JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2001, p. 3952–3954 Vol. 39, No. 11
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.11.3952–3954.2001
Copyright © 2001, American Society for Microbiology. All Rights Reserved.

Evaluation of Etest Method for Determining Posaconazole MICs for 314 Clinical Isolates of *Candida* Species

M. A. PFALLER,[1]* S. A. MESSER,[1] K. MILLS,[2] A. BOLMSTRÖM,[2] AND R. N. JONES[1,3]†

CAST Laboratories and Department of Pathology, University of Iowa College of Medicine, Iowa City,[1] and The JONES Group, North Liberty,[3] Iowa, and AB BIODISK, Solna, Sweden[2]

Received 11 June 2001/Returned for modification 27 July 2001/Accepted 12 August 2001

The performance of the Etest for posaconazole (SCH 56592) susceptibility testing of 314 isolates of *Candida* spp. was assessed against the National Committee for Clinical Laboratory Standards (NCCLS) microdilution broth method. The NCCLS method employed RPMI 1640 broth medium, and MICs were read after incubation for 48 h at 35°C. MICs were determined by Etest for all 314 isolates with RPMI agar containing 2% glucose (RPG agar) and were read after incubation for 48 h at 35°C. The *Candida* isolates included *C. albicans* (*n* = 174), *C. glabrata* (*n* = 57), *C. tropicalis* (*n* = 31), *C. parapsilosis* (*n* = 39), *C. krusei* (*n* = 5), *C. guilliermondii* (*n* = 6), and *C. lusitaniae* (*n* = 2). The Etest results correlated well with reference MICs. Overall agreement was 95%, and agreements for individual species were as follows: *C. krusei*, 100%; *C. albicans*, 98%; *C. tropicalis*, 97%; *C. glabrata*, 93%; *C. parapsilosis*, 85%; *C. guilliermondii*, 83%; and *C. lusitaniae*, 50%. The problem of trailing end points was minimized with RPG agar, and good agreement with broth dilution MICs was obtained when discernible growth within an established ellipse was ignored. The Etest method using RPG agar appears to be a useful method for determining posaconazole susceptibilities of *Candida* species.

Agar-based methods for antimicrobial susceptibility testing include agar dilution, disk diffusion, and the Etest and are used widely in clinical laboratories due to flexibility and ease of performance (9). The Etest stable agar gradient method has been shown to provide reference quality MIC determinations for a variety of pathogens and antimicrobial agents, including *Candida* spp. and filamentous fungi (7, 9, 12–17; M. A. Pfaller, S. A. Messer, K. Mills, A. Bolmström, and R. N. Jones, submitted for publication). Studies have shown that when performed according to the manufacturer's instructions, the Etest provides excellent performance for testing *Candida* spp. against a variety of antifungal agents, including polyenes, flucytosine, and azoles (4, 6, 12–16, 18). Recent studies have demonstrated that the Etest method is suitable for testing the investigational antifungal agents voriconazole and caspofungin against *Candida* (15; Pfaller et al., submitted).

Another investigational triazole antifungal agent, posaconazole, has potent activity against pathogenic yeasts, including most species of *Candida* (1, 3, 5, 8, 11, 12). This agent has been widely tested in broth according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines but has not yet been evaluated using an agar-based method. Given the success in testing other triazoles using the Etest, it is reasonable to assume that posaconazole may be tested by this method as well. The availability of a variety of Etest reagents for antifungal testing will provide great flexibility for laboratories that wish to perform quantitative antifungal susceptibility testing using selected antifungal agents.

In previous evaluations of the Etest for testing amphotericin B (14), fluconazole (13), voriconazole (15), and caspofungin (Pfaller et al., submitted), we have utilized three different media, RPMI 1640 agar supplemented with 2% glucose (RPG agar), Casitone agar, and Antibiotic Medium 3 agar. In every instance we have found performance to be best with RPG agar. Thus, in the present study we evaluated the Etest for posaconazole using only RPG agar in comparison to the NCCLS reference microdilution broth method for testing 314 clinical isolates of *Candida* spp.

MATERIALS AND METHODS

Test organisms. Three hundred fourteen clinical isolates of *Candida* species were selected for testing. The collection included 174 *Candida albicans*, 57 *Candida glabrata*, 39 *Candida parapsilosis*, 31 *Candida tropicalis*, six *Candida guilliermondii*, five *Candida krusei*, and two *Candida lusitaniae* isolates. The members of this collection were all recent clinical isolates from geographically diverse medical centers in North and Latin America. The majority were isolated from blood or normally sterile body fluids (12). The isolates were identified by standard methods (19) 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 optimal growth characteristics.

