida* biofilms are difficult to treat due to reduced susceptibility in biofilm. We compared the activity of amphotericin B (AMPB) and two echinocandins, caspofungin (CAS) and anidulafungin (AFG), against planktonic and biofilm *Candida* spp. using microcalorimetry, a real-time and highly sensitive assay for measurement of growth-related heat production. **Methods:** *C. albicans* (ATCC 90028), *C. krusei* (ATCC 6258) and *C. glabrata* (DSY 562) were tested. Minimal inhibitory concentration (MIC) was determined by microbroth dilution according to the EUCAST guidelines (EDef 7.1) and confirmed by E-test. Microcalorimetry was performed using an isothermal calorimeter (TAM III, TA Instruments) at 37°C in sealed glass ampoules containing 3 ml RPMI. Planktonic *Candida* species (5×10^5 CFU) were added to RPMI containing serial dilution of AMPB, CAS or AFG (0.125-512 µg/ml). *Candida* biofilm was formed on porous glass beads (diameter 4 mm, pore size 60 µm) and incubated for 24 h at 37° C in RPMI. Beads were washed and incubated for 24 h in RPMI containing serial dilution of AMPB, CAS or AFG (0.5-1024 µg/ml). Beads were then washed and placed in 3ml of RPMI into the calorimeter to quantify recovering yeasts. The minimal heat inhibitory concentration (MHIC) was defined as the lowest antifungal concentration reducing the heat-flow peak by $\geq 50\%$. **Results:** The MICs for tested *Candida* spp. ranged from 0.5-2 µg/ml for AMPB, 0.125-0.5 µg/ml for CAS and 0.03-0.015 µg/ml for AFG. Table shows MHICs (in µg/ml) for planktonic and biofilm *Candida*. Compared to planktonic counterparts, the activity of AMPB was significantly reduced against *Candida* biofilms (MHIC ≥ 32 µg/ml). CAS showed antibiofilm activity against *C. albicans* and *C. glabrata* (MHIC = 2-4 µg/ml), but not against *C. krusei* (MHIC = 32 µg/ml). AFG showed good activity against all *Candida* biofilms (MHIC ≤ 1 µg/ml). At high concentrations (>8 µg/ml), CAS and AFG induced paradoxical growth of planktonic *C. albicans* and CAS induced the same effect on *C. glabrata*. **Conclusions:** Microcalorimetry allows real-time evaluation of antifungal activity on planktonic and biofilm *Candida* spp. AFG showed better activity against *Candida* biofilms than AMPB, whereas CAS showed variable results. In future, microcalorimetry might be used to study novel antifungals (alone or in combination) to determine the optimal treatment strategies.

Species	AMPB (µg/ml)		CAS (µg/ml)		AFG (µg/ml)	
	Planktonic	Biofilm	Planktonic	Biofilm	Planktonic	Biofilm
<i>C. albicans</i>	0.5	64	0.125	4	0.03	0.5
<i>C. glabrata</i>	2	32	0.125	2	0.015	1
<i>C. krusei</i>	1	32	0.5	32	0.03	1