Use of Micafungin as a Surrogate Marker To Predict Susceptibility and Resistance to Caspofungin among 3,764 Clinical Isolates of Candida by Use of CLSI Methods and Interpretive Criteria

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Due to unacceptably high interlaboratory variation in caspofungin MIC values, we evaluated the use of micafungin as a surrogate marker to predict the susceptibility of Candida spp. to caspofungin using reference methods and species-specific interpretive criteria. The MIC results for 3,764 strains of Candida (eight species), including 73 strains with fks mutations, were used. Caspofungin MIC values and species-specific interpretive criteria were compared with those of micafungin to determine the percent categorical agreement (%CA) and very major error (VME), major error (ME), and minor error rates as well as their ability to detect fks mutant strains of Candida albicans (11 mutants), Candida tropicalis (4 mutants), Candida krusei (3 mutants), and Candida glabrata (55 mutants). Overall, the %CA was 98.8% (0.2% VMEs and MEs, 0.8% minor errors) using micafungin as the surrogate marker. Among the 60 isolates of C. albicans (9 isolates), C. tropicalis (5 isolates), C. krusei (2 isolates), and C. glabrata (44 isolates) that were nonsusceptible (either intermediate or resistant) to both caspofungin and micafungin, 54 (90.0%) contained a mutation in fks1 or fks2. An additional 10 C. glabrata mutants, two C. albicans mutants, and one mutant each of C. tropicalis and C. krusei were classified as susceptible to both antifungal agents. Using the epidemiological cutoff values (ECVs) of 0.12 μg/ml for caspofungin and 0.03 μg/ml for micafungin to differentiate wild-type (WT) from non-WT strains of C. glabrata, 80% of the C. glabrata mutants were non-WT for both agents (96% concordance). Micafungin may serve as an acceptable surrogate marker for the prediction of susceptibility and resistance of Candida to caspofungin.

The echinocandins caspofungin and micafungin are now well established as first-line agents for the treatment of candidemia and other forms of invasive candidiasis (IC) (1–3). The in vitro activities of caspofungin and micafungin against Candida spp. have been documented using broth dilution, agar disk diffusion, and Etest methods (4–8), and most recently, clinically relevant interpretative breakpoints for broth microdilution (BMD) MIC testing of Candida spp. were established by the Clinical and Laboratory Standards Institute (CLSI) (9). Whereas the CLSI has developed clinical breakpoints (CBPs) for anidulafungin, caspofungin, and micafungin against the six most common species of Candida (9), the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has elected to establish CBPs for anidulafungin and micafungin but not for caspofungin (10). Furthermore, the EUCAST does not currently recommend caspofungin MIC testing for clinical decision making due to unacceptably high variation among the caspofungin MIC values obtained from different centers (4, 5, 11, 12). This variation is evident not only using the EUCAST BMD method (4, 11) but also using the CLSI method (13). A recent CLSI analysis of the MIC distributions from 16 different laboratories showed variation in the caspofungin wild-type (WT) modal MIC values of as much as five doubling dilutions (e.g., 0.015 to 0.5 μg/ml) (13). In contrast, the variation in micafungin WT modal MIC values was within ±1 doubling dilution step for various species (M. A. Pfaller et al., unpublished data). The reasons for such variation in the caspofungin MIC values from center to center remain unclear but may involve the lot-to-lot variation in the potency of caspofungin powders, the use of dimethyl sulfoxide (DMSO) versus water as a solvent, storage conditions, or the determined MIC endpoints (4, 11–13).

Previously, we demonstrated that in vitro cross-resistance between micafungin and caspofungin does exist among clinical isolates of Candida (9, 14) (M. A. Pfaller et al., unpublished data). The clinical relevance of this cross-resistance has been documented in studies of the resistance mechanisms (detection of fks mutations) (15–17), in animal models of IC (18–21), and in case series where clinically significant resistance to one or more echinocandins, marked by the acquisition of a resistance mutation in the fks gene, has been reported in immunocompromised patients (e.g., those in intensive care, stem cell transplant patients, and solid organ transplant patients) with a high level of prior echinocandin exposure (11, 22–27). Although many of the resistant isolates and cases of breakthrough IC were due to Candida glabrata, a number of Candida albicans, Candida tropicalis, Candida krusei, and Candida lusitaniae isolates have also been observed to have reduced susceptibility or resistance to both micafungin and caspofungin (17, 22–24, 26, 28, 29). It should be noted that isolates of C. glabrata with paradoxical reduced caspofungin susceptibility but increased micafungin susceptibility have been reported (30, 31). Although the clinical importance of such differential sensitivities to the echinocandins is not clear, two recent studies found such differences to be meaningful in murine models of IC, irrespective of the presence or absence of fks mutations (19, 21).

