Comparison of Broth Macrodilution, Broth Microdilution, and E Test Antifungal Susceptibility Tests for Fluconazole

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A comparison of the E test, the broth microdilution test, and the reference broth macrodilution susceptibility test of the National Committee for Clinical Laboratory Standards for fluconazole susceptibility testing was performed with 238 clinical isolates of Candida species and Torulopsis (Candida) glabrata. An 80% inhibition endpoint MIC was determined by the reference broth macrodilution method after 48 h of incubation. The MICs obtained by the two study methods were read after 24 and 48 h of incubation. Overall, excellent agreement within 2 doubling dilutions was obtained between the broth microdilution and the broth macrodilution methods for the combined results for all species at both 24 h (93%) and 48 h (94%). The correlation of 24-h MIC endpoints between the E test and the broth macrodilution methods was 37% for T. glabrata, 56% for Candida tropicalis, 93% for Candida albicans, and 96% for other Candida species. The percent agreement at 48 h ranged from 34% for T. glabrata to 97% for Candida species other than C. albicans and C. tropicalis. These initial results support the further evaluation of the E test as an alternative method for fluconazole susceptibility testing of Candida species.

The incidence of serious yeast infections has increased because of the widespread use of broad-spectrum antibacterial agents in severely immunocompromised patients and patients with AIDS (1, 2, 15). The treatment of these infections with the less toxic azole agents has created an increased demand for in vitro antifungal susceptibility testing.

Over the past decade, numerous susceptibility test methods were evaluated for the testing of Candida and other yeast isolates. The major variables of antifungal susceptibility testing were the composition and the pH of the test medium, inoculum size, incubation temperature and duration, and endpoint definition (5, 9, 11, 12). As a result of collaborative studies on the factors responsible for test variation, the National Committee for Clinical Laboratory Standards (NCCLS) has proposed a standardized reference broth macrodilution method for the antifungal susceptibility testing of yeasts (7).

The determination of the endpoint for the azole antifungal agents remains a problem because partial inhibition of growth often occurs over a range of drug concentrations, especially with Candida albicans (8). The proposed NCCLS broth macrodilution method addresses this problem by defining the MIC endpoint for fluconazole and other azole compounds as the lowest concentration of drug that inhibits growth by 80% relative to the growth of the control, as measured by turbidity (7).

A broth microdilution method performed according to NCCLS guidelines demonstrated good agreement with the reference broth macrodilution method (10, 14). A recent study comparing colorimetric broth microdilution testing of fluconazole with reference broth macrodilution and broth microdilution methods demonstrated excellent agreement among the three methods (10). In addition, agar dilution was an acceptable alternative to the broth microdilution method for fluconazole susceptibility testing when a standardized inoculum and a defined medium were used (14).

Although standardized broth dilution methods for antifungal susceptibility testing are available, easier testing procedures are desirable. Recently, the E test (AB Biodisk, Solna, Sweden) has been introduced as a means of producing an accurate quantitative MIC result by an agar diffusion format (13). Although the E test has been successfully applied to the susceptibility testing of bacteria, experience with antifungal testing is limited. This report describes a comparative evaluation of the E test versus broth macrodilution and broth microdilution susceptibility testing of fluconazole with C. albicans, C. tropicalis, other Candida species, and Torulopsis (Candida) glabrata. The MICs obtained by the proposed NCCLS reference broth macrodilution method (read at 48 h) were compared with those of a broth microdilution method performed according to NCCLS guidelines and the E test agar diffusion method. The purpose of the study was to provide additional data comparing broth microdilution and agar diffusion antifungal susceptibility tests for fluconazole with the reference broth macrodilution method.

MATERIALS AND METHODS

Two hundred thirty-eight yeast isolates were tested for their susceptibilities to fluconazole by the broth macrodilution, broth microdilution, and E test (agar diffusion) methods. Multiple medical centers contributed the yeast isolate collection, which included C. albicans (n = 122), Candida krusei (n = 4), Candida lusitaniae (n = 3), Candida parapsilosis (n = 23), C. tropicalis (n = 45), and T. glabrata (n = 41). All isolates were recovered from clinical specimens (primarily blood and body fluids) by standard culture methods (6). Each isolate was identified by using the API 20C kit (Analytab Products, Plainview, N.Y.) and was then stored as a suspension in water at ambient temperature until used in the study. Prior to testing, each isolate was passaged at least twice on Sabouraud dextrose.
TABLE 1. In vitro activity of fluconazole determined by NCCLS broth macrodilution, broth microdilution, and E test methods

