

Comparison of EUCAST and CLSI Broth Microdilution Methods for the Susceptibility Testing of 10 Systemically Active Antifungal Agents against Candida spp.

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AMENDED ABSTRACT

Background: The need for reproducible, clinically relevant antifungal susceptibility testing of Candida spp. has been promoted by the increasing number of infections, the expanding use of new and established antifungal agents, and recognition of antifungal resistance or mechanism as an important clinical problem.

Methods: Due to the international importance of these two methods for surveillance of antifungal resistance there is a need to continue the process of harmonization for the testing of other new and established antifungal agents.

Results: C. albicans (CA; 114 isolates), C. glabrata (CG, 73 isolates), C. parapsilosis (CP, 76 isolates), C. tropicalis (CT, 60 isolates) and C. krusei (CK, 34 isolates) were concurrently tested by both methods.

Conclusions: These results suggest that the CLSI and EUCAST methods provide comparable results for a wide range of antifungal agents and may be used effectively in resistance surveillance and clinical testing of the five most common species of Candida.

METHODS

Organisms. A total of 357 clinical isolates of Candida spp. were selected from global surveillance collections to represent both WT and non-WT MIC results for the azoles (12.6% of fluconazole and micafungin results were non-WT).

Antifungal susceptibility testing. All isolates were tested for in vitro susceptibility to amphotericin B, flucytosine, anidulafungin, caspofungin, micafungin, fluconazole, isavuconazole, itraconazole, posaconazole, voriconazole [VRC], and isavuconazole [ISA] against a collection of 357 clinical isolates of Candida spp.

CLSI BMD testing was performed exactly as outlined in the CLSI document M27-A3 by using round-bottom trays and RPMI 1640 medium with 0.2% glucose, inocula of 0.5 x 10^5 to 2.5 x 10^5 cells/ml, and incubation at 35°C.

EUCAST BMD testing was performed exactly as outlined in document EDef 7.2 by using flat-bottom trays and RPMI1640 medium with 2.0% glucose, inocula of 0.5x10^5 to 2.5 x 10^5 cells/ml, and incubation at 35°C.

Quality control. Quality control was performed as recommended in CLSI document M27-A3 using C. krusei/ATCC 6258 and C. parapsilosis ATCC 22019.

Analysis of results. The MIC results for each triazole obtained with the EUCAST method were compared to those of the CLSI BMD method. High off-scale BMD MIC results were converted to the next highest concentration and low off-scale MIC results were left unchanged.

RESULTS

- The overall EA between the EUCAST and CLSI methods ranged from 78.9% (posaconazole) to 99.6% (flucytosine).
MIC values generated by the CLSI method were higher than those obtained by the EUCAST method for 60% (fluconazole) to 100.0% (amphotericin B, flucytosine, anidulafungin, micafungin, itraconazole) of results for all agents with the exception of caspofungin where 15 of 16 discrepancies (93.8%) were due to EUCAST MICs that were higher than CLSI MIC results.
The largest number of discrepancies observed with the EUCAST and CLSI comparison occurred with C. glabrata tested against anidulafungin (40 discrepant results), with C. parapsilosis tested against posaconazole (25 discrepant results) and with C. albicans and C. parapsilosis tested against itraconazole (21 and 22 discrepant results, respectively).

Table 1. Epidemiological cutoff values (ECVs) for systemically active antifungal agents and Candida spp. determined by 24-h CLSI broth microdilution methods. Table with columns for Species, Antifungal agent, ECV (µg/ml) WT and non-WT, and Quality Control (QC) results.

Table 2. In vitro susceptibilities of Candida spp. to 10 systemically active antifungal agents as determined by the CLSI and EUCAST broth microdilution methods. Detailed table with columns for Antifungal agent, Species (no. tested), Test method, MIC (µg/ml) Range and Mode, and EA (%).

Table 3. Categorical agreement between the results of the CLSI and EUCAST broth microdilution methods for nine systemically active antifungal agents and Candida spp. using epidemiological cutoff values. Summary table with columns for Species (no. tested), Antifungal agent (ECV µg/ml), Test method, and agreement metrics (sECV, >ECV, CA (%), VM, M).

CONCLUSIONS

- Overall, the EUCAST MIC results tended to be ≤1 2-fold dilution lower than those determined by the CLSI method for most agents and species with the exception of caspofungin where the EUCAST MIC results tended to be 1 dilution higher than the CLSI results.
The VM and M discrepancy rates may seem higher than normally seen in methods comparison studies due in part to the use of ECVs to assess the CA where only two categories, WT and non-WT, were employed as opposed to the use of clinical breakpoints with other comparisons where the susceptible and resistant categories are buffered by the intermediate or susceptible dose dependent categories.

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INTRODUCTION

The Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Antifungal Susceptibility Tests has standardized the broth microdilution (BMD) reference method for testing amphotericin B, flucytosine, the triazoles (including the investigational agent isavuconazole), and the echinocandins, against Candida spp.; and most recently has validated 24-h MIC readings for all agents and has developed new species-specific clinical breakpoints (CBPs) and epidemiological cutoff values (ECVs) for these agents and several species of Candida.

In the present study, we examine the essential agreement (EA; MIC ± 2 log2 dilutions) between the two methods for testing 10 antifungal agents (amphotericin B, flucytosine, anidulafungin, caspofungin, micafungin, fluconazole, isavuconazole, itraconazole, posaconazole, and voriconazole) against a collection of 357 clinical isolates of Candida selected to provide both WT and non-WT MIC phenotypes (using CLSI methods and ECVs) for most agents and species.