Evaluation of the Etest Method Using Mueller-Hinton Agar with Glucose and Methylene Blue for Determining Amphotericin B MICs for 4,936 Clinical Isolates of Candida Species

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The Etest stable agar gradient MIC method (AB BIODISK, Solna, Sweden) has been established as an excellent method for testing the susceptibilities of 4,936 clinical isolates of Candida spp. to amphotericin B. Previously, we have confirmed this work and have shown that RPMI agar with 2% glucose (RPG) was superior to other media, including antibiotic medium 3, Casitone, and unsupplemented Mueller-Hinton agar (MHA), for testing amphotericin B against Candida spp (7). Barry et al. (2) have shown that MHA supplemented with 2% glucose and methylene blue (0.5 μg/ml) (MH-GMB) supported the growth of essentially all species of Candida and was superior to RPG for fluconazole disk diffusion testing. The National Committee for Clinical Laboratory Standards (NCCLS) has adopted MH-GMB as the medium of choice for disk diffusion testing of Candida spp. (6). As both the disk diffusion and Etest methods are agar based, it is important to determine whether both methods can be performed on the same agar medium. Although we have shown that MH-GMB works well for determining MICs of fluconazole and voriconazole by the Etest (10), similar data for amphotericin B have not been published. In the present study we compared MICs obtained with the Etest using MH-GMB with those obtained with RPG by testing the susceptibilities of 4,936 clinical isolates of Candida spp. to amphotericin B.

The Etest results with MH-GMB correlated well with those with RPG. Overall agreement was 92.9%, and agreements for individual species were as follows: C. lusitaniae, 98.1%; C. albicans, 95.1%; C. glabrata, 94.3%; C. krusei, 91.6%; C. parapsilosis, 86.6%; and C. tropicalis, 86.4%. The Etest method using MH-GMB appears to be a useful method for determining amphotericin B susceptibilities of Candida species.

MATERIALS AND METHODS

Organisms. A total of 4,936 clinical isolates of Candida spp. were tested on both RPG and MH-GMB. The collection included 2,728 C. albicans isolates, 722 C. glabrata isolates, 666 C. parapsilosis isolates, 528 C. tropicalis isolates, 143 C. krusei isolates, 54 C. lusitaniae isolates, 39 C. guilliermondii isolates, 17 C. pelliculosa isolates, 15 C. kefyr isolates, 11 C. rugosa isolates, 5 C. dubliniensis isolates, 4 C. zeylanoides isolates, 3 C. lipolytica isolates, and 1 C. famata isolate. The members of this collection were all recent clinical isolates from geographically diverse medical centers throughout the world (11). The majority were isolated from blood or normally sterile body fluids (11). The isolates were identified by standard methods (3) and were stored as suspensions in water at ambient temperature until used in the study. Prior to testing, each isolate was subcultured at least twice onto potato dextrose agar (Remel, Lenexa, Kans.) to ensure purity and viability.

Susceptibility testing. Etest strips for amphotericin B were purchased from AB BIODISK. MICs were determined by Etest as described previously (7) with RPMI (Remel) and an inoculum suspension adjusted to the turbidity of a 0.5 McFarland standard (~10⁶ cells/ml). In addition, each isolate was tested on MH-GMB. The MICs of amphotericin B on both RPG and MH-GMB were read after 48 h of incubation at 35°C and were determined to be at 100% inhibition of growth where the border of the elliptical inhibition zone intersected the scale of the strip edge (7, 9, 12). Quality control was ensured by testing the NCCLS-recommended strains C. krusei ATCC 6258 and C. parapsilosis ATCC 22019 (1, 5).

Analysis of results. The Etest MICs of amphotericin B determined on MH-GMB were compared to those determined on RPG. Since the Etest scale has a continuous gradient of concentrations, the MICs between twofold dilutions were raised to the next twofold level for comparison (8). Discrepancies between MICs of no more than two dilutions were used to calculate the percent agreement.

RESULTS AND DISCUSSION

Table 1 summarizes the in vitro susceptibilities of 4,936 Candida isolates to amphotericin B as determined by Etest using RPG and MH-GMB. The MICs obtained with RPG are consistent with those reported previously for the individual Candida spp. (9). MICs obtained with MH-GMB were generally twofold lower than those with RPG.

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Overall, 92.9% of the amphotericin B MICs obtained by the Etest in MH-GMB were within ±2 dilutions of those obtained in RPG (Table 1). The agreement was >90% for all species, with the exception of C. parapsilosis (86.6%) and C. tropicalis (86.4%). When a discrepancy was observed between the results obtained with the two media, the MIC obtained with MH-GMB was always lower. The modal MICs obtained with both media ranged from 0.25 to 1 μg/ml for all species, with the exception of C. krusei, where the MICs tended to be elevated (2 to 4 μg/ml) with both RPG and MH-GMB.

The results of this study provide the first documentation of the applicability of MH-GMB for determining the in vitro susceptibilities of Candida spp. to amphotericin B by using the Etest stable agar gradient MIC method. As in previous studies (10), we found that MHA supplemented with glucose (2%) and methylene blue (0.5 μg/ml) supported optimal growth of all species of Candida and provided excellent agreement with MICs obtained on RPG. As was the case with fluconazole and voriconazole (11), the elliptical zones obtained on MH-GMB were sharp and easy to read. As with RPG, amphotericin B MICs obtained with the Etest in MH-GMB encompassed a much broader MIC range (0.002 to >32 μg/ml) than those obtained with the reference broth microdilution method (0.12 to 4 μg/ml) (data not shown).

In summary, we have provided the first evidence of the ability of the Etest using MH-GMB to generate amphotericin B MIC data that are comparable to those obtained with RPG. The importance of this finding is that one may use the same agar medium to perform disk testing of fluconazole and voriconazole and to obtain amphotericin B MICs by using the Etest. The use of agar-based technology for performing antifungal susceptibility testing of Candida provides a simple and flexible option for laboratories that wish to test only a few agents selectively and do not want to invest in broth microdilution testing.

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REFERENCES


