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Comparison of Etest and National Committee for Clinical Laboratory Standards Broth Macrodilution Method for Azole Antifungal Susceptibility Testing

ARNALDO L. COLOMBO,[1,2]* FRANCESCO BARCHIESI,[1,3] DEANNA A. MCGOUGH,[1] AND MICHAEL G. RINALDI[1,4]

Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center at San Antonio,[1] and Audie L. Murphy Memorial Veterans' Hospital,[4] San Antonio, Texas; Escola Paulista de Medicina, São Paulo, Brazil[2]; and Istituto di Malattie Infettive e Medicina Pubblica, Università degli Studi di Ancona, Ancona, Italy[3]

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The use of Etest strips for antimicrobial susceptibility testing is a new and promising method with broad applications in microbiology. Since these strips contain a predefined continuous gradient of a drug, it is possible to obtain a reproducible, quantitative MIC reading. We performed a prospective and double-blinded study to compare the Etest and National Committee for Clinical Laboratory Standards (Villanova, Pa.) broth macrodilution methods for determining the MICs of fluconazole, itraconazole, and ketoconazole for 100 clinical isolates (25 *Candida albicans*, 25 *Cryptococcus neoformans* var. *neoformans*, 20 *Torulopsis* [*Candida*] *glabrata*, 15 *Candida tropicalis*, and 15 *Candida parapsilosis*). The Etest method was performed according to the manufacturer's instructions, and the reference method was performed according to National Committee for Clinical Laboratory Standards document M27-P guidelines. Despite differences between results for some species-drug combinations, Etest and macrobroth MICs were, in general, in good agreement. The MIC agreement rates for the two methods, within ±1 dilution, were 71% for ketoconazole, 80% for fluconazole, and 84% for itraconazole. According to our data, Etest has potential utility as an alternative method.

The last 40 years have seen major changes in the available health care technology. The development of antibiotics and antineoplastic drugs, advances in intensive care support, progress in immunomodulation, and the advent of organ transplantation have all contributed to change the prognosis for degenerative diseases. Unfortunately, this prolonged survival of immunocompromised populations also makes them highly susceptible to invasive fungal infections (3, 5, 16).

None of these advances, however, has had much impact on the dramatic pandemic of AIDS. The World Health Organization estimates that a cumulative total of >13,000,000 persons has been infected by human immunodeficiency virus since the beginning of the epidemic. Candidiasis is the most common opportunistic infection in human immunodeficiency virus patients, and almost all these patients will develop oral candidiasis in the advanced stage of the disease (4, 10).

Paralleling the increased prevalence of fungal infections has been the introduction of new antifungal agents and the recognition of isolates resistant to antifungal drugs. The chronic use of azoles in the prophylaxis of systemic mycoses in bone marrow transplant patients and for long-term suppressive therapy in AIDS patients is a factor in the selection of isolates that are more resistant to azole therapy (13, 20, 23–25).

Consequently, there is a greater need for a reproducible in vitro susceptibility testing method as a guide to selecting and monitoring antifungal therapy. Despite advances represented by the recent standardization of a macrobroth procedure by the National Committee for Clinical Laboratory Standards (NCCLS), Villanova, Pa., additional efforts are necessary for the development of simpler and more economical methods (14).

The Etest (AB BIODISK, Solna, Sweden) is a novel susceptibility testing method which involves the placement of a plastic strip containing a defined continuous gradient of an antimicrobial drug on the surface of an inoculated agar. Preliminary AB BIODISK in-house studies with antifungal strips have shown good reproducibility (6, 7).

The Etest and the reference NCCLS macrobroth procedure were used to determine the MICs of ketoconazole, itraconazole, and fluconazole for 100 yeasts representing five different species of relevant human pathogenic fungi. The main purpose of this study is to compare the results obtained by the two methods.

MATERIALS AND METHODS

Organisms. The 100 yeast isolates were chosen from among 250 clinical isolates from the Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center at San Antonio. These isolates have been tested before by the authors, employing the NCCLS reference macrobroth method. Every effort was made to provide organisms with various susceptibility patterns.

The 100 yeast isolates consisted of 25 *Candida albicans*, 25 *Cryptococcus neoformans* var. *neoformans*, 20 *Torulopsis* (*Candida*) *glabrata*, 15 *Candida tropicalis*, and 15 *Candida parapsilosis*. The organisms were identified to the species level by the API-20C system after they underwent triage for *Candida albicans* identification by the germ tube test.

