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Recurrent episodes of Candidemia due to *Candida glabrata, Candida tropicalis* and *Candida albicans* with acquired echinocandin resistance

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ABSTRACT

Mixed fungal infection and acquired echinocandin resistance of *Candida* spp. remain infrequent. In this study we have reported the case of a patient hospitalized for tuberculosis who experienced multiple infections due to three common *Candida* species (*C. albicans, C. glabrata, C. tropicalis*). Furthermore, consecutive isolates from blood cultures and heart valve were found resistant to azoles (*C. tropicalis*) and to echinocandin with either novel (*C. tropicalis*) or previously described (*C. albicans*) missense mutations in the *Fks* gene.

1. Introduction

Candidemia is the fourth most common microbial bloodstream infection. Since the 2000s, caspofungin and micafungin have been employed as first-line treatment and prophylaxis for invasive candidiasis. Increasing use of these drugs has led to the emergence of echinocandin resistance [1–3]. Even though several case reports have been written, acquired echinocandin resistance remains uncommon especially for *Candida tropicalis* [4–6]. Here, we report the case of a 37-year-old man, hospitalized for Extensively drug-resistant tuberculosis disease (XDR TB), who was diagnosed with candidemia due to three different *Candida* species (*C. glabrata, C. albicans* and *C. tropicalis*), bacteremia and fungal endocarditis due to *C. tropicalis*. Usually, the recommended treatment for candidemia due to *C. glabrata* is an echinocandin, the choice being due to the intrinsic fluconazole resistance of this species. But in cases of combined echinocandin-resistance, a switch to amphotericin B or the association of two antifungals could be necessary.

2. Case

The patient was hospitalized six month before candidemia (day 0) for XDR tuberculosis disease. A central venous catheter (CVC) was placed. The subsequent clinical history regarding fungal infections is shown in the figure. Of note, bacteremias were also diagnosed 4 month before day 0 by *Klebsiella pneumonia* and one month before day 0 by *Enterobacter cloacae*.

In brief, three peripheral blood cultures were positive for *C. glabrata* on day 0. The antifungal susceptibility profile of the isolate (CNRMA13.446) tested using EUCAST broth microdilution method was normal for the species [4–7]. On day 32, the patient developed a second infection caused by *C. tropicalis* isolated from blood cultures (seven positive blood cultures). This isolate recovered (CNRMA13.526) was resistant to azoles (Table 1).

On day 93 and 94, the patient experienced fever and respiratory distress. Three peripheral blood cultures and a broncho-alveolar lavage (BAL) were positive with *C. albicans* and *C. tropicalis*. Both *C. albicans* (CNRMA13.695) and *C. tropicalis* (CNRMA13.694) isolates were resistant (Table 1) to the three echinocandins tested [4–8]. A missense mutation S645P in the Hot spot (HS)1 region of the *Fks* gene (Table 2), coding the betaglucan synthase, target enzyme of the echinocandins, was found for both isolates [4–13] .

On day 139, a trans-thoracic cardiac ultrasound confirmed an infective endocarditis, with large vegetation > 15 mm. Culture of the vegetations was positive with *C. albicans* (CNRMA13.779) and *C. tropicalis* (CNRMA13.778). Both isolates had the same antifungal susceptibility profiles and the same missense mutation as the previous isolates (Table 1). The three consecutive isolates of *C. tropicalis* (CNRMA13.778, CNRMA13.694, CNRMA13.526) were genotyped using MultiLocus Sequence Typing [14]. All shared the same genetic profiles suggesting that they were genetically linked.

History of the antifungal treatment is represented in the figure.

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Table 1
MICs and Fks mutation for isolates of *Candida* species studied.

Isolate	Species	Day of isolation	Site of isolation	MIC[a] (mg/L)								*Fks* mutation
				Fluco	Vori	Posa	Caspo	Mica	Anidula	5FC	AmphoB	
CNRMA13.446	*C. glabrata*	day 0	blood	8	0,5	1	0,06	0,03	0,06	0,124	0,125	ND
CNRMA13.526	*C. tropicalis*	day 32	blood	1	8	0,125	0,03	0,03	0,03	0,124	0,125	WT
CNRMA13.694	*C. tropicalis*	day 93	blood	0,5	0,06	0,06	4	0,5	0,5	0,124	0,06	S645P (HS1)
CNRMA13.778	*C. tropicalis*	day 139	heart valve	0,5	0,06	0,03	4	0,5	0,5	0,124	0,06	S645P (HS1)
CNRMA13.695	*C. albicans*	day 94	blood	0,124	0,014	0,014	4	2	0,25	0,124	0,06	S645P (HS1)
CNRMA13.779	*C. albicans*	day 139	heart valve	0,124	0,014	0,014	4	1	0,25	0,124	0,03	S645P (HS1)

