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Categorizing Susceptibility of Clinical Isolates of *Candida auris* to Amphotericin B, Caspofungin, and Fluconazole by Use of the CLSI M44-A2 Disk Diffusion Method

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ABSTRACT We evaluated the CLSI M44ed3E disk diffusion method compared with the CLSI M27ed4 broth microdilution method for caspofungin and fluconazole and the Etest method for amphotericin B to categorize susceptibility of 347 clinical isolates of *Candida auris*. Utilizing the zone diameter cutoffs established here, we observed overall categorical agreement between the two methods. For caspofungin, concordant results were observed for 98% of isolates, with <1% very major and 1% major errors. For fluconazole, concordant results were observed for 91% of isolates, with 1% very major and 8% major errors. For amphotericin B, concordant results were observed for 74% of isolates, with <1% very major errors and 25% major errors. The disk diffusion approach provides an accurate method for determining the susceptibility of *C. auris* for caspofungin and fluconazole and for identification of at least 75% of amphotericin B-susceptible isolates.

KEYWORDS *Candida auris*, disk diffusion, fluconazole, amphotericin B, caspofungin, antifungal susceptibility, antifungal, mycology, susceptibility

Candida auris is an emerging multidrug-resistant yeast that causes invasive infections with high mortality (1). Within a short time, *C. auris* has emerged in health care settings in more than 30 countries (2). Transmission of *C. auris* is aided by its ability to colonize skin and persist on surfaces for weeks (3). The infection is challenging to treat, as resistance to fluconazole is widespread and susceptibility to other azoles, amphotericin B and the echinocandins, is variable (4). There is an additional encumbrance to susceptibility testing in resource-limited countries due to a paucity of facilities able to afford or to perform the testing (5).

Disk diffusion is a cost-effective susceptibility testing method conventionally used in clinical laboratories for bacterial pathogens (6). This method is reproducible, easy to interpret, and the least costly of susceptibility methods. Conversely, the standard broth microdilution method requires expertise and specialized equipment and can be laborious; both of these limitations pose challenges to clinical laboratories, especially in resource-limited countries (7).

The purpose of this study was to evaluate the M44ed3E disk diffusion method to determine the *in vitro* susceptibility of *C. auris* to 10 μg amphotericin B, 5 μg caspofungin, and 25 μg fluconazole. For each isolate, the disk diffusion zone diameters were compared to the MICs determined by the M27ed4 broth microdilution method or by Etest for amphotericin B.

MATERIALS AND METHODS

We tested 347 clinical isolates of *C. auris* from various body sites (Table 1). Isolates were identified by matrix-assisted laser desorption ionization–time of flight (Bruker Daltonics) using a laboratory-developed database (8). Isolates were stored at −80°C in 20% glycerol until used.

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TABLE 1 *Candida auris* isolate data[a]

Antifungal	Zone diam cutoff, in mm	Susceptible isolates	Indeterminate isolates	Resistant isolates
Caspofungin	≤12	315 (91)		24 (7)
Fluconazole	≤12	48 (14)		267 (77)
Amphotericin B	≤17	191 (55)	66[b] (19)	66

[a]The isolates were tested using the disk diffusion method. Except for zone diameter cutoffs, data are presented as number (%).
[b]With the high number of major errors, this category should be indeterminate rather than resistant.

Reference antifungal susceptibility testing of *C. auris* for fluconazole and caspofungin was performed according to the broth microdilution method described in CLSI document M27ed4. The MIC endpoints were read visually after 24 h of incubation. Gradient diffusion susceptibility testing for amphotericin B was performed as previously described (4). Disk diffusion testing was performed as described in CLSI document M44ed3E. Briefly, 150-mm-diameter plates containing Mueller-Hinton agar (made in-house) supplemented with 2% glucose and methylene blue were used. A swab dipped in a cell suspension adjusted to 0.5 McFarland standard turbidity was used to inoculate the agar. The plates were subsequently incubated at 35°C and read at 24 h. The zone diameter endpoints were read at 100% growth inhibition with calipers. Amphotericin B (10 μg), caspofungin (5 μg), and fluconazole (25 μg) disks were purchased from Liofilchem, Inc. Isolates were tested in duplicate, and two different lot numbers were used for each antifungal tested. Isolates ATCC 22019 and ATCC 6258 were tested multiple times and always found to be in range, as defined in CLSI M60ed2 (9).

The CDC-developed breakpoints were used for this study: caspofungin resistance, MIC of ≥2 μg/ml; fluconazole resistance, MIC of ≥32 μg/ml; and amphotericin B resistance, MIC of ≥2 (1.5 by Etest) μg/ml (10). The breakpoints were developed based on MIC distribution, known molecular mechanisms of resistance, and pharmacokinetic/pharmacokinetic data based on a mouse model of infection (11). The breakpoints were used to determine categorical agreement between the two methods. Major errors were identified as a classification of resistant by the disk test and susceptible by broth microdilution. Very major errors were identified as a classification of susceptible by the disk diffusion test and resistant by broth microdilution.

