In invasive candidiasis, there has been an epidemiological shift from *Candida albicans* to non- *albicans* species infections, including infections with *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*. Although the prevalence of *C. krusei* remains low among yeast infections, its intrinsic resistance to fluconazole raises epidemiological and therapeutic concerns. Echinocandins have *in vitro* activity against most *Candida* spp. and are the first-line agents in the treatment of candidemia. Although resistance to echinocandin drugs is still rare, individual cases of *C. krusei* resistance have been reported in recent years, especially with strains that have been under selective pressure. A total of 15 *C. krusei* strains, isolated from the blood, urine, and soft tissue of an acute lymphocytic leukemia patient, were analyzed. Strains developed echinocandin resistance during 10 days of caspofungin therapy. The molecular epidemiology of the isolates was investigated using two different typing methods: PCR-based amplification of the species-specific repetitive polymorphic CKRS-1 sequence and multilocus sequence typing. All isolates were genetically related, and the mechanism involved in decreased echinocandin susceptibility was characterized. Clinical resistance was associated with an increase in echinocandin MICs *in vitro* and was related to three different mutations in hot spot 1 of the target enzyme Fks1p. Molecular evidence of the rapid acquisition of resistance by different mutations in *FKS1* highlights the need to monitor the development of resistance in *C. krusei* infections treated with echinocandin drugs.

The incidence of opportunistic fungal infections has increased in recent decades due to the growing number of immunocompromised patients. Concomitantly, the number of fungal species identified as pathogenic for humans has also increased. *Candida albicans* is the most common cause of candidiasis, but there have been increased numbers of isolations of non- *albicans* species of *Candida* in recent years, with the most prominent being *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* (1).

*Candida krusei* has been described as a causative agent of disseminated fungal infections in susceptible patients, resulting in the lowest 90-day survival rates (33.6%) among common *Candida* spp. (2). Although the prevalence of *C. krusei* remains low (2%) among yeast infections (3), its intrinsic resistance to fluconazole raises epidemiological and therapeutic concerns (4). While antifungal drug resistance in *C. albicans* isolates continues to be low (3), *C. glabrata* and *C. krusei* have emerged as important and potentially antifungal (azole)-resistant causes of candidemia (6). Therefore, echinocandins are a good therapeutic option for the treatment of invasive candidiasis, despite their cost and the absence of an oral form. They (i) possess fungicidal activity against most species of *Candida*, including those resistant to polyenes and to azoles (7); (ii) lack major adverse effects; and (iii) show no interaction with other drugs (8). Furthermore, echinocandins have been approved for the treatment of several fungal infections, including invasive candidiasis and candidemia, and are also indicated for empirical therapy in select high-risk patients (those with persistent fever and neutropenia) (9–11).

Echinocandins interfere with fungal cell wall synthesis through noncompetitive inhibition of the β-1,3-glucan synthase (GS) complex, the enzyme required for the synthesis of glucan. Inhibition occurs by targeting the catalytic subunit, which is encoded by three related genes: *FKS1*, *FKS2*, and *FKS3* (12). This results in a weakening of the fungal cell wall, a breakdown of cellular integrity, and cell lysis (13). Reports of clinical isolates of *Candida* spp. with decreased *in vitro* echinocandin susceptibility are still sporadic (<1.7 to 2%), including among the vast majority of *C. krusei* isolates (14, 15). However, individual cases of *C. krusei* resistance to the echinocandins have been reported (16–21). Acquired resistance to echinocandin therapy has been associated with amino acid substitutions caused by mutations in specific hot spot regions (HS1, HS2, and the recently described HS3 region) of the conserved FKS1 target gene in different *Candida* species, such as *C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. lusitaniae*, and *C. kefyr* (19, 22–27), as well as in the FKS2 gene of *C. glabrata* (25, 28). These mutations have been related to prior echinocandin exposure and therapeutic failure in candidemic patients (18, 29–32). The most prominent changes occurred at the serine 645 position in Fks1p, with mutations to proline, tyrosine, and phenylalanine.
expressed either homozygously or heterozygously for the mutant allele, indicating that expression of the resistance phenotype is dominant (33).

In this work, we analyzed 15 isolates of *C. krusei* from a patient with acute lymphocytic leukemia (ALL) who suffered from a candidemia that rapidly developed echinocandin resistance during 10 days of caspofungin therapy. Molecular typing of isolates and characterization of the resistance mechanism that confers decreased *C. krusei* echinocandin susceptibility are both described.

