

Fluconazole vs Voriconazole: *Candida glabrata*'s biofilms response to different azoles

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Abstract

Candida glabrata is the second most prevalent yeast in fungal infections, especially in immunocompromised and/or hospitalized patients. The azole resistance within this species is very well-known and results in a low therapeutic response of *C. glabrata* infections, particularly when associated with biofilms.

Objective - To understand the different efficacies of two azoles against *C. glabrata* biofilms:

- fluconazole (Flu), a long time used drug
- voriconazole (Vcz), a latest drug used only in hospitals.

Results

ERG Genes Expression

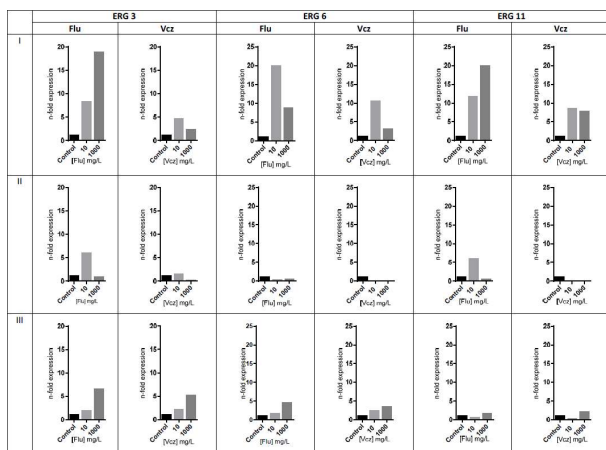


Figure 1. ERG 3, ERG 6 and ERG 11 expressions on biofilm cells for each strain, with different concentrations of fluconazole and voriconazole. (I) *C. glabrata* ATCC 2001; (II) *C. glabrata* 562123; (III) *C. glabrata* 534784

Overexpression of the three ERG genes in the presence of both azoles. ERG expression more dependent on the strain than on the agent.

% Cell death and Biomass reduction

Drug	<i>C. glabrata</i> ATCC 2001		<i>C. glabrata</i> 562123		<i>C. glabrata</i> 534784	
	% Cell death	% Biomass reduction	% Cell death	% Biomass reduction	% Cell death	% Biomass reduction
[Flu] mg/L						
10	0.75±0.01	0	0	16.27±0.01	3.54±0.01	6.93±0.15
100	2.34±0.01	18.77±0.01	0	27.06±0.01	4.62±0.02	8.27±0.01
500	2.16±0.03	18.21±0.01	0.05±0.06	25.10±0.10	5.08±0.10	6.40±0.01
1000	2.22±0.04	19.05±0.01	2.18±0.05	20.00±0.10	5.56±0.00	12.27±0.01
1250	1.21±0.06	15.69±0.01	13.46±0.00	23.14±0.01	4.94±0.00	25.87±0.00
2000	21.86±0.13	19.89±0.01	20.56±0.01	23.14±0.01	24.28±0.13	30.40±0.04
[Vcz] mg/L						
10	0.62±0.12	0	0	5.25±0.01	0	0
100	1.71±0.10	31.78±0.01	0	39.50±0.01	0	5.54±0.01
400	24.59±0.45	40.67±0.02	41.92±0.14	49.17±0.01	9.61±0.01	24.00±0.01
800	51.34±0.13	48.22±0.01	60.34±0.33	53.31±0.01	20.75±0.45	41.54±0.01
1000	57.32±0.21	52.44±0.01	70.57±0.14	61.33±0.02	43.37±0.20	35.23±0.02

bold: inhibition higher than 50%

Table 1. Ratio of cell death and biomass loss of biofilms of *Candida glabrata* strains with different concentrations of fluconazole and voriconazole, in comparison to the control sample

Flu was unable to eliminate the *C. glabrata*'s biofilm cells. Vcz showed to be much effective in the eradication of the three strains.

Acknowledgements

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Conclusions

- The pattern of *C. glabrata* biofilm resistance to Flu and Vcz is effectively different;
- The cell genetic alterations in genes encoding for the ergosterol biosynthesis exists but is still unable to explain the different resistance profiles obtained for these two agents;
- There were alterations in the matrix composition in the presence of the azoles, but there was not found a direct correlation with Flu and Vcz efficacy;
- The diffusion of the drugs within the biofilms is azole's dependent, with Vcz having a better diffusion and a better activity against *C. glabrata* biofilms than Flu, explaining its enhanced performance.