Despite the fact that CBPs for caspofungin and Candida spp.
were established by the CLSI (9), the extreme intra- and interlaboratory variations in caspofungin MIC results are of great concern (4, 11, 13). The fact that micafungin MIC values appear to be much less variable from laboratory to laboratory (M. A. Pf:\_{affer et al.}, unpublished data) suggests that this echinocandin may be the preferred reagent for the in vitro testing of echinocandins against Candida. Previously, we have shown that fluconazole may serve as a surrogate marker for evaluating the susceptibility of Candida to ravuconazole (32), voriconazole (33), and posaconazole (34). This same approach should be applicable to caspofungin, while the more reliable test reagent, micafungin, may be applied for the prediction of susceptibility and resistance to these echinocandins.

In the present study, we utilized a large database of susceptibility test results, all determined by CLSI BMD methods and that included results for 73 fks mutant strains, to provide a robust analysis of cross-resistance between the two agents and additionally to examine the usefulness of micafungin as a surrogate marker for evaluating caspofungin susceptibility and resistance among WT Candida spp.

**MATERIALS AND METHODS**

**Organisms.** We tested a total of 3,764 clinical isolates of Candida spp. obtained from >100 medical centers worldwide (8, 9, 34). The collection included 2,010 isolates of C. albicans, 566 isolates of C. glabrata, 539 isolates of Candida parapsilosis, 426 isolates of C. tropicalis, 105 isolates of C. krusei, 52 isolates of Candida guilliermondii, 42 isolates of C. lusitaniae, and 24 isolates of Candida kefyr. All were incident isolates from individual patients and were obtained from blood or other normally sterile body fluids. Among the included isolates of C. albicans, C. glabrata, C. tropicalis, and C. krusei were 73 isolates (11 C. albicans, 4 C. tropicalis, 3 C. krusei, and 55 C. glabrata) with documented fks resistance mutations. The isolates were identified by use of the Vitek and API yeast identification systems (bioM\_{\text{erieux}}, Inc., Hazelwood, MO, USA) supplemented with conventional methods as needed (35). The isolates were stored as water suspensions until use. Prior to testing, each isolate was passaged at least twice on potato dextrose agar (Remel, Lenexa, KS, USA) and CHROMagar Candida (Becton, Dickinson, Sparks, MD, USA) to ensure purity and viability. The presence or absence of a mutation in the hot spot (HS) regions of fks1 and fks2 (C. glabrata only) was determined as described previously (36, 37).

**Antifungal susceptibility testing.** All isolates were tested for in vitro susceptibility to caspofungin and micafungin using CLSI BMD methods (38, 39). The MIC results for the agents were read following 24 h of incubation. In all instances, the MIC values were determined visually as the lowest concentration of the drug that caused significant growth diminution levels (38, 39).

