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

Marine Grosseta,b,⁎ Marie Desnos-Ollivierb, Cendrine Godeta, Catherine Kauffmann-Lacroixc, France Cazenave-Roblota

a Service de Maladies Infectieuses et tropicales, CHU de Poitiers, 2 rue de la Milétrie, Poitiers 86021, France
b Institut Pasteur, Unité de Mycologie Moléculaire, Centre National de Référence Mycologie et Antifongiques, 25 rue du Dr Roux, Paris 75015, France
c Laboratoire de Parasitologie Mycologie, CHU de Poitiers, 2 rue de la Milétrie, Poitiers 86021, France

A R T I C L E   I N F O

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A B S T R A C T

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, 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 (CNRM1A3.446) 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 (CNRM1A3.526) 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 (CNRM1A3.695) and C. tropicalis (CNRM1A3.694) 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 (CNRM1A3.779) and C. tropicalis (CNRM1A3.778). 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 (CNRM1A3.778, CNRM1A3.694, CNRM1A3.526) 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.

⁎ Corresponding author.

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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 5-FC: 25 mg/kg/day) from day 107 to day 163.

Table 1

| Isolate       | Species      | Day of isolation | Site of isolation | MIC\(^{a}\) (mg/L) | Fks mutation |
|---------------|--------------|------------------|-------------------|---------------------|--------------|
| CNRMA13.446   | C. glabrata  | day 0            | blood             | 8                   | 0.5          |
| CNRMA13.526   | C. tropicalis| day 32           | blood             | 1                   | 0.06         |
| CNRMA13.694   | C. tropicalis| day 93           | blood             | 0.5                 | 0.03         |
| CNRMA13.778   | C. tropicalis| day 139          | heart valve       | 0.5                 | 0.03         |
| CNRMA13.695   | C. albicans  | day 94           | blood             | 0.124               | 0.014        |
| CNRMA13.779   | C. albicans  | day 139          | heart valve       | 0.124               | 0.014        |

\(^{a}\) MIC: Minimum Inhibitory Concentration, Fluco: fluconazole, Vori: voriconazole, Posa: posaconazole, Caspo: caspofungin, Mica: micafungin, Anidula: anidulafungin, 5FC: flucytosin, AmphoB: amphotericin B.

ND: not done, WT: wild-type protein sequence in comparison with type strain ATCC750, susceptible to echinocandins.

Table 2

| Species         | Région | Primes | Sequences 5’ 3’ | Ref. |
|-----------------|--------|--------|-----------------|------|
| Candida albicans| HS1    | GSC1f  | GAAATCGGCATATGCTGTGTC | Park et al. [9] |
|                 |        | GSC1r  | AATGAAAGCAATGAGAGAAG |
|                 | HS2    | CAS2f  | ACCACCAAGATGATGCTGCT | Desnos-Ollivier et al. [4,8,10] |
|                 |        | CAS2r  | TATCTAGACACCAACCCG |
| Candida tropicalis| HS1   | CTS1-1f | ATGGTTAGTATAGGTGGATG | Desnos-Ollivier et al. [4,8,10] |
|                 |        | CTS1-1r | AAGGAAACCAATGAGAGAAG |
|                 | HS2    | CTS1-2f | ACTACCAAGATTGTTGCTG | Desnos-Ollivier et al. [4,8,10] |
|                 |        | CTS1-2r | TAATCTAGACACCAACCCG | |
| Candida glabrata| FKS2-HS1 | CG1f  | GAAGGCTGGTCATGCTGCTAG | Katiyar et al. [11] |
|                 |        | CG1r   | AAGGATTTACCAACAGAGAAG |
|                 | FKS2-HS2 | CG2f  | ACAACTAAGATTGTTGACG | Blanchard et al. [12] |
|                 |        | CG2r   | TAACGACACACCCACA |
|                 | FKS1-HS1 | FKS1-2f | GTTGCAGTCTACTATGGCTA | Katiyar et al. [11] |
|                 |        | FKS1-2r | TAGTGTTCAGACATGGGAA |
|                 | FKS1-HS2 | FKS1HS2f | ATGGCTCAATTGTTGTTA | Zimbeck et al. [13] |
|                 |        | FKS1HS2r | CACAGACACATGACAA |
|                 | FKS3-HS1 | FKS3f  | TGGAGCAGCGACTTAAACA | Katiyar et al. [11] |
|                 |        | FKS3r  | GTTCATCTGCCAGTGTTGCTA |
|                 | FKS3-HS2 | CG3-HS2f | TTAAGCAGAGAAATGGCTC | Blanchard et al. 2011 [12] |
|                 |        | CG3-HS2r | GTGGCATGACAGTGAAGTA |

Table 3

| Strain          | MDR1 | XYR1 | SAPT2 | SAPT4 | ZWF1s | 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 (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 glucosynthase 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 (developed 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 trans-thoracic 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 vegetation 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 Oxygen and ISIS Medical.
Dr Kauffmann- Lacroix: travel support from MSD.
Pr Cazenave-Roblot is the head of French infectious diseases society and received consultancy or speaker fees, travel support from Janssen, Pfizer, Astellas, Gilead, MSD.

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