How do *Candida glabrata*'s biofilms respond to antifungal drugs?

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Abstract

Candida species are responsible for recurrent human infections, mostly in immunocompromised patients, due to their high vulnerability.

Candida glabrata has been shown to have a major role in these infections being the second most prevalent species involved in human fungemia.

Objective: To understand the effect of three different antifungal agents – Fluconazole (Flu), Amphotericin B (AmB) and Caspofungin (Csf) - in *C. glabrata*'s biofilm formation, specially their role on matrix composition.

Methods

Flu, AmB and Csf susceptibilities were determined in pre-formed 24-hour-biofilms of two clinical isolates and one reference strain of *C. glabrata*.

Concentrations of the drugs:

Flu – 1000 mg/L; **AmB** – 2 mg/L; **Csf** – *C. glabrata* ATCC 2001 – 3 mg/L; *C. glabrata* 562123– 0.5 mg/L; *C. glabrata* 534784– 2.5 mg/L.

1. ***Biofilm cell and biomass analysis***: biofilm cultivable cells (CFU) and biofilm total biomass quantification (Violet Crystal 1% v/v);
2. ***Biofilm matrix composition***: carbohydrates, proteins, β-1,3-glucans and ergosterol quantification;
3. ***Biofilm production***: dry weight.

Results

Antifungal Susceptibility

Table 1. *Candida glabrata's* % of viable cells and biomasse in presence of fluconazole, amphotericin B and caspofungin, comparing with the controls.

Candida glabrata strain	% Biofilm Cells (after 24h of drug exposure)	% Biomass (after 24h of drug exposure)
ATCC 2001	Flu: 104.5%	Flu: 81.8%
	AmB: 1.0%	AmB: 29.8%
	Csf: 48.1%	Csf: 23.1%
562123	Flu: 104.1%	Flu: 77.8%
	AmB: 1.0%	AmB: 35.7%
	Csf: 4.6%	Csf: 24.0%
534784	Flu: 99.0%	Flu: 87.6%
	AmB: 4.0%	AmB: 54.6%
	Csf: 65.1%	Csf: 19.6%

AmB and Csf showed the best performance in the reduction of biofilms formed by the three *Candida glabrata* strains, in opposition to Flu.

AmB, has a fungicidal, had a best CFU reduction performance, but Csf had a better biomass reduction.

β-1,3 glucans contents

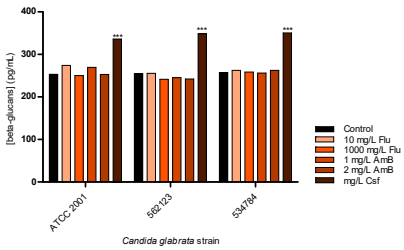

Table 2. β-1,3-glucans concentration on biofilm matrices of *Candida glabrata* strains in the presence of fluconazole (Flu 10 =10 mg/L; Flu 1000 = 1000 mg/L) , amphotericin B (AmB 1 = 1 mg/L; AmB 2 = 2 mg/L) and caspofungin (ATCC 2001 = 3 mg/L; 562123 = 0,5 mg/L; 534784 = 2,5 mg/L) (**** P<0.0001*).

The amount of β-1,3 glucans on the matrices did not show significant differences in the presence of the drugs, with the exception of Csf, which induced an increase of ≅20% of these compounds.

Carbohydrates and Proteins Contents

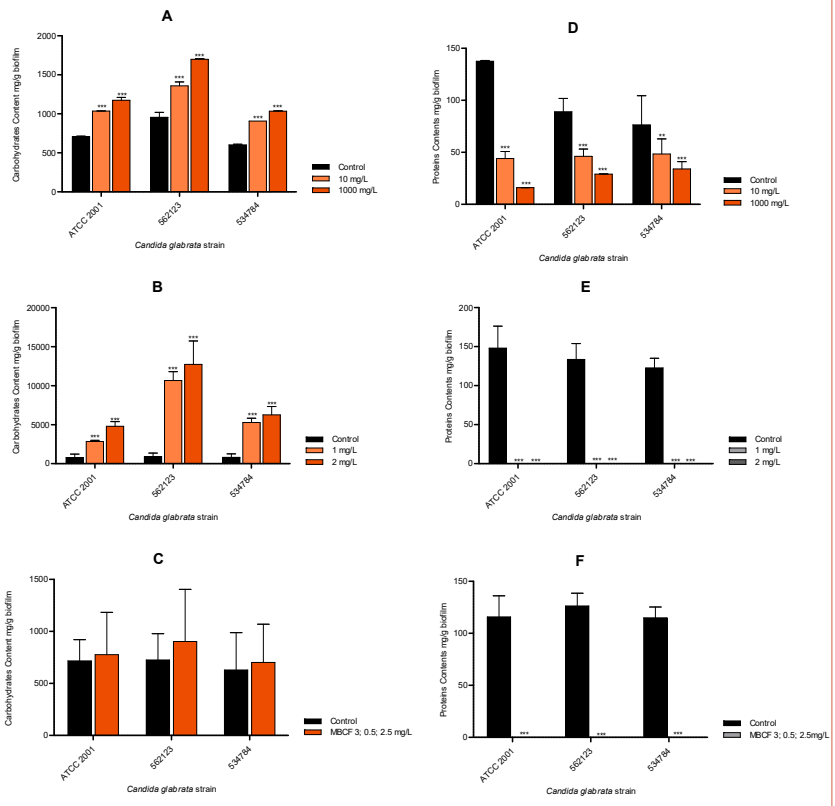

Figure 2. Ratio of carbohydrates (A, B and C) and proteins (D, E and F) content in biofilm matrices of *Candida glabrata* strains with different concentrations of fluconazole, amphotericin B and caspofungin (** P<0.05; ** P< 0.001; *** P<0.0001*)

There was an increase of carbohydrates and decrease of proteins, in the presence of the three antifungals

For AmB and Csf this decrease was very significative, being below the minimum detected value range of the BCA® proteins Kit.

Conclusions

- The pattern of *C. glabrata* biofilm response to Flu, AmB and Csf is clearly different;
- The three agents had diverse effects on *C. glabrata*'s biofilm formation;
- Matrices´ composition display variations when exposed to different antifungal agents, and these differences depend on the drug is used;
- AmB, and especially Csf, were confirmed in this study, to be the most effective pharmacoterapies for eradication of *C. glabrata* infections associated to biofilms.

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