Echinocandins are effective in patients with invasive candidiasis and recommended as first-line therapy, especially for patients with severe sepsis or those previously exposed to azoles or infected with Candida glabrata (1). Fewer than 50 persons infected with echinocandin-resistant species that are usually susceptible, such as C. albicans, C. glabrata, C. tropicalis, and C. krusei, have been described in limited series or case reports (2–4). All species were found in patients preexposed to echinocandins. The major mechanism of resistance is related to mutations in FKS genes coding for β-1,3-glucan-synthase (5), with almost 20 known FKS mutations. We describe the characteristics of infections from caspofungin-resistant Candida spp. isolates belonging to usually susceptible species recorded in France (2004–2010) and analyze their FKS mutations and effect on echinocandin susceptibility.

The Study

Isolates received at the French National Reference Center for Mycoses and Antifungals (NRCMA) are identified to the species level by standard mycologic procedures and routinely tested for susceptibility to caspofungin, micafungin, and anidulafungin by using European Committee for Antimicrobial Susceptibility Testing (EUCAST) methods (6) and AM3 medium (7). In addition, RPMI 1640 medium was used here for selected isolates and reference strains. For the clinical isolates with caspofungin MIC ≥0.5 μg/mL in AM3, nucleotide sequences of hot spot (HS) 1 and 2 regions of the FKS1 gene for C. albicans and C. krusei and of HS1 region of FKS1, FKS2, and FKS3 genes for C. glabrata were determined (7,8).

The resulting protein sequences were aligned with the BioloMics software (BioloMics, BioAware SA, Hannut, Belgium) and compared with reference strains (C. albicans, ATCC32354; C. krusei, ATCC6258; and C. glabrata, ATCC2001). Genetic relatedness of C. albicans and C. glabrata paired isolates was studied by using microsatellite-length polymorphism analysis (9–11). The Wilcoxon signed-rank test was used to compare echinocandin MICs of paired isolates. Surveillance for mycoses by the NRCMA has been approved by the Institut Pasteur Internal Review Board and by the Commission Nationale de l’Informatique et des Libertés.

During September 2004–April 2010, twenty proven infections caused by C. albicans (n = 10), C. glabrata (n = 8), or C. krusei (n = 2) with caspofungin MIC ≥0.5 μg/mL and a mutation in the target enzyme were reported to the NRCMA (Table 1). Nineteen of the isolates were recovered after caspofungin treatment for a median duration of 27 days (range 10–270 days; 13 of 19 patients received caspofungin at the time the resistant isolate was recovered). Caspofungin was prescribed for 14 patients with proven Candida spp. infection, 1 patient with proven invasive aspergillosis, and 2 patients with febrile neutropenia; for 2 persons with hematologic malignancies, caspofungin was prescribed prophylactically.

The geometric mean MIC for C. glabrata and C. albicans were 2.8 and 1.7 μg/mL for caspofungin, 0.4 and 0.7 μg/mL for micafungin, and 0.2 and 0.09 μg/mL for anidulafungin, respectively (Table 2). Of the 20 mutated isolates found resistant to caspofungin in AM3 by using the EUCAST method, 19 also were resistant to caspofungin (1 intermediate), 18 to micafungin (1 intermediate and 1 susceptible), and 9 to anidulafungin (5 intermediate and 6 susceptible) according to Clinical Laboratory Standards Institute (CLSI) breakpoints and RPMI 1640 medium (Table 2).

We report 20 episodes of infection caused by acquired echinocandin-resistant Candida spp. harboring diverse and new Fksp mutations. For 12 patients, initial isolates (low MIC, wild-type Fksp sequence) and subsequent isolates (after caspofungin treatment, high MIC, mutated Fksp) were genetically related.

**Candida spp. with Acquired Echinocandin Resistance, France, 2004–2010**

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We report 20 episodes of infection caused by acquired echinocandin-resistant Candida spp. harboring diverse and new Fksp mutations. For 12 patients, initial isolates (low MIC, wild-type Fksp sequence) and subsequent isolates (after caspofungin treatment, high MIC, mutated Fksp) were genetically related.
Candida spp. and Echinocandin Resistance

2. According to EUCAST breakpoints, 19 isolates also were resistant to anidulafungin, and 1 isolate was almost resistant (MIC 0.03 μg/mL). We thus showed discrepancies between CLSI and EUCAST regarding anidulafungin susceptibility (www.srga.org/eucastwt/MICTAB/EUCAST%20clinical%20MIC%20breakpoints%20-%20antimicrobials%20for%20Candida%20infections.htm [V 3.0 2011–4-27]) (12,13).