<table>
<thead>
<tr>
<th>Species (no. tested)</th>
<th>Test method</th>
<th>MIC (µg/ml) at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Candida albicans (122)</td>
<td>Macrodiution</td>
<td>0.12–2.0</td>
</tr>
<tr>
<td></td>
<td>Microdiution</td>
<td>0.12–2.0</td>
</tr>
<tr>
<td></td>
<td>E test</td>
<td></td>
</tr>
<tr>
<td>Candida krusei (4)</td>
<td>Macrodiution</td>
<td>16–32</td>
</tr>
<tr>
<td></td>
<td>Microdiution</td>
<td>8–32</td>
</tr>
<tr>
<td>Candida lusitaniae (3)</td>
<td>Macrodiution</td>
<td>2.0–2.0</td>
</tr>
<tr>
<td></td>
<td>Microdiution</td>
<td>1.0–2.0</td>
</tr>
<tr>
<td>Candida parapsilosis (23)</td>
<td>Macrodiution</td>
<td>0.25–2.0</td>
</tr>
<tr>
<td></td>
<td>Microdiution</td>
<td>0.12–2.0</td>
</tr>
<tr>
<td>Candida tropicalis (45)</td>
<td>Macrodiution</td>
<td>0.12–&gt;512</td>
</tr>
<tr>
<td></td>
<td>Microdiution</td>
<td>0.03–2.0</td>
</tr>
<tr>
<td></td>
<td>E test</td>
<td></td>
</tr>
<tr>
<td>Torulopsis glabrata (41)</td>
<td>Macrodiution</td>
<td>2.0–256</td>
</tr>
<tr>
<td></td>
<td>Microdiution</td>
<td>2.0–128</td>
</tr>
<tr>
<td></td>
<td>E test</td>
<td>0.12–&gt;256</td>
</tr>
</tbody>
</table>

agar (Prepared Media Laboratories, Tualatin, Oreg.) at 35°C for 24 to 48 h.

Fluconazole was obtained as a reagent-grade powder from Roerig Division of Pfizer Pharmaceuticals (New York, N.Y.). Microdilution trays containing serial dilutions of fluconazole were prepared by Alamar Biosciences, Inc. (Sacramento, Calif.), dried, and stored at ambient temperature until used in the study. Fluconazole was dissolved in sterile water, 10× concentrations were dispensed in 12-by-75-mm clear plastic tubes, and the tubes were held at −40°C until they were used in the study. The E test strips contained a gradient of 0.064 to 256 µg of fluconazole per ml and were stored at −20°C until use.

The broth microdilution and broth macrodilution tests were performed according to the NCCLS guidelines (7) by using the spectrophotometric method of inoculum preparation, an inoculum concentration of 0.5 × 10⁶ to 2.5 × 10⁵ cells per ml, and RPMI 1640 medium buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (American Biorganics, North Tonowanda, N.Y.). The wells of the broth microdilution trays were reconstituted by the addition of 100 µl of an inoculum suspension which yielded a final concentration of 0.16 to 512 µg of fluconazole per ml. An inoculum suspension (0.9 ml) was added to the broth macrodilution tubes to yield a final concentration range of 0.0625 to 256 µg of fluconazole per ml. The trays and tubes were incubated at 35°C. The microtitre trays were read at 24 and 48 h. The 48-h MIC endpoint was determined according to NCCLS recommendations (80% reduction in turbidity).

The E test was performed by inoculation of a 150-mm petri plate containing 60 ml of RPMI 1640 agar with a nontoxic swab by using a cell suspension adjusted to a 0.5 McFarland standard. After allowing excess moisture to be absorbed by the agar, the E test strips were applied. The plates were incubated at 35°C and were read at 24 and 48 h.

Quality control was performed by testing the following strains according to the recommendations of NCCLS Document M27-P (7): C. albicans ATCC 90028, C. albicans ATCC 90029, C. parapsilosis ATCC 90018, and T. glabrata ATCC 90030. Tests performed on at least eight different occasions with each of the strains from the American Type Culture Collection (ATCC) were within the control limits stated for fluconazole (data not shown).