RESULTS

Most isolates had a low MIC value to caspofungin. Figure 1 shows the relationship between the MIC determined using broth microdilution with ≥2 μg/ml as a breakpoint for resistance and zone diameters using 5-μg caspofungin disks. Selection of ≤12 mm as the zone diameter cutoff resulted in 98% categorical agreement between the results obtained by the two methods (339 of 347 isolates had concordant results). With this cutoff, 5 (1%) major and 3 (<1%) very major errors were observed.

Most of the isolates had a high MIC value to fluconazole. Figure 2 shows the relationship between broth microdilution MICs, with ≥32 μg/ml as the breakpoint for resistance and zone diameters using 25-μg fluconazole disks. Selection of ≤12-mm zone diameter cutoff resulted in 91% categorical agreement between the two methods (315 of 347 isolates were concordant). Using these parameters, 26 (7%) major errors but only 6 (2%) very major errors were observed.

Most of the isolates had a low MIC value to amphotericin B. Figure 3 shows the correlation between MICs using ≥1.5 μg/ml as the breakpoint for resistant isolates and 10-μg amphotericin B disk zone diameters. Selection of a ≤17-mm zone diameter as the cutoff resulted in 74% categorical agreement between the two methods: 257 of 347 isolates had concordant results with 88 (25%) major and 2 (<1%) very major errors observed. When the zone diameter cutoff was changed to ≤15 mm, the categorical agreement was increased to 82% with 45 (13%) major errors, but the very major errors increased to 16 (5%). When the zone diameter cutoff was increased to ≤20 mm, there were no very major errors, but 182 major (52%) errors were observed.

DISCUSSION

Although there are no established *Candida auris*-specific susceptibility breakpoints, the CDC has established tentative breakpoints based on MIC distributions, molecular mechanisms of resistance, and pharmacokinetic and pharmacodynamic values in a mouse model of infection (10). These tentative breakpoints were used here to define

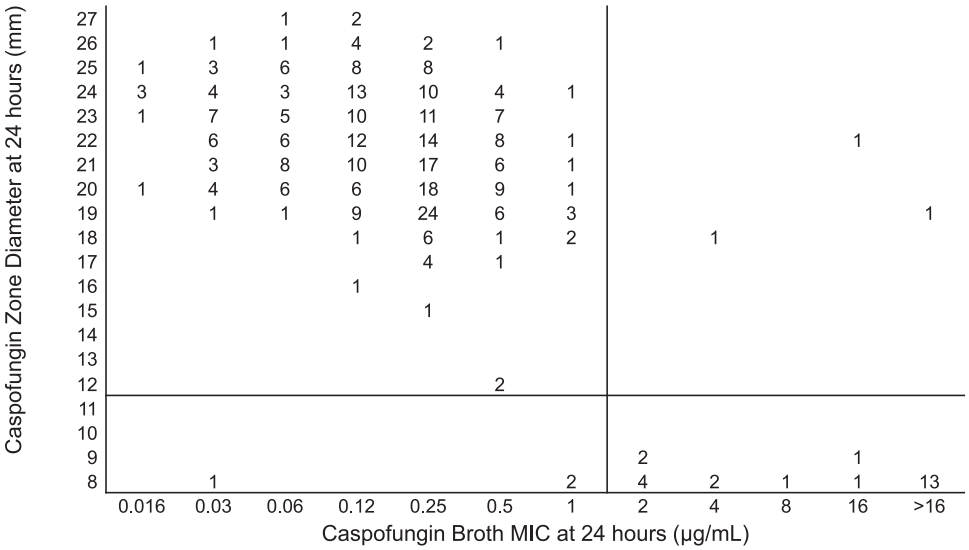

FIG 1 Zones of inhibition for 347 isolates of *C. auris* around 5-μg caspofungin disks on Mueller-Hinton methylene blue agar plotted against the 24-h MICs determined by the reference broth microdilution method. The numbers inside the graph indicate the number of isolates. The black horizontal line is the suggested breakpoint for the disk diffusion method.

zone diameter cutoffs for the disk diffusion method that distinguish between susceptible and resistant isolates for caspofungin and fluconazole and identified 75% of isolates susceptible to amphotericin B.

Selection of a ≥12-mm zone diameter cutoff for fluconazole and caspofungin generated highly concordant results between the broth microdilution and disk diffusion

