

DIFFUSION, AND MICRODILUTION METHODS FOR ANTIFUNGAL SUSCEPTIBILITY TESTING OF CANDIDA SPECIES

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Agar-based susceptibility testing methods have been a focus of interest to many researchers and include the classical disk diffusion methods and the E-test method. Those tests are very attractive due to their simplicity, reproducibility, and lack of requirements for specialized equipment. The correlation between the microdilution, E-test, and disk diffusion methods was determined for amphotericin B, itraconazole and fluconazole against *Candida* spp. The minimal inhibitory concentration (MIC) of those antifungal agents was established for a total of 70 *Candida* spp. isolates from colonization and infection using the microdilution method based on the Clinical and Laboratory Standards Institute (CLSI) M27-A2 method. The M-44A (disk diffusion) and E-test methods for agar-based testing on Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg/ml of methylene blue were also assayed. MIC and inhibitory zone diameters at the prominent growth reduction endpoint were recorded at 48 h. *Candida* isolates identified included *C. albicans* (n = 27), *C. tropicalis* (n = 17), *C. glabrata* (n = 16), *C. parapsilosis* (n = 8) and *C. lusitanae* (n = 2). Non-*Candida albicans* *Candida* species showed significantly higher MICs ($p=0.0141$) for the three antifungal agents when compared to *C. albicans* isolates. The overall concordance (based on the MIC value obtained within two dilutions) between the E-test and microdilution was 90% for amphotericin B, 67.14% to itraconazole and 58.5% to fluconazole. Considering the breakpoint, the agreement between the disk diffusion and microdilution methods was 71% to itraconazole and 67% to fluconazole. The E-test and microdilution MICs shown good concordance and interpretative agreement. The disk diffusion zone diameters are highly reproducible and correlate well with the microdilution method, making agar-based methods a viable alternative to microdilution for susceptibility testing. Although there is little data on agar-based tests for itraconazole and amphotericin B, results for E-test and

disk diffusion methods provide a good correlation with the microdilution method. However, further research must still be carried out to ensure the standardisation of other antifungal agents.