Of the 10 caspofungin-resistant C. glabrata isolates, 8 harbored a mutation in Fks2p only, 1 isolate had a mutation in Fks1p, and 1 had mutations in Fks1p and Fks2p (Table 2). Of the 8 caspofungin-resistant C. albicans isolates, 1 had a missense mutation in HS2, and 1 had a combination of 2 heterozygous mutations in HS1. The other 6 isolates harbored 4 different mutations in HS1 (Table 2). Finally, the 2 C. kruusei isolates had 2 different mutations in HS1 region. Of the 20 mutated isolates, 6 harbored 7 mutations not yet described in the literature (Table 2) (13).

Prior initial isolates available for 12 patients had the wild-type sequence for the HS regions that were mutated in the paired resistant isolate. All initial isolates were susceptible to anidulafungin and to micafungin and anidulafungin according to EUCAST and CLSI, respectively (data not shown). According to CLSI caspofungin breakpoints, 5 of 6 initial isolates of C. albicans were susceptible, and 1 was intermediate; 4 of 5 C. glabrata isolates were resistant (0.5 μg/mL), and 1 was intermediate; and the C. kruusei isolate was resistant (1 μg/mL). For each of the 12 pairs, MICs increased significantly (from 3 to 8 dilutions for caspofungin and micafungin and from 1 to 8 dilutions for anidulafungin) between the wild-type and the mutant isolate (Figure; p<0.001). Genetic

Table 1. Characteristics of 20 patients with infections caused by a non–parapsilosis/guilliermondii Candida spp. Fks mutation, France, 2004–2010*

| Patient no. | Age, y/sex | Underlying condition | Neutropenia | Species | Site of infection | Duration of caspofungin exposure, d† | Outcome at 30 d‡ |
|-------------|------------|---------------------|-------------|---------|------------------|-------------------------------------|-----------------|
| 1           | 34/M       | HIV positive        | No          | C. albicans | Esophagus        | 21                                   | Alive           |
| 2           | 20/M       | Hematologic malignancy: familial lymphohistiocytosis | Yes | C. albicans | Blood | 17 | Alive           |
| 3           | 77/M       | Hematologic malignancy: AML | Yes | C. albicans | Blood | 25 | Alive           |
| 4           | 46/M       | Hematologic malignancy: AML | Yes | C. albicans | Blood, peritoneum, pleural fluid | 26 | Dead           |
| 5           | 34/F       | Liver transplant: cirrhosis | No | C. albicans | Hepatic abscess, peritoneum | 60 | Alive           |
| 6           | 64/F       | Hematologic malignancy: AML; breast cancer | No | C. albicans | Blood | 25 | Alive at 17 d   |
| 7           | 59/M       | Teratocarcinoma | No | C. albicans | Pharynx | 35 | Dead           |
| 8           | 28/M       | Chronic mucocutaneous candidiasis | No | C. albicans | Pharynx, nails | 270 | Alive           |
| 9           | 14/F       | Hematologic malignancy: ALL | Yes | C. kruusei | Lung | 45 | Alive           |
| 10          | 79/M       | Hematologic malignancy: non-Hodgkin lymphoma | Yes | C. kruusei | Blood | 10 | Dead           |
| 11          | 46/M       | Hematologic malignancy: Burkitt lymphoma; HSCT | Yes | C. glabrata | Blood | None | Dead           |
| 12          | 85/M       | Gastric ulcer; CVC | No | C. glabrata | Blood | 32 | Alive           |
| 13          | 28/M       | Hematologic malignancy: non-Hodgkin lymphoma; HSCT | No | C. glabrata | Palate§ | 135 | Alive           |
| 14          | 48/M       | Esophageal cancer | No | C. glabrata | Blood | 12 | Alive           |
| 15          | 41/M       | Liver transplant: fulminant hepatitis | No | C. glabrata | Blood, peritoneum | 37 | Dead           |
| 16          | 38/F       | Hematologic malignancy: AML; HSCT | Yes | C. glabrata | Blood | 51 | Dead           |
| 17          | 60/M       | Acute pancreatitis; GI tract surgery | No | C. glabrata | Bile | 34 | Alive           |
| 18          | 39/M       | Hematologic malignancy: AML; HSCT | No | C. glabrata | Sinus§ | 15 | Alive           |
| 19          | 55/F       | Lock-in syndrome; neurogenic bladder | No | C. glabrata | Urine¶ | 27 | Alive           |
| 20          | 63/M       | Colon cancer | Yes | C. glabrata | Blood | 14 | Alive           |

*AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; HSCT, hematopoietic stem cell transplantation; CVC, central venous catheter; GI, gastrointestinal.
†Duration of caspofungin exposure before isolation of the first resistant Candida isolate.
‡Outcome 30 d after isolation of the first resistant Candida isolate.
§From a biopsy specimen.
¶With sepsis.
relatedness was demonstrated for all *C. albicans* and *C. glabrata* paired isolates.