We used the recently revised CBPs to identify the strains of the 6 most common species of Candida (C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. krusei, and C. guilliermondii) that were susceptible (S), intermediate (I), or resistant (R) to caspofungin and micafungin (40, 41): caspofungin and micafungin MIC values of ≤0.25 μg/ml, 0.5 μg/ml, and ≥1 μg/ml were considered to be susceptible, intermediate, and resistant, respectively, for C. albicans, C. tropicalis, and C. krusei, and MIC results of ≤2 μg/ml, 4 μg/ml, and ≥8 μg/ml were categorized as susceptible, intermediate, and resistant, respectively, for C. parapsilosis and C. guilliermondii; caspofungin MIC values of ≤0.12 μg/ml, 0.25 μg/ml, and ≥0.5 μg/ml and micafungin MIC values of ≤0.06 μg/ml, 0.12 μg/ml, and ≥0.25 μg/ml were considered to be susceptible, intermediate, and resistant, respectively, for C. glabrata. In addition to the CBPs for these species, epidemiological cutoff values (ECVs) were established in order to provide a sensitive means of separating WT from non-WT strains (those that possess an intrinsic or acquired resistance mutation). The ECVs for caspofungin and micafungin for each species are 0.12 μg/ml and 0.12 μg/ml for C. tropicalis, 0.25 μg/ml and 0.12 μg/ml for C. krusei, and 2 μg/ml and 2 μg/ml for C. guilliermondii, respectively (41). CBPs have not been established for caspofungin and micafungin and less common species, such as C. lusitaniae and C. kefyr. The caspofungin and micafungin ECVs for these two species are 1 μg/ml and 0.5 μg/ml for C. lusitaniae and 0.03 and 0.12 μg/ml for C. kefyr, respectively (41). Candida spp. isolates for which caspofungin or micafungin MICs exceed the ECVs are considered to be non-WT and may harbor acquired mutations in the fks gene (17).

**Quality control.** Quality control was performed as recommended in CLSI documents M27-A3 (39) and M27-S4 (38) using C. krusei strain ATCC 6258 and C. parapsilosis strain ATCC 22019. **Analysis of results.** All MIC results (in μg/ml) for micafungin were directly compared with those for caspofungin by regression statistics and by scattergram (data not shown). The error rate bounding method to minimize intermethod interpretive error was also applied using the interpretive criteria described above. The acceptable error rate limits used in this comparison were those cited in CLSI document M23-A3 (40, 42) and in other studies (43, 44). The definitions of the errors used in this analysis are as follows: a very major error (VME), or a false-susceptible error, was a susceptible result for the surrogate marker micafungin and a resistant result for caspofungin; a major error (ME), or a false-resistant error, was a resistant result for micafungin and a susceptible result for caspofungin; and minor errors occurred when the result for one of the agents was susceptible or resistant and that for the other agent was intermediate. In general, for an agent to be considered a reliable surrogate marker, the VME rate should be ≤1.5% of all results, and the absolute categorical agreement (CA) between methods should be ≥90% (33, 40, 42). In addition to the above analysis, we will also examine the detectability of fks mutants of C. albicans and C. glabrata using the CBPs and ECVs for each echinocandin.

**RESULTS AND DISCUSSION**

Table 1 depicts the MIC distribution profiles for micafungin and caspofungin determined for 3,764 strains of Candida spp. using BMD methods validated by the CLSI (39). Overall, 3,694 isolates (98.2%) were susceptible, 20 isolates (0.5%) were intermediate, and 50 isolates (1.3%) were categorized as resistant to micafungin. Similarly, 3,682 isolates (97.8%) were susceptible, 20 isolates (0.5%) were intermediate, and 62 isolates (1.7%) were resistant to caspofungin. The modal MIC for micafungin was 0.015 μg/ml (1,323 results [35.1%]) compared to 0.03 μg/ml (1,362 results [36.2%]) for caspofungin. There was a strong positive correlation (r = 0.84) between the micafungin and caspofungin MIC values (data not shown). Overall, the essential agreement (MIC ±2 dilutions) was 97.2%. Decreased potencies of both micafungin and caspofungin were observed among C. parapsilosis (modal MICs, 1 μg/ml and 0.5 μg/ml, respectively) and C. guilliermondii (modal MICs, 0.25 μg/ml and 0.5 μg/ml, respectively). The highest rates of resistance to both agents were observed with C. glabrata: 5.8% were resistant to micafungin and 7.9% were resistant to caspofungin. Among the 33 isolates of C. glabrata that were resistant to micafungin, 30 isolates (90.9%) possessed a mutation in fks1 or fks2, and among 45 isolates that were resistant to caspofungin, 40 (88.9%) possessed a mutation in the fks gene (Table 1).

