

Rapid Emergence of Echinocandin Resistance during *Candida kefyr* Fungemia Treatment with Caspofungin

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Echinocandin drugs are widely used for the treatment of candidemia. Resistance is considered rare, and only a few cases of breakthrough candidiasis in patients receiving echinocandin have been reported worldwide. We report here for the first time a *Candida kefyr* isolate that acquired echinocandin resistance very rapidly after the initiation of caspofungin treatment for candidemia. We characterized the *FKS* gene mutation responsible for the resistance via the comparison of isolates sampled before and during treatment.

A 61-year-old woman was admitted to the hematological unit at Pitié-Salpêtrière Hospital (Paris, France) for acute myeloid leukemia. The patient was not then receiving and had no history of receiving antifungal drugs. During chemotherapy-induced aplasia, a blood culture sampled at day 1 yielded positive for yeast at day 3. Considering the clinical condition of the patient, echinocandin antifungal therapy was chosen. Treatment by caspofungin was initiated on day 3 (70 mg the first day and then 50 mg/day; patient weight, 60 kg). Analysis of the strain with API 20C (bioMérieux, France) strips and mass spectrometry (Bruker Daltonics, Germany) clearly identified *Candida kefyr* (teleomorph, *Kluyveromyces marxianus*). Before the determination of the MICs, caspofungin treatment was left unchanged. The MICs for caspofungin, micafungin, and anidulafungin as determined by Etest (bioMérieux) were 0.06, 0.03, and 0.03 µg/ml, respectively. For fluconazole, the MIC was 0.25 µg/ml. As the patient's condition did not improve, treatment was switched to fluconazole at day 10. A blood culture sampled the same day was positive for yeast at day 11. Surprisingly, this culture gave *C. kefyr* again. For this isolate, the MICs for caspofungin, micafungin, and anidulafungin by Etest were 2, 4, and 8 µg/ml, respectively. It should be noted that the determination of MIC by the CLSI reference method showed similar results (Table 1), but considering the recently proposed epidemiological cutoff value (ECV) for echinocandins against *C. kefyr* (1), for the initial isolate with low MIC echinocandin values, the MIC for caspofungin was above the ECV, whereas those for anidulafungin and micafungin were below. While receiving fluconazole, a blood culture sampled at day 19 was positive for yeast at day 21, and this time *Candida krusei* was identified. Treatment was then switched to voriconazole at day 23. The patient died at day 30. Of note, the two candidemias were surely of digestive origin; a stool sample collected a few days before the first candidemia was positive for both *C. kefyr* and *C. krusei*.

Methods, results, and discussion. Faced with both microbiological resistance and clinical therapeutic failure, we decided to compare the hot spots (HS) of the *FKS* gene sequences of the two *C. kefyr* strains, the first identified in the blood culture sampled at day 1 (low echinocandin MIC) and the second at day 10 during caspofungin treatment (high echinocandin MIC). Resistance to echinocandin consistently involves these short *FKS* sequences that code for the glucan synthase complex enzyme, the latter being the

target of echinocandins (2, 3). No information on *C. kefyr* *FKS* gene sequences was found in databases such as NCBI GenBank. Therefore, for amplification of the HS regions of the *C. kefyr* *FKS* gene, primers were chosen by homology after alignment of different *FKS* gene sequences from different *Candida* species. For hot spot 1 (HS1), the forward primer had the sequence 5'-GGTGGT TTATTCACCTTCCTACA-3' and the reverse primer had the sequence 5'-GCGTAGCCAAAGATTGAGCA-3'. For HS2, the forward primer had the sequence 5'-AAGATTGGTGCYGGTATGG G-3' and the reverse primer had the sequence 5'-RGTDGCAAA ACCTCTAGCAGT-3'. For HS1, amplification resulted in a 729-bp DNA fragment and a consequent 243-deduced-amino-acid sequence. The HS1 amino acid sequence identified for the isolate with the low echinocandin MIC was strictly identical to that of the *Candida albicans* ATCC 90028 strain, as well as that of a *Candida tropicalis* isolate (4), both susceptible to echinocandin (Table 1). In contrast, analysis of the sequence of the isolate with high echinocandin MIC showed a T→A mutation resulting in an F→Y amino acid substitution of the deduced sequence. This substitution occurred at a position frequently involved in echinocandin resistance (5–8) and may be compared to the F641Y substitution of *C. albicans*, which involved the same single-nucleotide change (9), or the F639Y substitution of *Saccharomyces cerevisiae*, which is phylogenetically closer (10). For HS2, amplification resulted in a 496-bp fragment, for which no difference was detected between the susceptible and the resistant isolates. Thus, the HS2 sequence was identical to those of other echinocandin-susceptible yeasts, such as the *Candida glabrata* ATCC 90030 strain (Table 1). The analysis of sequences was thereafter enlarged, and longer sequences (3,628 bp) were deposited in the NCBI database. Several attempts to amplify a potential *FKS2* gene were unsuccessful. To give more relevance to our finding, we studied 6 other *C. kefyr*

