Development of Echinocandin Resistance in *Clavispora lusitaniae* during Caspofungin Treatment

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*Clavispora lusitaniae* is an opportunistic human pathogen responsible for 0.6 to 2% of candidemia. This species is intrinsically susceptible to echinocandins. Nevertheless, in this study, development of echinocandin resistance in *C. lusitaniae* isolates was observed during caspofungin treatment. This resistance resulted from missense mutation in the echinocandin target Fks1 gene.

*Candida albicans* remains the most common pathogen responsible for invasive candidiasis. However, increasing rates of candidemia caused by other species, including *Clavispora lusitaniae*, are reported worldwide (13, 23). *Clavispora lusitaniae* (anamorph: *Candida lusitaniae*) is an opportunistic haploid ascomycetous yeast (12, 25), recovered worldwide from plants, animals, and humans (4). This species is able to grow at 37°C and accounts for 0.6 to 2% of the isolates recovered during candidemia (12, 18, 23). Caspofungin, a member of the echinocandin class, demonstrates fungicidal activity against *C. albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, *Candida glabrata*, and *C. lusitaniae*. Beta-1,3-glucan synthase encoded by Fks genes is the target of the echinocandins (1, 5). Missense mutations in the hot spot 1 (HS1) and/or HS2 regions, resulting in increased MICs of echinocandins, had already been described in clinical isolates of *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. krusei* from patients treated with caspofungin (2, 3, 7, 11, 14, 15, 20, 21). *Clavispora lusitaniae* is known for its propensity to develop amphotericin B resistance during therapy (9). It is not intrinsically resistant to echinocandins, and modal caspofungin MIC was 0.25 μg/ml and 0.06 μg/ml (22) (NRCMA, unpublished data). Caspofungin can be used as first-line therapy for candidemia due to *C. lusitaniae* and is even recommended for patients preexposed to azoles.

Here, we report the first case of clinical isolates of *C. lusitaniae* with high echinocandin MICs recovered from a patient treated with caspofungin associated with a missense mutation localized in the HS1 region of hypothetical beta-1,3-glucan synthase.

A 77-year-old man was admitted to the intensive care unit after coloanal anastomosis and total cystectomy with bilateral nephrostomy for advanced rectal cancer. On day 7 after admission, the patient developed fever, dyspnea, and polypnea with high echinocandin MICs recovered from a patient treated with caspofungin and micafungin (7). Decreased susceptibility to caspofungin was defined by a MIC

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C. lusitaniae genes is also observed for clinical isolates of C. albicans Fks1p. The initial isolates (10BL1-59 and 10BL1-61) had low caspofungin MICs, whereas isolates recovered later from urine (10BL1-60) and peritoneal fluid (10BL1-62) had high MICs (Table 1). Of note, Pfaffer et al. recently defined epidemiological cutoff values for high MICs (Table 1). The 4 isolates had a wild-type protein sequence for the HS2 region (GenBank JF304614). This is the first time that clinical isolates of C. lusitaniae with high echinocandin MICs were recovered 2 weeks after initiation of caspofungin treatment, and these isolates exhibited missense mutation S645F in the HS1 region. Of note, among C. albi cans isolates, amino acid changes at Ser 645 are more common and lead to the most significant MIC echinocandin increases (24).