### MATERIALS AND METHODS

**Strains.** Fifteen *Candida krusei* isolates were serially recovered from a hematologic patient with catheter-related candidemia secondary to neutropenia due to chemotherapy treatment. Isolates were obtained before and after caspofungin therapy, as follows: (i) before, blood culture 1 (BC1); and (ii) after, BC2 to BC8, soft tissue culture (STC), and urine culture (UC) (Table 1). These isolates were first identified as *C. krusei* by morphological features (BBL CHROMagar *Candida* medium and corn meal agar with Tween 80; Sigma-Aldrich, Madrid, Spain) and then confirmed by DNA internal transcribed spacer (ITS) sequencing. *Candida krusei* ATCC 6258 (A) and five unrelated *C. krusei* strains (B, CNM-CL5642; C, CNM-CL5724; D, CNM-CL6695; E, CNM-CL6709; and F, CNM-CL0757) were used as control strains where required.

**Antifungal susceptibility testing.** Antifungal susceptibility was determined by the broth microdilution (BMD) and Etest methods. MICs were determined by following the recommendations proposed by AFST-EUCAST. BC, blood culture; CC, catheter culture; STC, soft tissue culture; UC, urine culture; CTC, catheter tip culture; wt, wild type; P, proline; Q, glutamine; F, phenylalanine; L, leucine; S, serine; AmB, amphotericin B; FLC, fluconazole; VRC, voriconazole; CSF, caspofungin; MCF, micafungin; ANF, anidulafungin.

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<td>0.5</td>
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</table>

*MICs were determined by following the recommendations proposed by AFST-EUCAST. BC, blood culture; CC, catheter culture; STC, soft tissue culture; UC, urine culture; CTC, catheter tip culture; wt, wild type; P, proline; Q, glutamine; F, phenylalanine; L, leucine; S, serine; AmB, amphotericin B; FLC, fluconazole; VRC, voriconazole; CSF, caspofungin; MCF, micafungin; ANF, anidulafungin.

**Amplification and sequencing of the FKS1 gene.** Genomic DNAs of all *C. krusei* isolates were extracted using DNEasy plant mini kits (Qiagen Inc., Valencia, CA) and used as templates for PCR amplification. PCR amplification and full sequencing of the three hot spots of the FKS1 gene (HS1, nucleotides 1963 to 1989; HS2, nucleotides 4090 to 4113; and newly described HS3, nucleotides 2116 to 2148) for all isolates were achieved using a panel of oligonucleotide primers (synthesized by Sigma Genosys, Madrid, Spain) (see Table S1 in the supplemental material). Amplification conditions consisted of 2 min of denaturation at 95°C, followed by 40 cycles of 30 s at 95°C for denaturation, 30 s at 55°C for annealing, and 60 s at 72°C for elongation, with a final elongation step of 2 min at 72°C. PCR products were purified with High Pure PCR product purification kits (Roche Diagnostics, Madrid, Spain) according to the manufacturer's recommendations. PCR products were semi-quantitated by agarose gel electrophoresis and used as templates for sequencing, which was performed with a dye terminator cycle sequencing quick-start kit (Beckman Coulter Inc., Fullerton, CA). The sequencing PCR products were then purified through G50 Sepharose columns (Amersham Biosciences) and, finally, sequenced on a CEQ8000 DNA analysis system (Beckman Coulter) with the forward and reverse primers used for PCR amplification. Sequences from all 15 *C. krusei* isolates were compared with that of the caspofungin-susceptible *C. krusei* (ATCC 6258) control strain.

The amino acid sequences of all three hot spot regions of the catalytic subunit of the 1,3-β-D-glucan synthase complex (Fks1p) were deduced from the nucleotide sequences and analyzed using the MegAlign software (DNAstar, Inc., Lasergene, Madison, WI). The amino acid sequence alignments were derived by CLUSTAL analysis (40).
Sequence chromatograms (43). The one-letter code for nucleotides from
fined by the presence of two coincident peaks in the forward and reverse
ers that were used in the PCR step. The sequence data were analyzed with
CEQ8000 DNA analysis system (Beckman Coulter), using the same prim-
(41). Each reaction mixture (50 μl) contained 5 μM (each) primers, PCR
master mix (Life Science), and 1 μl of template DNA. Cycling conditions
consisted of 4 min at 92°C followed by 32 cycles of 30 s at 92°C, 30 s at
55°C, and 2 min at 72°C, with a final step of 10 min at 72°C. Amplification
products were resolved by electrophoresis through a 1.0% agarose gel in
Tris-acetate-EDTA (TAE) buffer for 4 h at 50 V, stained with ethidium bromide, and photographed under UV illumination. A 1-kb
ladder (Promega) was used as a molecular size marker.