Drugs. The Etest strips were provided by the manufacturer and had drug concentrations ranging from 0.02 to 32 μg/ml for ketoconazole and itraconazole and from 0.016 to 256 μg/ml for fluconazole. The strips were stored at −20°C until used. All Etest strips at the time of this study were in the research and development stage (i.e., they were not commercially available). Reference grade powders of fluconazole (Pfizer, Inc., New York, N.Y.) and ketoconazole and itraconazole (Janssen Pharmaceutica, Titusville, N.J.) were used to obtain drug dilutions ranging from 0.03 to 32 μg/ml. A stock solution of fluconazole at 16,000 μg/ml was prepared with sterile distilled water; the stock solution of itraconazole, at 5,000 μg/ml, was prepared with polyethylene glycol 400 (Union Carbide, Danbury, Conn.) by heating at 70°C for 40 to 60 min. The stock solution of ketoconazole, at 5,000 μg/ml, was prepared with 0.2 N HCl. Different concen-

* Corresponding author. Mailing address: Disc. Doenças Infecciosas e Parasitárias, Escola Paulista de Medicina, Rua Botucatu, 740, CEP: 04023-062, São Paulo-SP, Brazil. Phone: (5511) 572 6348. Fax: (5511) 549 2127.

TABLE 1. Antifungal susceptibilities of 100 pathogenic yeasts and 2 control organisms to fluconazole, ketoconazole, and itraconazole as determined by the reference and Etest methods

Fungus (no. of isolates tested)	MIC range (μg/ml) of:					
	Fluconazole		Ketoconazole		Itraconazole	
	Reference[a]	Etest	Reference	Etest	Reference	Etest
Candida albicans (25)	0.125–>32	0.25–>32	≤0.03–>32	≤0.03–>32	≤0.03–>32	≤0.03–>32
Cryptococcus neoformans (25)	0.5–>32	0.25–>32	≤0.03–0.5	≤0.03–0.5	≤0.03–0.5	≤0.03–0.5
Torulopsis glabrata (20)	1–>32	0.5–>32	0.125–8	≤0.03–4	0.06–2	≤0.03–4
Candida tropicalis (15)	0.5–>32	0.125–>32	≤0.03–4	≤0.03–1	0.125–1	≤0.03–2
Candida parapsilosis (15)	0.5–4	0.25–2	0.06–0.25	≤0.03–0.125	0.06–0.25	≤0.03–0.5
Candida albicans ATCC 90029	0.25–0.5	0.5–1	≤0.03–0.125	≤0.03	≤0.03	≤0.03–0.06
Torulopsis glabrata ATCC 90030	8–16	8–16	0.25–0.5	0.06–0.25	0.5–1	0.25–0.5

[a] The reference method is the NCCLS macrobroth dilution method described in reference 14.

trations of the drugs were dispensed in 0.1-ml aliquots into polystyrene screw-cap tubes (13 by 100 mm) and frozen at −70°C until use.

Susceptibility medium. RPMI 1640 with L-glutamine but without bicarbonate was buffered with MOPS (morpholinepropanesulfonic acid) at pH 7.0 (American Biorganics, Niagara Falls, N.Y.) and used to perform the broth macrodilution. RPMI 1640 with L-glutamine but without bicarbonate was buffered with potassium phosphate at pH 7.0, and it and agar-BACTO (Difco, Detroit, Mich.) were used to prepare Etest RPMI-agar (1.5%) plates.

Susceptibility testing procedure. All 100 isolates were tested by the two methods, and different concentrations of the same standardized inoculum were used. To accomplish the study, 10 different experiments testing 12 organisms (10 new organisms plus 2 control organisms) were carried out simultaneously by the two methods each day. All readings for which the investigator was in doubt about the end point definitions were rechecked by duplicate testing of the isolates. In order to confirm the MIC results, the MIC determination was repeated until the investigator felt secure with the end point definitions, with at least two results in agreement.