We then looked for a mutation within the putative FKS1 gene to help explain the high MIC values. In the genome of C. lusitaniae (ATCC 42720) currently annotated in the Candida database on the Broad Institute website (http://www.broadinstitute.org/annotation genome/candida_lusitaniae/MultiHome.html), one hypothetical beta-1,3-glucan synthase protein of 688 amino acids (CLUG_01702 transcript 1, supercontig 2: 964000-967862) was 83% similarity with the HS2 region of C. lusitaniae Fks1p. The DNA sequence localized upstream from this sequence (supercontig 2: 964000-967862) was compared with the nucleotide sequence of the coding region of the C. albicans Fks1 gene (orf19-2929, GenBank D88815.1) and had 79% similarity. Resulting protein sequences of C. lusitaniae and C. albicans (GenBank BAA21535.1) were compared, and 83% similarity was observed for the 867-amino-acid sequence. For C. lusitaniae, protein regions (FFLTLSLRD and WIRRTLSIF) similar to HS1 and HS2 regions of C. albicans (FFSTLSLRD and WIRRTLSIF, respectively) were localized. Primers were designed to amplify these hypothetical HS1 and HS2 regions of C. lusitaniae (Table 2). The sequences were translated with the standard genetic code (http://bioinformatics.org/sms/index.html), and resulting protein sequences were compared (Biolimics, v7.2.5; BioAware SA, Hannut, Belgium). Numbering of the protein sequence was based on C. albicans Fks1p. The initial isolates (10BL1-59 and 10BL1-61, GenBank JF304615) showed a protein sequence for the HS1 region identical to that of ATCC 42720 and CBS 4413 and were considered wild type. The subsequent isolates shared similar nucleotide sequences (GenBank JF304613), leading to a missense mutation, S645F, localized in the HS1 region (Table 1). The 4 isolates had a wild-type protein sequence for the HS2 region (GenBank JF304614). A mutation within the putative FKS1 gene to help explain the high MIC values. In the genome of C. lusitaniae (ATCC 42720) currently annotated in the Candida database on the Broad Institute website (http://www.broadinstitute.org/annotation genome/candida_lusitaniae/MultiHome.html), one hypothetical beta-1,3-glucan synthase protein of 688 amino acids (CLUG_01702 transcript 1, supercontig 2: 964000-967862) was 83% similarity with the HS2 region of C. lusitaniae Fks1p. The DNA sequence localized upstream from this sequence (supercontig 2: 964000-967862) was compared with the nucleotide sequence of the coding region of the C. albicans Fks1 gene (orf19-2929, GenBank D88815.1) and had 79% similarity. Resulting protein sequences of C. lusitaniae and C. albicans (GenBank BAA21535.1) were compared, and 83% similarity was observed for the 867-amino-acid sequence. For C. lusitaniae, protein regions (FFLTLSLRD and WIRRTLSIF) similar to HS1 and HS2 regions of C. albicans (FFSTLSLRD and WIRRTLSIF, respectively) were localized. Primers were designed to amplify these hypothetical HS1 and HS2 regions of C. lusitaniae (Table 2). The sequences were translated with the standard genetic code (http://bioinformatics.org/sms/index.html), and resulting protein sequences were compared (Biolimics, v7.2.5; BioAware SA, Hannut, Belgium). Numbering of the protein sequence was based on C. albicans Fks1p. The initial isolates (10BL1-59 and 10BL1-61, GenBank JF304615) showed a protein sequence for the HS1 region identical to that of ATCC 42720 and CBS 4413 and were considered wild type. The subsequent isolates shared similar nucleotide sequences (GenBank JF304613), leading to a missense mutation, S645F, localized in the HS1 region (Table 1). The 4 isolates had a wild-type protein sequence for the HS2 region (GenBank JF304614).

Development of antifungal resistance has been described for yeasts and filamentous fungi after environmental exposure or clinical treatment (15, 21, 26, 28). Although specific data on caspofungin are lacking, antimicrobial drug distribution has been shown to be potentially impaired in critically ill patients. This could have resulted in subinhibitory levels of caspofungin in the patient’s peritoneal fluid and subsequently selection of the resistant mutant. Fluconazole-clarithromycin cross-resistance due to nonsense and missense mutations in FCY2 and FCY1 genes is also observed for clinical isolates of C. lusitaniae (10). In the present case, isolates of C. lusitaniae with increased echinocandin MICs were recovered 2 weeks after initiation of caspofungin treatment, and these isolates exhibited missense mutation S645F in the HS1 region. Of note, among C. albicans isolates, amino acid changes at Ser 645 are more common and lead to the most significant MIC echinocandin increases (24).

This is the first time that clinical isolates of C. lusitaniae with high echinocandin MICs due to mutation in hypothetical Fksp after caspofungin treatment are described. There is no available typing method for C. lusitaniae, which prevented analysis of the genetic relatedness between the 4 clinical isolates. However, this species is a rare human pathogen and its recovery from multiple anatomical sites and over time in the same patient makes it likely that the isolates are genetically linked. The recent demonstration that exposure to caspofungin influences the epidemiology of candidemia, the potential for C. lusitaniae to become an emerging pathogen in this setting (16), and the development of echinocandin resistance after caspofungin treatment should be taken into account for future therapeutic management.

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