Nonrelated C. krusei strains (strains A to F; see above) were used as
control strains for typing. Two independent observers analyzed the pat-
tterns obtained with the polymorphic species-specific repetitive sequence
CKRS-1 by visual grouping of the patterns. Also, cluster analysis was done
by the unweighted-pair group method using average linkages (UPGMA),
by using the software PyElph, version 1.4 (http://pyelph.sourceforge.net/) (42).

For the second molecular typing method, multilocus sequence typing
(MLST) was used. PCRs were used to amplify gene fragments of six genes
(NMT1, ADE2, LEU2, HIS3, TRP1, and LYS2) by using previously de-
scribed primers (43). Reaction volumes of 50 μl contained 100 ng of
genomic DNA, 2.5 U Taq DNA polymerase (Applied Biosystems), 5 μl of
10 mM buffer (supplied with the enzyme), 2 mM MgCl2, a 0.5 mM concentra-
tion of each deoxynucleoside triphosphate (Roche), and 1 mM (each)
forward and reverse primers. A GeneAmp 9700 PCR system (Applied
Biosystems) was used with a first cycle of denaturation for 5 min at 94°C,
followed by 30 cycles of denaturation at 94°C for 1 min, annealing at
48°C/50°C for 1 min, and elongation at 72°C for 1 min, with a final exten-
sion step of 10 min at 72°C. The annealing temperature was 48°C for the
NMT1 and ADE2 genes and 50°C for the LEU2, HIS3, TRP1, and LYS2
genomes. The amplified products were purified using Illustra Exoprostar
1-Step kits (GE Healthcare Life Science, Little Chalfont, United King-
dom). Both strands of purified gene fragments were sequenced on a
CEQ8000 DNA analysis system (Beckman Coulter), using the same prim-
ers that were used in the PCR step. The sequence data were analyzed with
DNAStar software (LaserGene, Madison, WI). Heterozygosityes were de-
finned by the presence of two coincident peaks in the forward and reverse
sequence chromatograms (43). The one-letter code for nucleotides from
the International Union of Pure and Applied Chemistry (IUPAC) no-
omenclature was used to define results.

Finally, sequences were searched against those in the MLST C. krusei
consensus sequence database (http://pubmlst.org/crkrusei/) to obtain an
allele number for each locus. Once all six loci had allele numbers assigned,
these were entered into the online database to determine the diploid se-
dquence type (DST).

RESULTS

Candida krusei strain isolation. On 6 February 2013, a 63-year-
old female with acute lymphocytic leukemia (ALL) was admitted to
the hospital to begin chemotherapy. During the following 3 months, despite having received prophylaxis with a broad-spectrum
antibiotic (ciprofloxacin) and an antifungal drug (flucona-
zole), she developed three episodes of bacteremia (Staphylococcus
epidermidis and Escherichia coli) and two urinary infections (E.
coli) that were resolved by switching the antibiotic therapy to
cephelime and vancomycin (Fig. 1). During prolonged neutrope-
nia, the patient presented febrile relapse, so she received different
antibiotic and antifungal schemes (itraconazole, anidulafungin,
and caspofungin). On 31 May, the patient continued to have per-
sistent fever and neutropenia, and a set of blood cultures (BC) was
obtained. A Candida krusei (BC1) strain was isolated from these
blood cultures. Caspofungin therapy (70 mg on the first day fol-
lowed by 50 mg daily) was initiated. Blood cultures obtained during
the following week were all negative. After 10 days of caspo-
fungin therapy, a new set of blood cultures (BC2), catheter culture
(CC1), urine culture (UC), and soft tissue culture (STC) were all
positive for C. krusei. Caspofungin treatment was discontinued,
and liposomal amphotericin B (AmB) therapy was begun at a dose
of 5 mg/kg of body weight/day. The patient died 20 days later due
to persistent candidemia (BC2 to BC8, CC2, CC3, CTC1, and
CTC2) with multiple-organ failure (Fig. 1).