Antifungal agents. Etest strips containing posaconazole were supplied by AB BIODISK (Solna, Sweden). Posaconazole was obtained as a powder from Schering-Plough Research Institute (Kenilworth, N.J.). Stock solutions were prepared in polyethylene glycol. Serial twofold dilutions were prepared exactly as outlined in NCCLS document M27-A (10). Final dilutions were made in RPMI 1640 medium buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma). The final concentration of solvent did not exceed 1% in any well. Aliquots (0.1 ml) of each antifungal agent at a 2× final concentration were dispensed into the wells of plastic microdilution trays using a Quick Spense II System (Dynatech Laboratories, Chantilly, Va.). The trays were sealed and frozen at −70°C until they were used. The final concentrations of posaconazole were 0.007 to 8 μg/ml.

Media. The agar formulation used for the Etest was RPMI 1640 (American Biorganic, Buffalo, N.Y.) supplemented with 1.5% agar and 2% glucose (RPG agar) and buffered with MOPS. The RPMI 1640 broth medium used for the microdilution testing was buffered with MOPS in accordance with the NCCLS M27-A method (10).

Antifungal susceptibility testing methods. Broth microdilution tests were performed as described in NCCLS document M27-A (10). An inoculum concentra-

* Corresponding author. Mailing address: Medical Microbiology Division, C606 GH, Department of Pathology, University of Iowa College of Medicine, Iowa City, IA 52242. Phone: (319) 384-9566. Fax: (319) 356-4916. E-mail: michael-pfaller@uiowa.edu.

† Present address: 345 Beaver Kreek Centre, North Liberty, IA 52317.

TABLE 1. In vitro activity of posaconazole against 314 clinical isolates of *Candida* species as determined by the reference broth microdilution method[a]

Organism	No. of isolates tested	MIC (μg/ml)[b]		
		Range	50%	90%
C. albicans	174	0.007–>8	0.015	0.03
C. glabrata	57	0.015–>8	0.5	1
C. parapsilosis	39	0.015–0.12	0.03	0.12
C. tropicalis	31	0.015–0.5	0.06	0.12
C. quilliermondii	6	0.06–0.25	0.12	
C. krusei	5	0.25–0.5	0.25	
C. lusitaniae	2	0.015–0.03	0.015	
All	314	0.007–>8	0.03	0.5

[a] Performed as described in NCCLS document M27-A (10).
[b] 50 and 90%, MICs at which 50 and 90% of isolates tested, respectively, are inhibited.

tion of 0.5×10^3 to 2.5×10^3 cells per ml was standardized spectrophotometrically and validated by quantitative plate counts. Microdilution trays were incubated at 35°C and read after 48 h of incubation. For posaconazole, the MIC end point was defined as the lowest concentration that produced a prominent decrease in turbidity (approximately a 50% reduction in growth) compared with that of the drug-free control (10).

For the Etest, 90-mm-diameter plates containing agar at a depth of 4.0 mm were used. The agar surface was inoculated by using a nontoxic swab dipped in a cell suspension adjusted spectrophotometrically to the turbidity of a 0.5 McFarland standard. After excess moisture was absorbed into the agar and the surface was completely dry, an Etest strip was applied to each plate. The plates were incubated at 35°C and read at 48 h. The MIC was read at the lowest concentration at which the border of the elliptical inhibition zone intercepted the scale on the strip. Any growth, such as microcolonies, throughout a discernible inhibition ellipse was ignored.

QC. Quality control (QC) was performed in accordance with NCCLS document M27-A using *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 (10). QC determinations made on each day of testing were within the control limits for posaconazole as established by Barry et al. (2): *C. krusei* ATCC 6258, 0.12 to 1 μg/ml; and *C. parapsilosis* ATCC 22019, 0.06 to 0.25 μg/ml.

Analysis of results. Etest MICs read at 48 h were compared to reference microdilution MICs read at 48 h. Since the Etest scale has a continuous gradient of concentrations, the MICs between twofold dilutions were raised to the next twofold level of the reference method for comparison (13–15). Off-scale MICs at the upper limit were converted to the next higher concentration, and off-scale results at the lower limit were left unchanged. 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 314 *Candida* isolates to posaconazole as determined by the reference broth microdilution method. The posaconazole MICs obtained were consistent with values reported previously for the individual *Candida* spp. tested in RPMI 1640 medium (11, 12). Posaconazole MICs of >1 μg/ml were observed for only three isolates of *C. albicans* (MICs of >8 μg/ml) and five isolates of *C. glabrata* (MICs of 2, 4, and >8 [three isolates] μg/ml).