The extent of cross-resistance between micafungin and caspofungin can be seen more clearly in Table 2. For the 3,694 isolates that were susceptible to micafungin, 3,672 (99.4%) were also susceptible to caspofungin. There were seven isolates that were susceptible to micafungin and resistant to caspofungin; of those, four isolates were C. glabrata, two isolates were C. guilliermondii, and one isolate was C. krusei, and two of the four C. glabrata isolates contained an fks mutation. Among the 50 isolates that were resis-
tant to micafungin, 43 isolates (86.0%) were also resistant and seven isolates (14.0%) were susceptible to caspofungin. Similarly, 17 of the 20 (85.0%) isolates categorized as intermediate to micafungin were either intermediate or resistant to caspofungin. Thus, 99.4% of the micafungin-susceptible and 85.7% of the micafungin-nonsusceptible (I plus R) isolates were susceptible and nonsusceptible, respectively, to caspofungin.

When the micafungin test result category (S, I, or R) was used to predict the caspofungin category, the absolute categorical agreement (CA) between the test results was 98.8%, with only a 0.2% VME (false-susceptible error) and ME (false-resistant error) rate and a 0.8% minor error rate (Table 3). Among the eight species of Candida tested, the CA was 92% (range, 92.4% to 100.0%) for all species. Generally, the discrepancies in the categorical results were minor errors. VMEs were seen more often with C. glabrata, C. krusei, and C. guilliermondii; however, only the VME rate involving C. guilliermondii (3.8%) exceeded the allowable VME rate of ≤1.5% (Table 3) (40, 42, 43).

Clearly, it is important to detect those isolates of Candida that possess an acquired mutation in the fks gene (9, 17), and in that regard, micafungin performs quite well as a surrogate marker. Among all 73 fks mutant strains, 14 (19.2%) were susceptible to both caspofungin and micafungin, and 54 (74.0%) were intermediate or resistant to both, for an overall concordance of 93.2%. Furthermore, for the 11 isolates of C. albicans with a mutation in fks1, nine isolates (82.0%) were either intermediate or resistant to both micafungin and caspofungin and two isolates were susceptible to both agents (Tables 1 and 4). Using the micafungin ECV for C. albicans (0.03 µg/ml), all 11 isolates would be classified as non-WT, indicating that they were likely to contain an acquired resistance mutation. There were a total of 55 isolates of C. glabrata that contained a mutation in fks1 or fks2 (Tables 1 and 4). Of these, 10 isolates (18.2%) were susceptible to both micafungin and caspo-

### Table 1: MIC Distributions of Caspofungin and Micafungin Versus Candida spp., Including Strains with fks Mutations Using CLSI Methods

<table>
<thead>
<tr>
<th>Candida sp. (no. of isolates tested)</th>
<th>Echinocandins</th>
<th>Caspofungin (µg/ml)</th>
<th>Micafungin (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of isolates with an MIC</td>
<td>no. of isolates with fks mutation</td>
<td>No. of isolates with an MIC</td>
</tr>
<tr>
<td></td>
<td>0.007 0.015 0.03 0.06 0.12 0.25 0.5 1 2 4 ≥8</td>
<td></td>
<td>0.007 0.015 0.03 0.06 0.12 0.25 0.5 1 2 4 ≥8</td>
</tr>
<tr>
<td>C. albicans (2,010)</td>
<td>Caspofungin</td>
<td>33 539 892 502 22 12 (2) 3 (2) 4 (4) 3 (3)</td>
<td>Micafungin</td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>34 273 (1) 189 (6) 16 (3) 9 (5) 14 (11) 7 (6) 8 (8) 2 (2) 14 (13)</td>
<td>Micafungin</td>
</tr>
<tr>
<td>C. parapsilosis (539)</td>
<td>Caspofungin</td>
<td>1 1 1 17 35 208 219 49 7 1</td>
<td>Micafungin</td>
</tr>
<tr>
<td>C. tropicalis (426)</td>
<td>Caspofungin</td>
<td>4 137 191 82 3 (1) 4</td>
<td>Micafungin</td>
</tr>
<tr>
<td>C. krusei (105)</td>
<td>Caspofungin</td>
<td>1 44 32 19 (1) 6 2 (1) 1 (1) 1 (1)</td>
<td>Micafungin</td>
</tr>
<tr>
<td>C. guilliermondii (52)</td>
<td>Caspofungin</td>
<td>1 2 4 13 22 7 1 2</td>
<td>Micafungin</td>
</tr>
<tr>
<td>C. lusitaniae (42)</td>
<td>Caspofungin</td>
<td>1 1 20 18 2</td>
<td>Micafungin</td>
</tr>
<tr>
<td>C. kefyr (24)</td>
<td>Caspofungin</td>
<td>3 20 1</td>
<td>Micafungin</td>
</tr>
</tbody>
</table>