Antifungal susceptibility testing. Antifungal susceptibility
testing showed that all isolates were susceptible to amphotericin B
(MICAmB, 0.25 to 1 mg/liter) and voriconazole (MICVRC, 0.25 to
0.5 mg/liter) and had predictably high fluconazole MICs (MICFlc,
32 to 64 mg/liter). The first blood isolate (BC1), obtained pre-
caspofungin therapy, was susceptible to anidulafungin (MICAnf,
0.03 mg/liter) and had a caspofungin MIC of 0.5 mg/liter and a
micafungin MIC of 0.06 mg/liter. Isolates recovered after caspo-
fungin therapy (BC2, BC3, BC5 to BC8, UC, STC, CC1 to CC3,
and CTC1) showed elevated MICs for anidulafungin (0.125 to
1 mg/liter), caspofungin (1 to 4 mg/liter), and micafungin (0.5 to
1 mg/liter) (Table 1). Similar results were observed with the E-tests
casparofungin (Fig. 2) and anidulafungin. Curiously, echi-
ocandin MICs indicating susceptibility were observed for two

FIG 1 Time course of development of echinocandin resistance in different isolates from a patient suffering candidemia from Candida krusei. ALL, acute
lymphocytic leukemia; D, days; FLC, fluconazole; ANF, anidulafungin; ITC, itraconazole; CSF, caspofungin; AmB, amphotericin B; BC, blood culture; CC,
catheter culture; UC, urine culture; STC, soft tissue culture; CTC, catheter tip culture. The boxes represent the patient’s isolates.
isolates (BC4 and CTC2), both obtained post-caspofungin therapy.

**Amplification and sequencing of the FKS1 gene.** Sequence analysis of the FKS1-HS1 region in the *C. krusei* ATCC 6258 and BC1 strains showed no differences. In contrast, all isolates recovered after caspofungin treatment showed mutations in hot spot 1 of the FKS1 gene (FKS1-HS1) compared with the first isolate (BC1). *Candida krusei* strains isolated from blood (BC3 and BC6) and urine (UC) presented a missense mutation corresponding to an amino acid substitution of phenylalanine 655 to leucine (F655L) in the FKS1-HS1 region. Similarly, isolates recovered from blood (BC2, BC5, BC7, and BC8), catheter (CC1, CC2, and CC3), catheter tip (CTC1), and soft tissue (STC) cultures showed an amino acid change from proline 663 to glutamine (P663Q) compared with BC1. The analysis of the chromatograms generated from DNA sequencing revealed a single nucleotide base change at the site of the mutation, which is consistent with a homozygous change at FKS1-HS1 in mutant clinical isolates. A heterozygous mutation corresponding to an amino acid substitution of serine 659 to proline (S659P) was observed in the FKS1-HS1 region of blood culture 4 (BC4) and catheter tip culture 2 (CTC2). These isolates, along with BC1, presented an amino acid change from lysine 701 to methionine (L701M) as a result of a heterozygous mutation in a region outside the hot spots.

No changes were observed in hot spot 2 or 3 of FKS1 in any of the isolates.

**Molecular typing of Candida krusei.** Given the diverse mutations found among isolates, molecular typing of all isolates was performed, using two different methods, in order to discern if the patient was infected by more than one *C. krusei* strain (41, 43).

**PCR-based amplification of the species-specific repetitive polymorphic CKRS-1 sequence.** The banding patterns obtained after polymorphic species-specific repetitive sequence CKRS-1 (*C. krusei* repeated sequence 1) amplification were analyzed visually. Band sizes ranged from 0.7 to 2 kb among all isolates tested. A unique band pattern was obtained with each of the six control isolates, showing a high discriminatory power of the technique (Fig. 3). However, no differences were observed in the band patterns obtained for the 15 strains isolated from the patient. Isolates showing only one band difference were considered identical. To confirm the visual reading of the gel, computer-assisted cluster analysis of profiles was performed with PyElph software, version 1.4. The dendrogram derived from the UPGMA clustering algorithm showed that all the patient isolates appeared to have the same genotype (genetic distance of 0.0) and were different from the nonrelated control strains (Fig. 3; see Fig. S1 in the supplemental material).

**Multilocus sequence typing.** For the 15 isolates of *C. krusei* obtained from different anatomical locations and with different FKS1 mutations, the obtained DSTs were identical (DST 187) but were different from that of the ATCC 6258 control strain (DST 188) (Table 2).