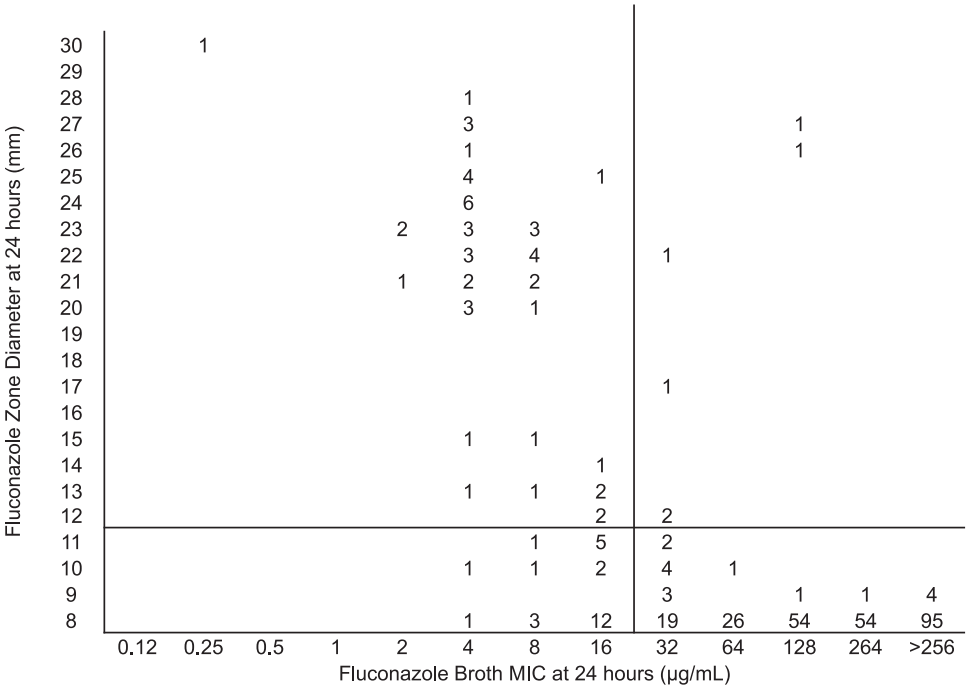

FIG 2 Zones of inhibition for 347 isolates of *C. auris* around 25-μg fluconazole disks on Mueller-Hinton methylene blue agar plotted against the 24-h MICs determined by the reference broth microdilution method. The numbers inside the graph indicate the number of isolates. The black horizontal line is the suggested breakpoint for the disk diffusion method.

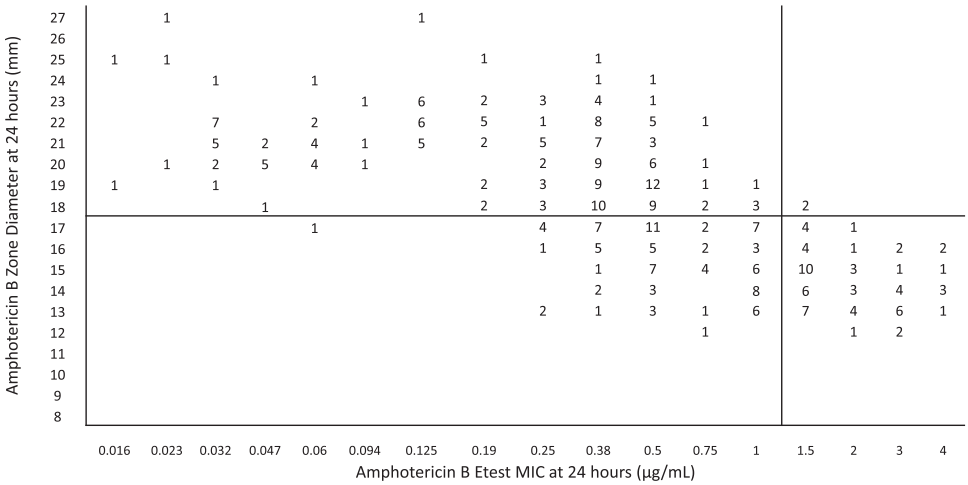

FIG 3 Zones of inhibition for 347 isolates of *C. auris* around 10-μg amphotericin B disks on Mueller-Hinton methylene blue agar plotted against the 24-h MICs determined by Etest. The numbers inside the graph indicate the number of isolates. The black horizontal line is the suggested breakpoint for the disk diffusion method. Zone diameters of ≤17 mm must be reported as indeterminate.

methods, with 91 and 98% categorical agreement, respectively. However, for amphotericin B it was difficult to establish a zone diameter that had an acceptable amount of both major and very major errors, most likely because the difference between resistant and susceptible isolates was within the testing margin of error for broth microdilution. One possible solution to this problem may be to select the largest zone diameter with an acceptable amount of very major errors and define it as the zone of susceptibility without defining a zone of resistance. Selection of a ≤17-mm zone diameter for amphotericin B generated <1% very major errors but 25% major errors. These numbers are unacceptable. However, if we use ≥18 mm as the cutoff for susceptible isolates, with no definition for resistant isolates, the categorical agreement is 99% for the single category. The caveat to this method is that 24% (the major errors) of the susceptible isolates will not be categorized as susceptible. Zone diameters of ≤17 mm must be reported as indeterminate.

Antifungal susceptibility testing is an important tool for guiding antifungal therapy for multidrug-resistant organisms. However, most methods of antifungal susceptibility testing are costly or require acquired expertise. The Clinical and Laboratory Standards Institute has developed a standard for disk diffusion testing of *Candida* species (12). This method could greatly facilitate testing in resource-limited settings, as disk diffusion has proven to be an economical and reliable option (13). This study expands the application of caspofungin, fluconazole, and amphotericin B disk diffusion tests to include *Candida auris*. Utilizing the disk diffusion method with Mueller-Hinton agar supplemented with glucose and methylene blue appears to be a useful alternative for determining *Candida auris* resistance to caspofungin and fluconazole and susceptibility to amphotericin B.

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