### Table 2: Use of Micafungin to Predict Susceptibility Patterns of Caspofungin Employing 3,764 Clinical Isolates of Candida spp. from a Global Surveillance Program

<table>
<thead>
<tr>
<th>Candida sp. (no. of isolates tested)</th>
<th>Micafungin susceptibility category</th>
<th>No. (%) of isolates in caspofungin category</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans (2,010)</td>
<td>S 1,994 (99.2)</td>
<td>1 (0.1) 2 (0.1) 6 (0.3) 5 (0.2)</td>
</tr>
<tr>
<td>C. glabrata (566)</td>
<td>S 509 (90.0)</td>
<td>6 (1.1) 4 (0.7)</td>
</tr>
<tr>
<td>C. parapsilosis (539)</td>
<td>S 538 (99.8)</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>C. tropicalis (426)</td>
<td>S 420 (98.6)</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>C. krusei (105)</td>
<td>S 96 (91.4)</td>
<td>6 (5.6) 1 (1.0)</td>
</tr>
<tr>
<td>C. guilliermondii (52)</td>
<td>S 49 (94.2)</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>C. lusitaniae (42)</td>
<td>S 42 (100.0)</td>
<td></td>
</tr>
<tr>
<td>C. kefyr (24)</td>
<td>WT 24 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

* S, susceptible; I, intermediate; R, resistant; WT, wild type.

MIC interpretive criteria for each species as shown in reference 41.
fungin, 5 isolates (9.1%) were susceptible to micafungin and either intermediate or resistant to caspofungin, and 40 (72.7%) were intermediate or resistant to both agents (Table 4). The overall concordance between the two methods (testing of micafungin versus caspofungin) using the CLSI CBPs to classify fks mutant strains of C. glabrata as susceptible or nonsusceptible was 90.9%. Using the ECVs of 0.03 μg/ml for micafungin and 0.12 μg/ml for caspofungin to differentiate WT from non-WT strains of C. glabrata, nine strains (16.4%) were WT and 44 strains (80.0%) were non-WT for both agents (overall concordance, 96.4%).

There were four strains of C. tropicalis and three of C. krusei that harbored an fks mutation. Of these, 3 strains of C. tropicalis (75.0%) and 2 strains of C. krusei (66.7%) were intermediate or resistant to both agents, whereas one strain of each species was susceptible to both agents. All of these would be considered non-WT using the micafungin ECV of 0.12 μg/ml for each species.

The most frequently encountered mutations in this collection occurred at position S663 (18 isolates), followed by F659 (nine isolates), S645 (seven isolates), and S629 (six isolates), and four isolates each contained mutations at positions F625, F641, and S629 (six isolates), and four isolates (seven isolates), and S629 (six isolates), and four isolates (five isolates), and S629 (ten isolates), and S629 (six isolates, and four isolates each contained mutations at positions F625, F641, and S629 (Table 4). Previous reports indicate that isolates of C. glabrata with the S663F mutation respond in vivo to high doses of either micafungin or caspofungin, whereas isolates with the S629P mutation fail to respond to even the highest dose of either agent (21). Notably, the pharmacodynamic target for the isolate with the S663F mutation was below the human area under the concentration curve (AUC) using standard doses of micafungin but not caspofungin, suggesting that differences in the echinocandin MIC values observed with individual fks mutations may be associated with differential antifungal activity in vivo (21). These findings are supported by those of Spreghini et al. (19), who found in a comparison between micafungin, caspofungin, and anidulafungin that micafungin showed the best in vitro and in vivo activities against two resistant mutants of C. glabrata bearing specific mutations in the fks2 HS region. Mutations at positions S663 and F659 in C. glabrata have been associated with breakthrough infections in patients receiving echinocandin therapy (22, 23, 27), whereas patients infected with C. glabrata strains containing the I137V and I634V mutations (i.e., susceptible to both micafungin and caspofungin) tended to respond to therapy with either agent.
TABLE 4 (Continued)