**DISCUSSION**

Echinocandins are antifungal drugs that inhibit the enzyme 1,3-β-D-glucan synthase (GS) complex, whose catalytic subunit is encoded by three related genes: FKS1, FKS2, and FKS3 (12). This mode of action makes them particularly interesting against yeasts with reduced susceptibility to azoles, such as *Candida glabrata* and *C. krusei*. The rising use of echinocandins, however, plays an important role in the species distribution of invasive infections (44) and in decreased echinocandin susceptibility after prolonged exposure to the drug, primarily in severely immunocompromised patients, in whom infections manifest as recurrent episodes of candidemia or invasive candidiasis (45, 46).

Currently, reduced echinocandin susceptibility remains rare and has been shown to occur through the following three mechanisms: (i) acquired FKS mutations, which confer reduced glucan synthase sensitivity and elevated echinocandin MICs and are associated with clinical failure (23); (ii) adaptive stress responses that result in elevated cell wall chitin content and paradoxical growth in vitro (47); and (iii) intrinsic FKS mutations, which are naturally occurring in *C. parapsilosis* and *C. guilliermondii* and result in elevated MICs but a lower level of reduced glucan synthase sensitivity than that with acquired FKS mutations (23, 48, 49). In most cases, alterations in FKS genes confer cross-resistance to all three echinocandins. However, some alterations cause more moderate MIC elevations, and not always for all related compounds. Additionally, several clinical studies have shown that the presence of an FKS mutation is the most important independent risk factor in predicting echinocandin therapeutic responses among patients with invasive candidiasis (31, 32, 50).

In the present case, rapid development of resistance was demonstrated in sequential and genetically related isolates of *C. krusei* recovered from a patient under caspofungin treatment. Clinical resistance was associated with increased echinocandin MICs and
was ultimately related to new mutations of the target enzyme (FKS1).

Sequencing of FKS1 hot spot 1 showed that the C. krusei strain isolated prior to caspofungin therapy presented no amino acid substitutions compared to the FKS1 sequence of the reference strain (ATCC 6258). However, all isolates of C. krusei recovered after only 10 days of treatment with caspofungin presented three different amino acid substitutions in hot spot 1 of Fks1p: F655L, S659P/S, and P663Q. This shows the rapid selection of mutant strains during antifungal treatment. None of the three amino acid changes have been reported previously for C. krusei. So far, only three clinical isolates of C. krusei with mutations in FKS1 associated with reduced echinocandin susceptibility and treatment failure have been reported (18, 20, 21). As in a previous report on C. albicans (51), we found that isolates of C. krusei with the F655L change (corresponding to F641 in C. albicans) showed the highest anidulafungin MIC. In fact, the effect on echinocandin susceptibility produced by this mutation (F655L) has already been demonstrated for a clinical isolate of C. tropicalis with reduced echinocandin MICs through an elevated 50% inhibitory concentration (IC50) in a kinetic enzyme assay (24), a method that has been critical for revealing the relationship between reduced whole-cell susceptibility and target site inhibition (19). Additionally, a heterozygous mutation at this position (F655C) was reported by Kahn et al. for a C. krusei isolate recovered post-caspofungin treatment, which presented an increase in the echinocandin MIC from 16- to 32-fold with respect to the first susceptible isolate (18).

Heterozygous amino acid changes within the FKS-HS region have been associated with caspofungin resistance in several yeast species, such as a Saccharomyces cerevisiae laboratory strain, C.

### TABLE 2 Full multilocus sequence typing results for Candida krusei isolates

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<th>Isolate</th>
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</table>
albicans (18, 19), and C. tropicalis clinical isolates (24), indicating that the phenotypic expression of the mutation is dominant (33). In this regard, it is important that the degree of MIC elevation depends not only on ploidy but also on (i) the Candida species (20) and (ii) the position and specific amino acid substitution in the hot spot. These considerations may help to explain the anidulafungin-susceptible phenotype (MIC, 0.06 mg/liter) observed for two isolates of C. krusei (BC4 and CTC2) with an S659P/S heterozygous amino acid change. Further studies are under way in our laboratory to determine the impact of an S659P/S mutation in C. krusei.