(i) Etest. The Etest was performed according to the manufacturer's instructions (1). In brief, the inoculum concentration was adjusted with the aid of a spectrophotometer (530 nm wavelength) to correspond to a 0.5 McFarland standard for the *Candida* species and *T. glabrata* and a 1.0 McFarland standard for *Cryptococcus neoformans* var. *neoformans*. A cotton swab was used to apply a previously pipetted volume of 0.6 ml of the inoculum-adjusted solution onto the 150-mm-diameter RPMI-agar (1.5%) surface. The plate was allowed to dry for at least 15 min before the three strips of Etest were placed on the medium surface. The incubation time was overnight or 24 h for the *Candida* species and *T. glabrata* and from 48 to 72 h for *Cryptococcus neoformans* var. *neoformans*. The plates were read as soon as observable growth was noted. The MIC was read where the border of the elliptical inhibition zone intersected the scale on the strips. No information about MIC determinations by the reference method was allowed during the Etest reading of the isolates and vice versa, i.e., the testing was blinded. As the Etest scale has a continuous gradient of concentrations instead of the twofold dilutions that are tested by the macrobroth dilution method, the MIC determined by the Etest was raised to the next twofold dilution level of the reference method in such cases for the sake of comparison. MICs of fluconazole which were higher by the Etest than those by the reference method were identified as >32 μg/ml.

(ii) Reference method. The reference method was the macrobroth dilution method, and it was performed according to the proposed NCCLS standard guidelines (14). The isolates were tested by the two methods, with different concentrations of the same standardized inoculum being used. The inoculum was prepared from Sabouraud dextrose agar subcultures incubated at 35°C. Five colonies of ≥1 mm in diameter from 24-h-old subcultures of the *Candida* species and *T. glabrata* and from 48-h cultures of *Cryptococcus neoformans* var. *neoformans* were suspended in 5 ml of sterile distilled water. The resulting suspension was adjusted with the aid of a spectrophotometer to a cell density of 0.5 McFarland standard at 530 nm wavelength. A working suspension was made by diluting the original suspension to 1:100 and then to 1:20 with RPMI broth medium. A 0.9-ml volume of the adjusted solution of inoculum and medium was dispensed into each plastic tube containing 0.1 ml of the various 10× concentrations of the drugs to be tested. The growth control tube received 0.1 ml of drug diluent, plus 0.9 ml of the adjusted inoculum-medium solution. The test tubes were incubated at 35°C for 48 h for all isolates except *Cryptococcus neoformans* var. *neoformans*, which was incubated for 72 h. The reading criterion was the lowest concentration that produced at least an 80% inhibition compared with that for the growth control tube. In case of doubt, a precise dilution of the control tube producing an 80% inhibition standard was prepared.

(iii) Quality control. Quality control organisms (*Candida albicans* ATCC 90029 and *T. glabrata* ATCC 90030) were included on each day of repeated testing to check the accuracy of the drug dilutions and the reproducibility of the results. The inoculum size, purity, and viability of all tested organisms were checked by subculturing the inoculum suspension in Sabouraud dextrose agar.

Analysis of the results. The 600 MICs obtained by both methods were analyzed according to their distribution at different concentrations tested for by the reference method: 0.03 to 32 μg/ml. Cumulative MIC percentage curves were used to permit a visual analysis of this distribution. The MICs at which 50% (MIC_{50}) and 90% (MIC_{90}) of the 100 isolates tested were inhibited were determined for each azole. A head-to-head comparison of azole MICs as determined by both methods was performed. Essential agreement (EA) occurred when the MIC results by the Etest and reference methods were in exact agreement or were within ±1 twofold dilution.

RESULTS

The MIC ranges as determined by both the reference method and the Etest of the three drugs tested against the 100 yeasts and two control organisms are summarized in Table 1. The MICs for the two control organisms tested by the reference method in the 13 sets of experiments were consistently in agreement with those from the NCCLS reference results (14), confirming the reproducibility of the results as well as the proper drug concentration preparation. The control organism MICs determined by the Etest also exhibited results in the range reported in Table 1. The inoculum sizes of the original suspension used to perform the testing ranged from 1×10^6 CFU/ml to 8×10^6 CFU/ml, with purity and viability confirmed by subculturing on Sabouraud dextrose agar.