TABLE 2. Agreement between Etest and reference posaconazole MICs for 314 clinical isolates of *Candida* species

Organism	No. of isolates tested	% Agreement[a]
C. albicans	174	98
C. glabrata	57	93
C. parapsilosis	39	85
C. tropicalis	31	97
C. quilliermondii	6	88
C. krusei	5	100
C. lusitaniae	2	50
All	314	95

[a] Percentage of Etest MICs (read at 48 h) determined with RPG agar medium that are within ± 2 $\log_2$ dilutions of the reference microdilution MICs (RPMI broth, 48 h).

Table 2 summarizes the percentages of 48-h posaconazole MICs obtained by the Etest in RPG agar that were within two dilutions of the reference method result. Overall, the agreement was 95%. The agreement between Etest and microdilution MICs was >90% for *C. albicans* (98%), *C. glabrata* (93%), *C. tropicalis* (97%), and *C. krusei* (100%). With the exception of *C. glabrata*, when a discrepancy was observed between the results obtained by the Etest and the reference method, the Etest provided a lower MIC. In the case of *C. glabrata*, discrepant MICs determined by the Etest were always higher than those determined by the reference method.

The results of this study provide the first documentation of the applicability of the Etest method for determining the in vitro susceptibilities of *Candida* species to the investigational triazole posaconazole. As in previous studies, we found that RPMI agar with glucose (2% final concentration) supported optimal growth of all species tested and provided excellent agreement with the MICs obtained with the broth microdilution method (Table 2). Similar to the case with the other triazoles, fluconazole (13) and voriconazole (15), the problem of trailing end points due to partial inhibition of growth by azoles was minimized by use of RPG agar and strict adherence to specific criteria for reading Etest MICs as described in the Etest package insert and technical guide for yeasts (AB BIODISK). Good agreement with broth dilution MICs was observed when discernible growth within an established ellipse was ignored.

In summary, we have provided the first evidence of the ability of the Etest to generate posaconazole MIC data that are comparable to those obtained by the NCCLS microdilution method. RPMI agar with 2% glucose may be used to determine reference quality MICs with the new investigational triazole (voriconazole and posaconazole) and echinocandin (caspofungin) Etest reagents in tests with *Candida* spp. (15; Pfaller et al., submitted). The availability of Etest reagents for these new antifungal agents will be useful to clinical laboratories because it will provide the flexibility to test one or more of these agents selectively as they are introduced into clinical practice and as the clinical situation dictates.

ACKNOWLEDGMENTS

The excellent secretarial support of Kay Meyer and Linda Elliott is greatly appreciated.

This study was supported in part by Schering-Plough Research Institute and by AB BIODISK.

REFERENCES

1. **Barchiesi, F., D. Arzeni, A. W. Fothergill, L. F. DiFrancesco, F. Caselli, M. G. Rinaldi, and G. Scalise.** 2000. In vitro activities of the new antifungal triazole SCH56592 against common and emerging yeast pathogens. Antimicrob. Agents Chemother. **44:**226–229.
2. **Barry, A. L., M. A. Pfaller, S. D. Brown, A. Espinel-Ingroff, M. A. Ghannoum, C. Knapp, R. P. Rennie, J. H. Rex, and M. G. Rinaldi.** 2000. Quality control limits for broth microdilution susceptibility tests of 10 antifungal agents. J. Clin. Microbiol. **38:**3457–3459.
3. **Cacciapuoti, A., D. Loebenberg, E. Corcoran, F. Menzel, Jr., E. L. Moss, Jr., C. Norris, M. Michalski, K. Raynor, J. Halpern, C. Mendrick, B. Arnold, B.**

Antonacci, R. Parmegiani, T. Yarosh-Tomaine, G. H. Miller, and R. S. Hare. 2000. In vitro and in vivo activities of SCH56592 (posaconazole), a new triazole antifungal agent, against *Aspergillus* and *Candida*. Antimicrob. Agents Chemother. **44:**2017–2022.