Received 8 October 2012 Returned for modification 24 November 2012

Accepted 14 February 2013

Published ahead of print 25 February 2013

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doi:10.1128/AAC.02037-12

TABLE 1 Modification of the amino acid sequence of *fks* genes and MICs for echinocandins for the susceptible and nonsusceptible *C. kefyr* isolates

Strain	Origin	GenBank accession no.	Caspofungin exposition (days)	Genotype (wild type or <i>FKS</i> mutation)	Fksp sequence ^a		MIC (μg/ml) by ^b :					
					HS1	HS2	Etest			CLSI		
							Csp	Mic	Anf	Csp	Mic	Anf
<i>C. kefyr</i>												
PSL11051268	Blood	JX899422	0	Wild type	FLTLSLRDPI	DWVRRYTL	0.06	0.03	0.03	0.125	0.125	0.06
PSL1106226	Blood	JX899423	7	T→A in <i>FKS1</i> HS1	<u>Y</u> LTLSLRDPI	DWVRRYTL	2	4	8	2	4	2
PSL1104459	Blood	NA ^c	0	Wild type	FLTLSLRDPI	DWVRRYTL	0.125	0.06	0.01	0.125	0.125	0.03
1202035978	Bile	NA	0	Wild type	FLTLSLRDPI	DWVRRYTL	0.125	0.06	0.01	0.125	0.125	0.06
1205072395	Stool	NA	0	Wild type	FLTLSLRDPI	DWVRRYTL	0.125	0.06	0.01	0.25	0.125	0.06
1206069902	Blood	NA	2	Wild type	FLTLSLRDPI	DWVRRYTL	0.125	0.03	0.01	0.125	0.125	0.06
1211088295	Bronchoalveolar lavage specimen	NA	0	Wild type	FLTLSLRDPI	DWVRRYTL	0.125	0.06	0.01	0.125	0.125	0.06
HEGP2734	Blood	NA	0	Wild type	FLTLSLRDPI	DWVRRYTL	0.06	0.06	0.03	0.25	0.125	0.125
<i>C. albicans</i> ATCC 90028	NA	GQ456066.1	NA	Wild type	FLTLSLRDPI	DWVRRYTL						
<i>C. glabrata</i> ATCC 90030	Blood	HM366440.1	NA	Wild type	FLTLSLRDPI	DWVRRYTL						
<i>C. tropicalis</i>	NA	EU676168.1	NA	Wild type	FLTLSLRDPI	DWVRRYTL						
<i>Saccharomyces cerevisiae</i>	NA	U12893.1	NA	Wild type	FLTLSLRDPI	DWVRRYTL						
<i>Kluyveromyces lactis</i> NRRL Y-1140	NA	XP_451796	NA	Wild type	FLTLSLRDPI	DWVRRYTL						

^a Sequences of *fks1* HS1 and HS2 were deduced by analogy with those of *C. albicans* (3). Changed residues are underlined.

^b Csp, caspofungin; Mic, micafungin; Anf, anidulafungin.

^c NA, not available.

isolates retrieved from clinical samples, including 3 blood cultures. Echinocandin MIC determinations performed by Etest and the CLSI method were indicative of isolates with relatively low MICs. Finally, molecular analysis of the *FKS1* gene showed that the HS1 sequences of these 6 isolates were strictly identical to that of the initial isolate with a low MIC. Concerning HS2, sequences were either identical ($n = 3$) to the initial isolate or very similar, with only a single-amino-acid modification ($n = 3$). In the latter case, the HS2 sequence was thus identical to the HS2 sequences of other echinocandin-susceptible *Candida* isolates, such as *C. albicans* ATCC 90028 (Table 1).

Despite their cost and the absence of an oral form, echinocandin drugs continue to gain popularity for the treatment of invasive candidiasis; they offer an interesting anti-*Candida* spectrum, cause no major adverse effects, and do not interact with other drugs. Echinocandin resistance remains rare. In the literature, *C. albicans* and *C. glabrata* are the most frequent *Candida* species to develop echinocandin resistance, findings consistent with their predominance in invasive candidiasis (6, 11). *C. kefyr* is usually very sensitive to all antifungal drugs. This is especially true for echinocandins: in one study, the echinocandin MIC was ≤ 0.12 μg/ml for all of the 37 strains collected worldwide (12). We report here what is to our knowledge the first detection of echinocandin resistance in a clinical *C. kefyr* isolate that led to treatment failure. We provide molecular evidence of the acquisition of resistance via the illustration of a mutation in the *FKS* gene sequence, which has already been implicated in echinocandin resistance and clinical failure in patients infected by other *Candida* species.

Of particular interest in our case was the very rapid emergence of resistance, during the first days of treatment. Therefore, the surveillance of the susceptibility to echinocandin of this relatively scarce species, responsible for <1% to 1.6% of cases of candidemia (13, 14), may be of interest. Awaiting other reports of such

rapid resistance emergence, we suggest closer monitoring of *C. kefyr* in patients treated with echinocandins.

Nucleotide sequence accession numbers. The different sequences have been deposited in the NCBI database and are available under accession no. [JX899422](#) and [JX899423](#).

ACKNOWLEDGMENTS

We thank Pfizer, Astellas, and MSD for providing anidulafungin, micafungin, and caspofungin, respectively.

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