The two isolates mentioned above (BC4 and CTC2), together with the first, susceptible isolate of C. krusei (BC1), also showed an L701M change, a substitution already reported for C. krusei (21, 52). However, its implication in resistance is unclear, as it is located outside the hot spots of FKS1 and, in our case, was present in the initial isolate that showed lower echinocandin MICs (BC1). In C. parapsilosis, a substitution in the distal proline of hot spot 1 (P660A) is responsible for the decreased susceptibility to the echinocandin drugs that is intrinsically observed in this species and related species, demonstrating the importance of this amino acid in the drug-enzyme interaction (48). In our case, a change at this position (P663Q) was the most frequently found mutation in the isolates from the patient, regardless of the body site origin. This could be a consequence of the random effect at the time of strain storage, as a single colony is kept to ensure the purity of the stored isolate. Therefore, it is likely that all C. krusei isolates with different mutations were present in the patient during the course of the disease and antifungal therapy and that the catheter was the common reservoir of infection (biofilm), reflecting the emergence of different isolates with a decreased echinocandin susceptibility phenotype under selective pressure. However, the study of the relationships of the different mutations to the caspofungin concentrations achieved at different body sites is an interesting matter that deserves further research. Additionally, 2 months before the development of the C. krusei candidemia, the patient received a 4-day course of anidulafungin. However, as the first isolated strain was susceptible to all echinocandins, it seems clear that the development of resistance occurred during treatment with caspofungin. Nevertheless, we cannot disregard the possibility that the use of anidulafungin as a preemptive treatment may have conditioned the development of resistance in some way.

The genetic discrimination among C. krusei isolates could offer some important clues to understanding transmission, pathogenesis, and resistance development. Several techniques have been used to document the genetic diversity of C. krusei clinical isolates, such as those employed here (41, 43). Both PCR-based and MLST techniques are accurate and useful for the typing of C. krusei isolates to clarify the epidemiology of nosocomial infections (41, 43). In our clinical case, the analysis of C. krusei’s genomic DNA and the analysis of fragment sequences of six genes revealed that all isolates, wild type and mutated, were genetically indistinguishable and were different from control strains. It seems likely that the mutant isolates recovered from this patient arose from a wild-type progenitor strain. Furthermore, these results establish that strains with different FKS1 mutant alleles can be recovered from different sites in the same patient, as reported by Park et al. for a patient infected with the same strains of C. albicans but with different mutations in the FKS gene (19).

The best methodological parameters to be used for in vitro susceptibility testing of caspofungin are still under debate (38). The consequence is that susceptible strains are misclassified according to established clinical breakpoints, and this has led to limited therapeutic options, especially for Candida spp. with secondary or intrinsic resistance to fluconazole, such as C. glabrata and C. krusei (38).

At the time of isolate recovery, only the Etest for caspofungin was performed to test echinocandin susceptibility. The Etest method allowed us to easily determine the caspofungin MIC, and CLSI breakpoints helped to detect the MIC increase and the categorization of wild-type and mutant isolates. But previously published work by Arendrup and Pfäffler (38) found that the caspofungin Etest for susceptible isolates of C. glabrata and C. krusei presents the risk of misclassification if the CLSI breakpoints are used. Therefore, we confirmed our finding by using BMD to establish the echinocandin susceptibility profile and to detect possible mutated isolates.

In summary, we provide molecular evidence of the rapid acquisition of clinical echinocandin resistance associated with increased echinocandin MICs in vitro and related to different mutations in hot spot 1 of the target enzyme (FKS1). The risk factors present, such as the underlying disease, use of multiple antibiotics, and antifungal prophylaxis, were decisive for the acquisition of infection by C. krusei and for the rapid development of resistance during caspofungin therapy. Therefore, these results highlight the need to monitor the development of resistance in C. krusei infections treated with echinocandin drugs. The categorization of isolates through susceptibility testing is an important tool to minimize unnecessary echinocandin use and treatment duration, helping to reduce selection pressure and the rise in echinocandin resistance.

ACKNOWLEDGMENTS

E.M. is supported by a project from the Spanish Fondo de Investigacion Sanitaria (FIS; grant PI12_02376) and by the Instituto de Salud Carlos III, Subdirección General de Redes y Centros de Investigación Cooperativa, Ministerio de Economía y Competitividad, Spanish Network for Research in Infectious Diseases (grant REIPI RD 12/0015). M.C.M. is funded by the Spanish Fondo de Investigacion Sanitaria through a Sara Borrell fellowship (grant CD13/00198).

E.M. declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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