Caspofungin Disk Diffusion Breakpoints and Quality Control

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Interpretive disk diffusion breakpoints for caspofungin are proposed by evaluating 762 isolates of Candida spp., representing 10 different species, were provided by Merck & Co. The isolates were obtained as part of the caspofungin clinical trials. Standardized broth microdilution reference tests were compared to the zone diameters observed with 5-μg caspofungin disks produced by two different disk manufacturers. Disk diffusion breakpoints of ≥11 mm for susceptible are proposed. Compared to results from MIC testing, these zone diameters produced error rates that were ≤0.3% for all categories. In addition, an eight-laboratory disk diffusion quality control (QC) study was performed, and QC ranges are proposed for the four QC strains recommended by the CLSI.

The need for the early diagnosis and treatment of fungal infections has been well documented (13, 16, 19). Caspofungin, an FDA-approved antifungal agent of the echinocandin family, has been shown to be effective in the treatment of a variety of fungal infections, including those caused by Candida species (1, 2). It is the first drug of its class to be approved for use by the FDA. It also has been shown to be effective in vitro against isolates with decreased susceptibility to amphotericin B or to the FDA. It also has been shown to be effective in vitro against (1, 2). It is the first drug of its class to be approved for use by @clinmicroinst.com.

MATERIALS AND METHODS

Isolates. A total of 762 strains of Candida spp., representing 10 different species, were obtained during the caspofungin clinical trials. The species included 530 C. albicans, 72 C. glabrata, 27 C. guilliermondii, 1 C. krusei, 18 C. kefyr, 2 C. lipolytica, 2 C. lusitaniae, 52 C. parapsilosis, 1 C. neoformans, and 57 C. tropicalis strains. Prior to being tested, all strains were passaged at least twice on Sabouraud dextrose agar to ensure viability and purity.

Antimicrobial agent. Caspofungin (lot no. REK0070) was provided as a standardized powder by Merck & Co. Fluconazole powder (lot no. 02FLU-010-00) was obtained from Pfizer Pharmaceuticals and was used as the control drug for all testing. Stock solutions were prepared in water, and serial twofold dilutions were prepared as recommended by the CLSI (7).

Broth microdilution tests. The reference broth microdilution methodology described by the CLSI (7) was utilized as the reference method in this study. The medium was RPMI 1640 broth (lot no. 014K310; Sigma) supplemented with 1-glutamine (lot no. 94H01931; Sigma) and buffered to pH 7.0 with morpholinepropanesulfonic acid (Sigma) in a sterile phosphate buffer (lot no. 12SK0010; Sigma). The concentrations of caspofungin tested were serial twofold dilutions ranging from 0.004 to 128 μg/ml. The fluconazole concentrations tested ranged from 0.008 to 256 μg/ml. After 24 h of incubation at 35°C, MICs were read as the lowest concentration that showed a marked decrease in the density of growth (approximately 50% inhibition, as judged by the unaided eye). Colony counts were performed on the suspension in the growth control well from randomly selected tests, and the results ranged from 5 × 102 to 3.7 × 104 CFU/ml.

Disk diffusion test. Disk diffusion tests were performed by following the procedure outlined by the CLSI for yeasts (5) using Mueller-Hinton agar supplemented with 2% glucose and 0.5% methylene blue (MHA-GMB). Caspofungin-impregnated paper disks were provided by Merck. The disks were prepared to contain 5 μg of caspofungin and were manufactured by BBL (lot no. 6150854) and Oxoid (lot no. 372738). Twenty-five-microliter fluconazole disks (lot no. 6093051; BBL) were used as the internal control. Inhibitory zone diameters were measured after 24 h of incubation, at which point there was a sharp decline in the amount of growth (approximately 50% inhibition, as judged by the unaided eye).

Daily QC. Daily QC testing was performed using the CLSI-recommended strains of C. parapsilosis ATCC 22019 and C. krusei ATCC 6258 for the MIC portion of the study and C. parapsilosis ATCC 22019, C. krusei ATCC 6258, C. albicans ATCC 90028, and C. tropicalis ATCC 750 for the disk diffusion portion of the study. The fluconazole MIC and disk diffusion QC ranges utilized in this study were those recommended by the CLSI (6, 8).