ND: not done, WT: wild-type protein sequence in comparison with type strain ATCC750, susceptible to echinocandins.
[a] MIC: Minimum Inhibitory Concentration, Fluco: fluconazole, Vori: voriconazole, Posa: posaconazole, Caspo: caspofungin, Mica: micafungin, Anidula: anidulafungin, 5FC: flucytosin, AmphoB: amphotericin B.

Table 2
List of primers used for amplification and sequencing of Fks HS1 and 2 regions.

Species	Région	Primes	Sequences 5′ 3′	Ref.
Candida albicans	HS1	GSC1f GSC1r	GAAATCGGCATATGCTGTGTC AATGAACGACCAATGGAGAAG	Park et al. [9]
	HS2	CAS2f CAS2r	ACCACCAAGATTGGTGCTG TATCTAGCACCACCAACGG	Desnos-Ollivier et al. [4,8,10]
Candida tropicalis	HS1	CTS1-1f CTS1-1r	ATGGTTCAGTATAGGTGGATG AAGGAACGACCAATGGAGAAG	Desnos-Ollivier et al. [4,8,10]
	HS2	CTS1-2f CTS1-2r	ACTACCAAGATTGGTGCTG TATCTAGCACCACCAACAG	
Candida glabrata	FKS2-HS1	CG1f CG1r	GAAGGCTGGTCATGCTGTAG AAGGATTTACCAACAGAGAAG	Katiyar et al. [11]
	FKS2-HS2	CG2f CG2r	ACAACTAAGATTGGTGCAG TAACGAGCACCACCCACA	Blanchard et al. [12]
	FKS1-HS1	FKS1-2f FKS1-2r	GTTGCAGTCGCTACATTGCTA TAGCGTTCCAGACTTGGGAA	Katiyar et al. [11]
	FKS1-HS2	FKS1HS2f FKS1HS2r	ATTGGCTCAAATTGGTGGTA CACAGACCACGTTCAATCAA	Zimbeck et al. [13]
	FKS3-HS1	FKS3f FKS3r	TGGAGCCCAGCACTTAACAA GTCCATCTCGGATGTTGCTA	Katiyar et al. [11]
	FKS3-HS2	CG3-HS2f CG3-HS2r	TTATGCAGAGGAACCTGCTC GTGCCATCGACAGTAAGTGA	Blanchard et al. 2011 [12]

Table 3
MLST profiles of the three *Candida tropicalis* isolates.

Strain	MDR1	XYR1	SAPT2	SAPT4	ZWF1a	ICL1
CNRMA13.526	1	1	3	1	1	1
CNRMA13.694	1	1	3	1	1	1
CNRMA13.778	1	1	3	1	1	1

First, caspofungin was administered between day 3 and day 18 and then between day 33 and day 67 (70 mg loading dose, followed by 50 mg/day). The catheter was immediately removed on day 33. Third candidemia was treated with: From day 93 to day 97 by caspofungin, with MIC we decided switch by voriconazole during ten days (day107), treatment was changed to liposomal amphotericin B IV (3 mg/kg/day associated with flucytosin IV (25 mg/kg/day) from day 107 to day 163.

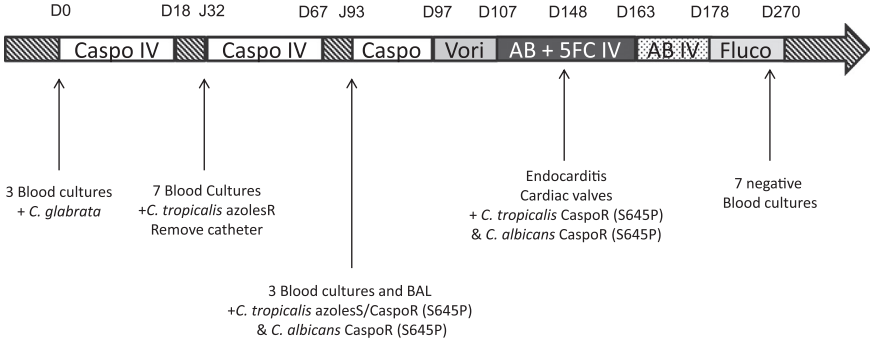

Fig. 1. History of fungal infection and antifungal treatment. Site and date of isolation, species recovered from samples and antifungal susceptibility are indicated.