**Conclusions**

We demonstrated that recent exposure to caspofungin altered the distribution of species causing *Candida* bloodstream infections (14), and that caspofungin exposure was independently associated with fungemia associated with intrinsically less-susceptible species in hematology (15). Echinocandin resistance in *Candida* spp. is still uncommon (4,13). Through our surveillance program, we estimated the incidence of decreased susceptibility to caspofungin associated with *FKS* mutations among *C. albicans*, *C. glabrata*, and *C. krusei* isolates responsible for candidemia in children and adults in Paris at 6 (0.4%) of 1,643 (NRCMA, unpub. data). We report proven caspofungin-resistant *Candida* spp. infections with none of the isolates belonging to the intrinsically less-susceptible species *C. parapsilosis* or *C. guilliermondii*.

We determined antifungal susceptibility testing by the EUCAST technique using AM3 because it enables better discrimination between susceptible wild-type and resistant mutant isolates (7). All isolates with high caspofungin MIC (≥0.5 μg/mL) had mutation in the HS1 and/or HS2 region of *FKS* genes. The mutations were not restricted to a given position but were diverse, especially for *C. albicans* with 6 different mutations among the 8 resistant isolates; 5 different mutations were observed among the 10 *C. glabrata* resistant isolates. Most mutations in *C. glabrata* isolates were in Fks2p. Two mutations in *C. albicans*, 2 patterns of mutation in *C. glabrata*, and 1 mutation in *C. krusei* had not been reported before, highlighting the great mutation diversity that could be responsible for echinocandin resistance (13).

All but 1 patient had received caspofungin (70 mg on day 1, then 50 mg/d) before recovery of the resistant isolate, with a variable duration of exposure (<10 days to >8 months), in agreement with the literature (5 [3] to 420 days). In addition, 13 of 19 patients received caspofungin at the time of recovery of the resistant isolate. Most patients had malignancy, but 7 intensive care unit hospitalizations also were recorded. Echinocandins MICs between the wild-type parent and the subsequent mutant isolate increased by up to 8 log, dilutions (Figure). The source of the resistant isolate is not unequivocal; it was acquired from the environment time of recovery of the resistant isolate. Most patients had malignancy, but 7 intensive care unit hospitalizations also were recorded. Echinocandins MICs between the wild-type parent and the subsequent mutant isolate increased by up to 8 log, dilutions (Figure). The source of the resistant isolate is not unequivocal; it was acquired from the environment.

**Table 2. In vitro susceptibility and Fksp mutations of 20 echinocandin-resistant Candida spp. isolates, France, 2004–2010**

