

# Twofold point mutations in *Candida albicans* FKS1 gene leading to echinocandin resistance after long-time treatment



K. Heidrich<sup>1</sup>, R. Krause<sup>2</sup>, M. Hönigl<sup>2</sup>, C. Lass-Floerl<sup>3</sup>, W. Buzina<sup>1</sup>

Medical University of Graz

<sup>1</sup> Medical University of Graz, Institute of Hygiene, Microbiology and Environmental Medicine, Graz, Austria.

<sup>2</sup> Medical University of Graz, Department of Internal Medicine, Section of Infectious Diseases and Tropical Medicine, Graz, Austria.

<sup>3</sup> Division of Hygiene and Medical Microbiology, Innsbruck Medical University, Innsbruck, Austria

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## Objectives

The development of pan-echinocandin resistance of *Candida albicans* isolates with the same MLST (multilocus sequence typing) profile isolated from a candidemic patient prior and after caspofungin treatment is shown.

## Materials and Methods

Two *C. albicans* isolates obtained from the same patient from blood prior to caspofungin treatment and from the oral cavity after caspofungin treatment, respectively, were investigated. Antifungal susceptibility tests for echinocandins, amphotericin B, flucytosine and azoles were performed both with Etest® and MICRONAUT susceptibility testing system. The β-1,3-glucan synthase catalytic subunit 1 (FKS1) gene, which is known as a hot spot for mutations leading to reduced susceptibility to echinocandins, was amplified and sequenced. MLST profiles were examined by comparison of the sequences of 7 DNA sites encoding housekeeping genes: AAT1a, ACC1, ADP1, PMI1b, SYA1, VPS13, and ZWF1b.

## Results (1)

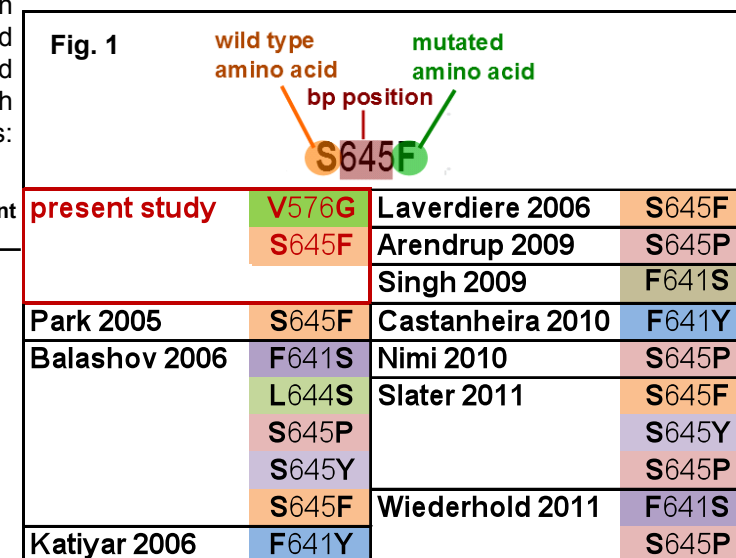
The colonizing isolate obtained after caspofungin treatment showed reduced pan-echinocandin susceptibility whereas the pre-treatment invasive isolate was susceptible to all antimycotics tested (tab. 1). Both isolates shared the same MLST profile, suggesting a high clonal homology.

## Results (2)

FKS1 sequencing of the pre-treatment strain showed a sequence corresponding to the wild type, whereas the post-treatment strain showed two point mutations in resistance hot spot 1. Both these mutations resulted in amino acid changes: V576G, S654F (fig. 1).

Tab. 1	EUCAST breakpoints	pre-treatment isolate	post-treatment isolate
<i>Amphotericin B</i>	1	0.5	0.5
<i>Flucytosine</i>	*	4	4
<i>Fluconazole</i>	4	1	1
<i>Voriconazole</i>	0.12	0.06	0.06
<i>Itraconazole</i>	*	0.125	0.125
<i>Posaconazole</i>	0.06	0.012	0.016
<i>Caspofungin</i>	*	0.38	1.5
<i>Anidulafungin</i>	0.03	0.032	1.5
<i>Micafungin</i>	0.016	0.032	0.5

**Tab. 1:** Susceptibility of the isolates obtained before and after treatment in comparison with the latest EUCAST susceptibility breakpoints. \*no EUCAST breakpoint established.



**Fig. 1:** Mutations in FKS1 hot spot 1 reported so far.

## Conclusion

This report documents the development of pan-echinocandin resistance after caspofungin treatment caused by two point mutations in FKS1 gene.

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