<table>
<thead>
<tr>
<th>Candida sp.</th>
<th>fks mutation</th>
<th>Micafungin (µg/ml) for:</th>
<th>Caspofungin (µg/ml) for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Micafungin</td>
<td>Caspofungin</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>F641S</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>F641S</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>S645P</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>S645P</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>C. krusei</td>
<td>F655C</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>R1361G</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>L701M</td>
<td>0.06</td>
<td>0.25</td>
</tr>
</tbody>
</table>

(22). Regarding the C. albicans mutants in this study, three of the 11 fks mutants contained the S645P mutation, a genotype that cannot be treated with conventional doses of micafungin or caspofungin (45). Taken together, these results suggest a linkage cannot be treated with conventional doses of micafungin or caspofungin (11) fks results to predict the susceptibilities of Candida spp., these results suggest a linkage between the increased echinocandin MIC results, specific fks mutations, and the potential for a successful clinical outcome (19, 21, 22, 27).

Previously, we conducted similar analyses using fluconazole results to predict the susceptibilities of Candida spp. to the newer triazoles as a proof of concept regarding the use of surrogate markers or class representatives for antifungal susceptibility testing (32–34). This strategy has been used for decades in antibacterial susceptibility testing to develop practical alternatives for the microbiology laboratory when specific diagnostic susceptibility reagents produce unreliable results or are limited or unavailable (40, 44). Given the issue of unacceptable variability in caspofungin MIC results, we concur with the recommendations of the EUCAST that the caspofungin MIC results for Candida spp. should not be used for clinical decision making at this time (10). As shown herein, MIC testing with micafungin is highly predictive of caspofungin categorical results, and it reliably detects clinically important fks mutations.

In addition to providing a strategy for predicting caspofungin susceptibility and resistance among Candida spp., these results provide strong support for concerns regarding the issue of cross-resistance between micafungin and caspofungin (5, 15, 16, 18, 22, 26). By using an extensive global collection of clinically important isolates, including fks mutant strains, we validate concerns originating from single-center case series and rightly focus attention on C. glabrata as the species most likely to demonstrate cross-resistance between these two studied echinocandins.

Micafungin functioned well as a surrogate marker for caspofungin susceptibility when applied to this large collection of clinically significant isolates of Candida spp. The absolute CA of 98.8%, with only 0.2% VMEs among the 3,764 isolates tested, easily meets the recognized criteria for a reliable surrogate marker in antibacterial susceptibility testing (40, 44). The excellent concordance between the micafungin and caspofungin results in categorizing the fks mutants also provides further validation of this approach. The major limitation of this study, given the interlaboratory variability of caspofungin MICs, is the fact that the data were obtained from only two laboratories. This may lead to concerns regarding the generalizability of the findings to other laboratories. These concerns are somewhat mitigated by the inclusion of a large number of fks mutants in the study.

In conclusion, we have demonstrated the existence of cross-resistance between micafungin and caspofungin, with the greatest emphasis on C. glabrata. Furthermore, we have shown that in the face of the unreliable caspofungin susceptibility in a medical center currently performing antifungal susceptibility testing of micafungin can be accomplished by using the micafungin result as a surrogate marker for caspofungin susceptibility and resistance. Arguably, the most important role of in vitro susceptibility testing is to predict the resistance of the infecting organism to the agent under consideration for use in a patient (46). The occurrence of false-resistant and false-susceptible errors with this application of the class representative concept to the echinocandin antifungal was low and was considered very acceptable for use as a surrogate marker. The excellent CA documented in this study was further supported by the high level of concordance in identifying strains of Candida with clinically important fks resistance mutations. Further efforts to clarify and correct the issues of caspofungin testing using the CLSI and EUCAST BMD methods should be a priority for future research.

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