4. **Colombo, A. L., F. Barchiesi, D. A. McGough, A. W. Fothergill, and M. G. Rinaldi.** 1995. Evaluation of the Etest system versus a microtitre broth method for antifungal susceptibility testing of yeasts against fluconazole and itraconazole. J. Antimicrob. Chemother. **36:**93–100.
5. **Espinel-Ingroff, A.** 1998. Comparison of in vitro activities of the new triazole SCH56592 and the echinocandins MK-0991 (L-743,872) and LY303366 against opportunistic filamentous and dimorphic fungi and yeasts. J. Clin. Microbiol. **36:**2950–2956.
6. **Espinel-Ingroff, A., M. Pfaller, M. E. Erwin, and R. N. Jones.** 1996. Interlaboratory evaluation of Etest method for testing antifungal susceptibilities of pathogenic yeasts to five antifungal agents by using Casitone agar and solidified RPMI1640 medium with 2% glucose. J. Clin. Microbiol. **34:**848–852.
7. **Espinel-Ingroff, A., T. White, and M. A. Pfaller.** 1999. Antifungal agents and susceptibility tests, p. 1640–1652. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. ASM Press, Washington, D.C.
8. **Galgiani, J. N., and M. L. Lewis.** 1997. In vitro studies of activities of the antifungal triazoles SCH56592 and itraconazole against *Candida albicans*, *Cryptococcus neoformans*, and other pathogenic yeasts. Antimicrob. Agents Chemother. **41:**180–183.
9. **Jorgensen, J. H., J. D. Turnidge, and J. A. Washington.** 1999. Antibacterial susceptibility tests: dilution and disk diffusion methods, p. 1526–1543. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. ASM Press, Washington, D.C.
10. **National Committee for Clinical Laboratory Standards.** 1997. Reference method for broth dilution antifungal susceptibility testing of yeast. Approved standard M27-A. National Committee for Clinical Laboratory Standards, Wayne, Pa.
11. **Pfaller, M. A., S. Messer, and R. N. Jones.** 1997. Activity of a new triazole, SCH56592, compared with those of four other antifungal agents tested against clinical isolates of *Candida* spp. and *Saccharomyces cerevisiae*. Antimicrob. Agents Chemother. **41:**233–235.
12. **Pfaller, M. A., S. A. Messer, R. J. Hollis, R. N. Jones, G. V. Doern, M. E. Brandt, and R. A. Hajjeh.** 1998. In vitro susceptibilities of *Candida* bloodstream isolates to the new triazole antifungal agents BMS-207147, SCH56592, and voriconazole. Antimicrob. Agents Chemother. **42:**3242–3244.
13. **Pfaller, M. A., S. A. Messer, A. Karlsson, and A. Bolmström.** 1998. Evaluation of the Etest method for determining fluconazole susceptibilities of 402 clinical yeast isolates by using three different agar media. J. Clin. Microbiol. **36:**2586–2589.
14. **Pfaller, M. A., S. A. Messer, and A. Bolmström.** 1998. Evaluation of Etest for determining in vitro susceptibility of yeast isolates to amphotericin B. Diagn. Microbiol. Infect. Dis. **32:**223–227.
15. **Pfaller, M. A., S. A. Messer, A. Houston, K. Mills, A. Bolmström, and R. N. Jones.** 2000. Evaluation of the Etest method for determining voriconazole susceptibilities of 312 clinical isolates of *Candida* species by using three different agar media. J. Clin. Microbiol. **38:**3715–3717.
16. **Pfaller, M. A., S. A. Messer, K. Mills, and A. Bolmström.** 2000. In vitro susceptibility testing of filamentous fungi: comparison of Etest and reference microdilution methods for determining itraconazole MICs. J. Clin. Microbiol. **38:**3359–3361.
17. **Szekely, A., E. M. Johnson, and D. W. Warnock.** 1999. Comparison of Etest and broth microdilution methods for antifungal drug susceptibility testing of molds. J. Clin. Microbiol. **37:**1480–1483.
18. **Wanger, A., K. Mills, P. W. Nelson, and J. H. Rex.** 1995. Comparison of Etest and National Committee for Clinical Laboratory Standards broth macrodilution method for antifungal susceptibility testing: enhanced ability to detect amphotericin B-resistant *Candida* isolates. Antimicrob. Agents Chemother. 39:2520–2522.
19. **Warren, N. G., and K. C. Hazen.** 1999. *Candida*, *Cryptococcus*, and other yeasts of medical importance, p. 1184–1199. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. ASM Press, Washington, D.C.