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Candida glabrata can acquire *in vitro* resistance to echinocandins after exposure to low micafungin concentrations

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Background and Objectives

- Echinocandin resistance in *Candida glabrata* isolates has emerged in some geographic areas. Resistant isolates show elevated echinocandin MICs and harbour mutations in *fks* genes.
- We studied the potential of *C. glabrata* to acquire *in vitro* resistance to echinocandins after exposing the isolates to low micafungin concentrations and the fitness of *fks* mutant isolates was studied by measuring growth kinetics and virulence in an *in vivo Galleria mellonella* model.

Methods

Candida glabrata isolates (n=5), from patients with candidemia, genetically unrelated.
Micafungin and anidulafungin MICs were determined according to EUCAST EDef 7.2 ($MIC_{initial}$).

Exposure to micafungin-containing agar plates:

- Isolates suspensions were adjusted to 4 – 6 x 10^9 (mean of 4.7±0.98 x10^9) CFU/mL, stroked on 0.031 mg/L micafungin-containing plates (100 µL), and incubated at 35ºC for 24 h.
- If growth was observed, a new adjusted suspension was propagated on 0.031 mg/L micafungin-containing plates up to nine propagation steps. The MICs of the echinocandins and *fks1* and *fks2* mutations were studied at each propagation step ($MIC_{subsequent}$).
- Geometric means of $MIC_{initial}$ and MIC_{final} (last propagation step) of micafungin and anidulafungin were compared. Genotyping proved the absence of contaminations.

Study of fitness of the wild type and the resulting *C. glabrata* mutant isolates:

- The *in vitro* growth kinetics of wild type isolates and mutant isolates were compared. Differences among the kinetics parameters (average growth rate, and time to maximum rate) were studied.
- We compared the mortality caused by wild type isolates and by *fks* mutant isolates (obtained in the last propagation step) on *Galleria mellonella*.
  - Ten *G. mellonella* larvae per isolate were infected with 10 µL of inocula ranging 3-7x 10^6 CFU per larva.
  - Larvae were incubated at 37º C up to 7 days post-infection and the number of dead larvae was scored daily.
  - Survival curves were obtained by the Kaplan–Meier method and differences were evaluated by Log-Rank; a *P* value of <0.05 was considered to be statistically significant.

Results

All isolates were initially echinocandin-susceptible (GM of micafungin/anidulafungin = 0.015 mg/L and 0.017 mg/L, respectively) but they became phenotypically echinocandin resistant after 2-4 days of exposure to micafungin (GM 2.64 mg/L and 2 mg/L, respectively) (*P* < 0.05) (Table).

Mutations in the HS1 of the *fks2* gene were found in all resistant isolates. S663P was the most frequent substitution, followed by other substitutions located outside the HS1 (deletion at F658), and a substitution newly described (W715L) (Table).

Table: Susceptibility and fks2 HS1 substitutions on the first phenotypic resistant propagation step

Isolates	Propagation day when the isolate become phenotypically resistant	EUCAST MIC (mg/L) MYC/AND			*fks2* HS1 substitution
		$MIC_{initial}$	$MIC_{subsequent}$	MIC_{final}	
1	2nd	0.015/0.03	2/2	2/2	S663P
2	4th	0.015/0.015	2/2	2/2	W715L
3	3rd	0.015/0.015	1/2	4/4	S663P
4	3rd	0.015/0.015	2/1	2/1	delF658
5	2nd	0.015/0.015	2/1	4/2	S663P

The kinetics parameters of wild type and *fks* mutant isolates were similar.

However, we found differences (*P* < 0.0001) in the median survival days of larvae infected by *fks* mutant isolates (5 days) and by wild type isolates (3 days) (Figure).

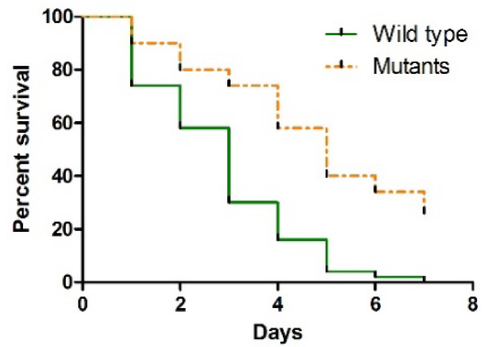

Figure. Survival curves of *G. mellonella* larvae infected by wild type isolates or infected by their corresponding mutant isolates.

Conclusions

- *C. glabrata* isolates can become resistant to echinocandins when exposed to low concentrations of micafungin. However, the resulting *fks* mutant isolates showed an attenuated virulence compared to the wild type isolates.
- These results suggest that the acquisition of resistance to echinocandins may hamper the pathogenicity of *C. glabrata*.