Disk diffusion QC ranges. The MIC and disk diffusion QC ranges were tested in eight laboratories collaboratively. The MICs were tested using the CLSI-recommended strain of C. parapsilosis ATCC 22019, C. krusei ATCC 6258, C. albicans ATCC 90028, and C. tropicalis ATCC 750 for the MIC portion of the study and C. parapsilosis ATCC 22019, C. krusei ATCC 6258, C. albicans ATCC 90028, and C. tropicalis ATCC 750 for the disk diffusion portion of the study. The fluconazole MIC and disk diffusion QC ranges utilized in this study were those recommended by the CLSI (6, 8).

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control. Readings were made at 24 h of incubation, as recommended by the CLSI (5).

The eight laboratories that participated in this study were the following: S. Brown, Clinical Microbiology Institute, Wilsonville, OR; D. Diekema, University of Iowa, Iowa City; M. Ghannoum, Case Western Reserve University, Cleveland, OH; G. Hall, Cleveland Clinic, OH; D. Hardy, University of Rochester Medical Center, NY; C. Knapp, TREK Diagnostic Systems, Cleveland, OH; L. Ostrosky-Zeichner, University of Texas-Houston Medical School; and R. Rennie, University of Alberta Hospital, Edmonton, Alberta, Canada. QC ranges are proposed based upon the method of Gavan et al. (10).

RESULTS AND DISCUSSION

Disk diffusion breakpoints. The MIC and disk diffusion results obtained as part of this investigation are presented in Fig. 1. Disk diffusion zone diameters were recorded for all strains using both BBL and Oxoid prepared disks (i.e., two disks per strain). There was good correlation between the zone diameters observed with the disks provided by the two different manufacturers, in that 99.1% of the values observed were ±3 mm of the mode. Fully 83.5% of the values observed were ±1 mm of the mode. The average zone of inhibition observed with the BBL disks was 20.47 mm; an average of 20.95 mm was observed with the Oxoid disks. The differences in zones of inhibition between the two manufacturers were consistently small for all species under study.

MIC breakpoints of ≤2 μg/ml for susceptible and >2 μg/ml for nonsusceptible have been previously described by Pfaller et al. (submitted) and approved by the CLSI. Figure 1 shows the 24-h MIC reading using these breakpoints. All of the MIC and disk diffusion values were able to be read at 24 h, and none required additional incubation.

Disk diffusion breakpoints of ≤11 mm for susceptible are proposed. These breakpoints are proposed on the basis of the combined data from both the BBL and Oxoid disks (i.e., two disks per isolate). The error rates are calculated for the combined disk data but not for the individual disk manufacturers. The proposed disk diffusion breakpoints produced 0 very major errors and 4 (0.3%) major errors. All error rates were well within the acceptable limits proposed by the CLSI (4). Unfortunately, isolates that were truly resistant to caspofungin were rarely encountered during the caspofungin clinical trials. The lack of these isolates severely limits our ability to propose reliable MIC or disk diffusion breakpoints. As with all other drugs, the accuracy of these breakpoints must be continually monitored in order to ensure that they continue to be effective.

![FIG. 1. Caspofungin MIC at the 24-h endpoint versus caspofungin zone diameters at 24 h using 5-μg BBL and Oxoid disks combined (two disks per strain). The MIC breakpoints were ≤2 μg/ml for susceptible and >2 μg/ml for nonsusceptible. R, resistant; S, susceptible; m, minor; M, major; VM, very major; n.a., not applicable.](http://jcm.asm.org/)

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* Recommended QC ranges are in boldface.

* Percentage of results that fall within the recommended range.
in the event that organisms with decreased susceptibility are encountered.

**QC ranges.** QC ranges are proposed according to the method of Gavan et al. (10), which is based upon the all-laboratory median plus or minus half the range of medians for each laboratory. The two lots of caspofungin disks gave essentially identical results, in that the eight laboratory means for the two disks were within 0.1 mm (data not shown). The proposed QC limits include an 8- to 10-mm range of zone diameters for each of the control strains (Table 1).

**Conclusions.** Disk diffusion breakpoints of ≥11 mm of inhibition for susceptible and ≤10 mm of inhibition for nonsusceptible and the proposed QC limits have now been approved by the CLSI subcommittee for antifungal susceptibility testing and will be presented in an upcoming printing of their documents. It is hoped that the addition of disk diffusion methodology will place antifungal susceptibility testing within the grasp of even the smallest clinical laboratory.

**ACKNOWLEDGMENT**

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**REFERENCES**