On day 148, a surgical resection of vegetation was performed and the patient underwent a surgical anuloplasty without replacement of the heart valve. On day 163, liposomal amphotericin B IV (3 mg/kg/day) was administered alone until day 178. Thereafter and for three months, oral fluconazole (400 mg/day) was administered.

At the end of day 270 and after seven sequential blood cultures were found negative, the patient was considered as cured.

3. Discussion

This case report illustrates the first mutation S645P in the hot spot coding the beta glucansynthase target enzyme of the echinocandin in *C. tropicalis* (Genbank accession number KP313858). On the identification of species, all isolates were subcultured on CHROMagar™ Candida medium (develeped by Becton Dickinson GmbH, Heidelberg, Germany) to ensure purity and viability. Isolates were identified at the species level by standard mycological procedures including the assimilation patterns obtained with the commercialized strips ID32C (developed by bioMérieux, Marcy-l'Etoile, France). For all *Candida albicans* isolates a specific PCR amplification [15] was performed to distinguish this species from *C. dubliniensis*. *About sequencing*, parts of the *Fks* ORF, containing Hotspot 1 and 2 regions, were sequenced and analyze by using primers previously described (Table 2).

The 7 MLST loci described by Tavanti et al. [12] for genotyping of *Candida tropicalis,* were sequenced for the three isolates (CNRMA13.526, CNRMA13.694, CNRMA13.778) (Table 3). Sequences were compared with sequences available in the online database [16]. The three isolates have same allelic profile suggesting that they are genetically linked (Table 3). This profile is not known in the MLST database.

In this case, a catheter was essential to the treatment of XDR TB. But the patient was used to having heroin injected.

The recurrence of candidemia and the multiple species involved were puzzling, probably explained by: a wide spectrum of antibiotics and careful questioning of the patient uncovered the fact that through the CVC, he had been injecting himself with heroin during hospitalization.

Caspofungin was administered first because of the intrinsic azole resistance of *C. glabrata*. It was prescribed again for the second candidemia due to *C. tropicalis*, because of the high azole MICs of the isolates and the absence of endocarditis based on normal transthoracic cardiac ultrasound. During the third episode of candidemia, a switch to voriconazole was decided because of the high caspofungin MICs of *C. albicans* and *C. tropicalis* isolates and their susceptibility to azoles. The discovery of fungal endocarditis led to a combination of liposomal amphotericin B and flucytosine 15 days after surgical anuloplasty, and then to liposomal amphotericin B alone (side effect at flucytosine). Finally, oral fluconazole was administered for 3 months.

Echinocandin-susceptible and resistant genetically linked isolates of *C. tropicalis* were recovered suggesting that the protein mutation was due to the caspofungin treatment as already described for *Candida spp* [17–19]. For *C. albicans*, this mutation has previously been described in the literature [3] and associated with decreased *in vitro* echinocandin susceptibility after caspofungin treatment but for *C. tropicalis*, it is a novel missense mutation.

Even though endocarditis was diagnosed in September 2013, we did not accept the indication for emergency operative intervention, the reasons being that the patient was hemodynamically stable and that his drug addiction and lack of cooperation during treatment rendered cardiac surgery in our opinion inadvisable. Our decision may be objectionable inasmuch as conventional recommendations on candidemia endocarditis specify that whenever feasible and whatever the size of vegetation on the heart valve, emergency surgery should be carried out to reduce inoculum levels and to restrict the growth of resistant mutants.

Furthermore a CVC thrombosis was discovered by ultrasound, and the heart vegetations probably provided an ideal environment for biofilm formation, which is known to contain isolates exhibiting antifungal drug resistance.

In conclusion, we describe a patient who experienced recurrent infections due to common *Candida* species. His drug addiction and prior antifungal treatments favored the emergence of resistance and of multiple and sometimes mixed infections. Novel missense mutation in the target *FKS* gene was uncovered for *C. tropicalis* isolates (Fig. 1).

Conflict of interest

Dr Grosset: None.

Dr Desnos-Ollivier: None.

Dr Godet: received consultancy or speaker fees, travel support from Pfizer, Astellas, Gilead, MSD, Basilea, SOS Oxygene and ISIS Medical.

Dr Kauffmann- Lacroix: travel support from MSD.

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