Methods

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

1. **Gene expression analysis:** ERG3, ERG6 and ERG11 expression by qRT-PCR;
2. **Biofilm matrix composition:** carbohydrates, proteins, β-1,3-glucans and ergosterol quantification;
3. **Biofilm production:** dry weight;
4. **Biofilm cell and biomass analysis:** biofilm cultivable cells (CFU) and biofilm total biomass quantification (Violet Crystal 1% v/v);
5. **Retention of the two azoles within the biofilm matrix:** HPLC analysis.

Carbohydrates and Proteins Contents

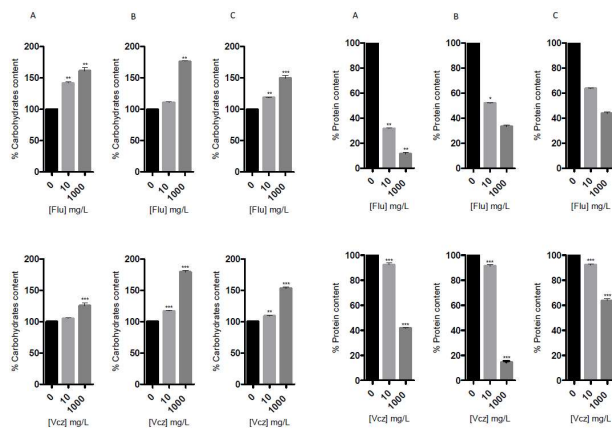


Figure 2. Ratio of carbohydrates content in biofilm matrices of *Candida glabrata* strains with different concentrations of fluconazole and voriconazole. (A) *C. glabrata* ATCC 2001; (B) *C. glabrata* 562123; (C) *C. glabrata* 534784 (* P<0.05; ** P<0.001; *** P<0.0001)

Figure 3. Ratio of protein production in biofilm matrices of *Candida glabrata* strains with different concentrations of fluconazole and voriconazole. (A) *C. glabrata* ATCC 2001; (B) *C. glabrata* 562123; (C) *C. glabrata* 534784 (* P<0.05; ** P<0.001; *** P<0.0001)

In presence of antifungal: increase of carbohydrates and decrease of proteins.

β-1,3 glucans and Ergosterol contents

<i>C. glabrata</i> strain	[Drug] mg/L	[β-glucan]/Carbohydrates [Flu]	[β-glucan]/Carbohydrates [Vcz]	[Ergosterol] mg/g biofilm [Flu]	[Ergosterol] mg/g biofilm [Vcz]
<i>C. glabrata</i> ATCC 2001	0	0.00012		1.17 ± 0.70	
	10	0.00098	0.00018	1.15 ± 0.77	0.58 ± 0.21
	1000	0.00074	0.00011	0.51 ± 0.14	0.79 ± 0.16
<i>C. glabrata</i> 562123	0	0.00013		1.48 ± 0.71	
	10	0.00088	0.00017	1.01 ± 0.09	0.95 ± 0.47
	1000	0.00043	0.00009	1.39 ± 0.25	1.45 ± 0.30
<i>C. glabrata</i> 534784	0	0.00012		1.32 ± 0.67	
	10	0.00114	0.00027	0.75 ± 0.05	1.04 ± 0.21
	1000	0.00075	0.00017	1.45 ± 0.07	2.16 ± 1.01

Table 2. β-1,3-glucans concentration and ergosterol concentration on biofilm matrices of *Candida glabrata* strains in the presence of fluconazole and voriconazole

In the presence of antifungal: increase of β-1,3 glucans for all strains. Generally: no alteration on the amount of ergosterol present in the matrices of biofilms exposed to agents, in comparison with the controls.

Antifungal diffusion through biofilm matrices

Drug	<i>C. glabrata</i> strain	[Drug] matrix (mg/L) ± SD	[Drug] supernatant (mg/L) ± SD
Fluconazole	ATCC 2001	670.80 ± 61.87	69.21 ± 21.49
	562123	551.87 ± 56.31	60.81 ± 20.10
	534784	702.29 ± 77.92	69.88 ± 17.06
Voriconazole	ATCC 2001	0.44 ± 0.20	5.38 ± 1.20
	562123	0.43 ± 0.21	5.27 ± 1.38
	534784	0.60 ± 0.25	5.85 ± 1.67

Table 3. Antifungal drug diffusion by *Candida glabrata* biofilm matrices and in supernatant

Higher capacity of Vcz to penetrate the biofilm net: Vcz diffused better through the biofilm net (96%) than Flu (90%) and with a better connection to the fungi cells.