A total of five MIC determinations was obtained for each isolate; two broth microdilution MICs and the E test were read at 24 and 48 h and one broth macrodilution MIC was read at 48 h. Both on-scale and off-scale results were included in the data. The high off-scale MICs were converted to the next higher concentration, and the low off-scale results were left unchanged. Discrepancies between MIC endpoint determinations of no more than 2 dilutions (two tubes or wells) were used to calculate the percent agreement. Because of insufficient growth at 24 h, data were not obtained for one strain of C. albicans (broth microdilution and E test), three strains of C. parapsilosis (broth microdilution), and five strains of T. glabrata (E test). Data for these isolates were not included in the final evaluation of the data.

RESULTS AND DISCUSSION

Table 1 summarizes the in vitro susceptibilities of 238 yeast isolates to fluconazole as measured by the broth macrodilution, broth microdilution, and E test (agar diffusion) methods. The data are reported as the concentrations of fluconazole necessary to inhibit 50% (MIC₅₀) and 90% (MIC₉₀) of the isolates tested. After 24 h of incubation, the MIC₅₀ results were comparable (± two dilutions) to the MICs obtained by the reference method at 48 h. The MIC₉₀s obtained by the test methods at 24 h were generally two to fourfold greater than the MIC₅₀s. After 48 h of incubation, the MIC₅₀s and MIC₉₀s were generally two- to fourfold greater than the comparable values obtained at 24 h. A large increase in the MIC₉₀ read at 48 h occurred with C. albicans and C. tropicalis, as measured by the
The present study confirmed the results of previous comparisons between the broth microdilution and the reference broth macrodilution methods for fluconazole testing when the tests are performed according to NCCLS recommendations (3, 4, 10). A large collaborative study performed by Espinel-Ingroff et al. (4) demonstrated excellent agreement between the two methods for fluconazole susceptibility testing at 24 h (90%) and poorer agreement at 48 h (77%). Pfaller et al. (10) obtained 94% agreement at 24 h between the NCCLS reference broth macrodilution method and two broth microdilution methods (turbidimetric and colorimetric). The agreement at 48 h was slightly lower for the colorimetric (91%) and turbidimetric (93%) methods. We found 93% agreement at 24 h and 94% agreement at 48 h between the reference broth macrodilution method and the broth microdilution procedure. These results further support the observation that the broth microdilution method is a comparable alternative to the NCCLS reference broth macrodilution method for fluconazole susceptibility testing of yeasts (4, 10).

This is the first reported comparison between the NCCLS reference broth macrodilution, broth microdilution, and E test (agar diffusion) methods for fluconazole susceptibility testing of Candida species and T. glabrata. The E test is based on the diffusion of a continuous concentration gradient of drug from a plastic strip into an agar medium and is comparable to both broth microdilution and disk diffusion tests used for antifungal susceptibility testing (13). The simplicity and familiarity of the E test methodology to personnel in most clinical microbiology laboratories makes it a potentially useful method for the azole susceptibility testing of yeasts.

The results of the present study demonstrate an excellent level of agreement at 24 h between the E test and the NCCLS reference broth macrodilution method as well as a broth microdilution method for some Candida species. The level of agreement between the E test and the broth macrodilution method was approximately 90% for Candida species (C. parapsilosis, C. krusei, and C. lusitaniae) and C. albicans. There was a poor correlation between the MICs obtained by the E test and broth macrodilution method for C. tropicalis (56%) and T. glabrata (37%). The overall agreement of MICs at 48 h was only 71%, in large part because of the poor comparison of values with T. glabrata (34%) and C. tropicalis (67%). In general, the MICs obtained by the E test at 24 and 48 h tended to be greater than those obtained by the broth macrodilution and broth microdilution methods for C. albicans and were less than those obtained by the broth macrodilution and broth microdilution methods for C. tropicalis and T. glabrata. The reasons for these discrepancies are unclear.

Overall, good agreement (84%) was obtained between the E test and the broth dilution methods for the fluconazole susceptibility testing of Candida isolates when endpoint determinations were recorded after 24 h of incubation. The preliminary data presented here support the continued evaluation of the E test as an alternative method for the azole susceptibility testing of Candida species.

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REFERENCES