| Patient no. | Strain | Species | MIC, μg/mL, AM3/RPMI 1640 medium | Fksp mutation |
|------------|--------|---------|----------------------------------|---------------|
| 1*         | 05BL1-38 | *C. albicans* | Caspofungin: 1/2, 0.25/1; Micafungin: 1/1, 0.125/0.25; Anidulafungin: 0.06/0.125 | *FKS1* (HS1) + *FKS2* (HS1) |
| 2*         | ODL1-1254 | *C. albicans* | Caspofungin: 1/2, 0.25/1; Micafungin: 1/1, 0.125/0.25; Anidulafungin: 0.06/0.125 | *FKS1* (HS1) + *FKS2* (HS1) |
| 3†         | 06BL2-127 | *C. albicans* | Caspofungin: 2/2, 0.25/1; Micafungin: 0.125/0.25; Anidulafungin: 0.06/0.125 | *FKS1* (HS1) + *FKS2* (HS1) |
| 4          | ODL19-1894 | *C. albicans* | Caspofungin: 1/2, 0.25/1; Micafungin: 0.06/0.125; Anidulafungin: 0.06/0.125 | *FKS1* (HS1) + *FKS2* (HS1) |
| 5*         | 08BL1-94  | *C. albicans* | Caspofungin: 4/2, 0.25/1; Micafungin: 0.125/0.25; Anidulafungin: 0.06/0.125 | *FKS1* (HS1) + *FKS2* (HS1) |
| 6*         | 08BL2-143 | *C. albicans* | Caspofungin: 8/4, 0.25/1; Micafungin: 0.125/0.25; Anidulafungin: 0.06/0.125 | *FKS1* (HS1) + *FKS2* (HS1) |
| 7*         | 09BL1-43  | *C. albicans* | Caspofungin: 1/2, 0.25/1; Micafungin: 0.125/0.25; Anidulafungin: 0.06/0.125 | *FKS1* (HS1) + *FKS2* (HS1) |
| 8*         | 09BL1-77  | *C. albicans* | Caspofungin: 0.5/0.5, 0.5/0.5; Micafungin: 0.015/0.03; Anidulafungin: 1/1 | *FKS1* (HS1) + *FKS2* (HS1) |
| 9          | 06BL1-34  | *C. krusei* | Caspofungin: 4/2, 0.25/1; Micafungin: 0.125/0.25; Anidulafungin: 0.06/0.125 | *FKS1* (HS1) + *FKS2* (HS1) |
| 10*        | 10BL1-50  | *C. krusei* | Caspofungin: 1/2, 0.25/1; Micafungin: 0.125/0.25; Anidulafungin: 0.06/0.125 | *FKS1* (HS1) + *FKS2* (HS1) |
| 11         | ODL7-647  | *C. glabrata* | Caspofungin: 8/8, 0.5/1; Micafungin: 0.25/0.125; Anidulafungin: 0.125/0.25 | *FKS1* (HS1) + *FKS2* (HS1) |
| 12*        | 07BL2-157 | *C. glabrata* | Caspofungin: 4/1, 0.25/1; Micafungin: 0.125/0.25; Anidulafungin: 0.06/0.125 | *FKS1* (HS1) + *FKS2* (HS1) |
| 13*        | 06BL1-33  | *C. glabrata* | Caspofungin: 8/8, 0.125/0.125; Micafungin: 0.25/0.25; Anidulafungin: 0.125/0.25 | *FKS1* (HS1) + *FKS2* (HS1) |
| 14*        | ODL21-2028 | *C. glabrata* | Caspofungin: 1/2, 0.25/0.25; Micafungin: 0.25/0.25; Anidulafungin: 0.125/0.125 | *FKS1* (HS1) + *FKS2* (HS1) |
| 15*        | ODL22-2183 | *C. glabrata* | Caspofungin: 8/2, 0.25/0.25; Micafungin: 0.25/0.25; Anidulafungin: 0.125/0.125 | *FKS1* (HS1) + *FKS2* (HS1) |
| 16         | ODL23-2221 | *C. glabrata* | Caspofungin: 1/2, 0.25/0.25; Micafungin: 0.125/0.125; Anidulafungin: 0.125/0.125 | *FKS1* (HS1) + *FKS2* (HS1) |
| 17*        | 08BL2-142 | *C. glabrata* | Caspofungin: 1/4, 0.25/0.25; Micafungin: 0.125/0.125; Anidulafungin: 0.125/0.125 | *FKS1* (HS1) + *FKS2* (HS1) |
| 18         | 09BL1-55  | *C. glabrata* | Caspofungin: 8/4, 0.125/0.125; Micafungin: 0.25/0.25; Anidulafungin: 0.125/0.125 | *FKS1* (HS1) + *FKS2* (HS1) |
| 19         | 10BL1-19  | *C. glabrata* | Caspofungin: 0.5/0.5, 0.5/0.5; Micafungin: 0.125/0.125; Anidulafungin: 0.125/0.125 | *FKS1* (HS1) + *FKS2* (HS1) |
| 20         | 10BL1-67  | *C. glabrata* | Caspofungin: 4/4, 0.125/0.125; Micafungin: 0.125/0.125; Anidulafungin: 0.125/0.125 | *FKS1* (HS1) + *FKS2* (HS1) |

*Parentage of initial isolate available.
†In this patient, another isolate with reduced susceptibility to echinocandin was retrieved. This isolate harbored an S645P mutation in *FKS1*.
‡Heterozygous mutation.
§Mutations not already described (13).
¶Strains had also an L701M mutation.
*Strains had also an L701M mutation.
#Deletion.
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Figure. Corresponding caspofungin (A), micafungin (B), and anidulafungin (C) MICs in 12 Fksp mutant Candida spp. isolates and their wild-type parent isolates, France, 2004–2010. Susceptibility testing was performed by using the European Committee for Antimicrobial Susceptibility Testing method (6) and AM3 medium (7).
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