Etest reading. The reading of the Etest plates could be done according to the manufacturer's instructions (1) for the vast majority of the isolates. Five isolates (two *T. glabrata* and three *Candida parapsilosis*) had insufficient growth after 24 h of incubation and consequently were read at 36 h. All *Cryptococcus neoformans* var. *neoformans* isolates were read after 48 or 72 h of incubation. It was possible to identify four different reading patterns for the Etest MICs, all of which are illustrated in Fig. 1. The growth of microcolonies inside all or almost all of the inhibition zone, which presents the most difficult end point reading, was associated with the tests involving isolates of *Candida albicans* and *Candida tropicalis* more than with those involving the other organisms and was present with the three drugs tested in the experiment. This specific pattern was seen for 11 of 25 (44%) of the *Candida albicans* isolates and 9 of 15 (60%) of the *Candida tropicalis* isolates tested. The double halo, which was considered a relatively clear MIC intersection, and the sharp MIC end point reading were each seen more frequently among isolates of *Cryptococcus neoformans* var. *neoformans*, *Candida parapsilosis*, and *T. glabrata.*

Fluconazole MICs. The MICs as determined by both methods for the 100 isolates tested cover a broad range, as illustrated in Tables 1 and 2, with high MICs for several isolates.

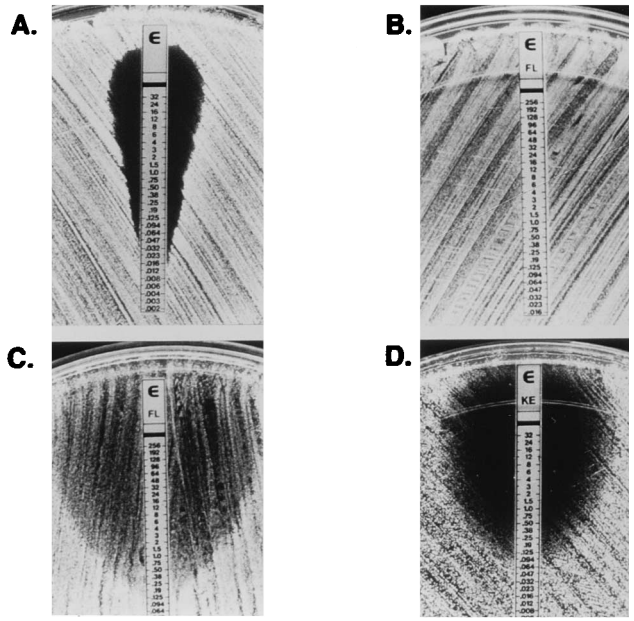

FIG. 1. Etest reading patterns. (A) Sharp end point reading. (B) Resistant isolate with homogeneous growth around all the strip. (C) Growth of microcolonies inside all the inhibition zone. (D) Double halo, illustrated by the growth of microcolonies just close to the border of the inhibition zone.

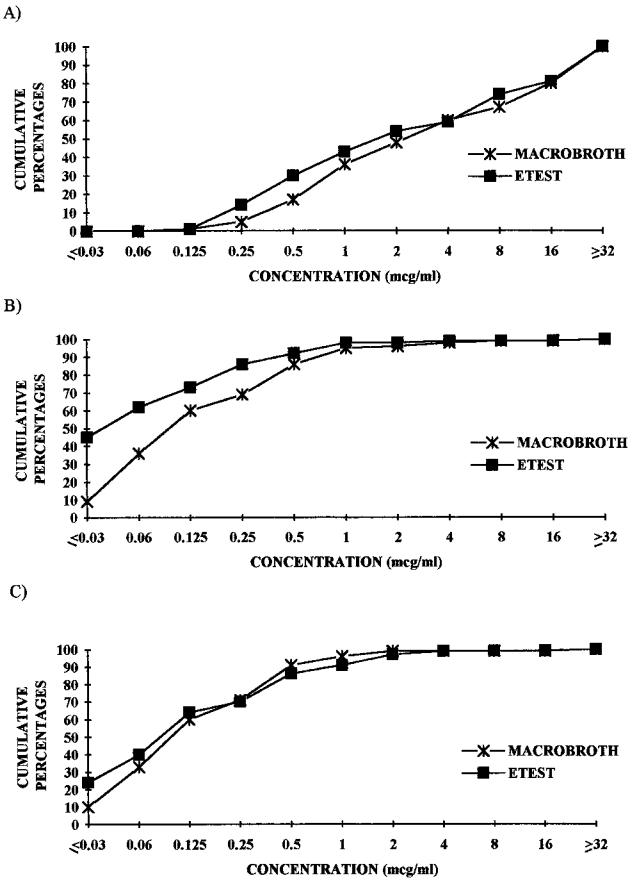

FIG. 2. Cumulative MIC percentages of fluconazole (A), ketoconazole (B), and itraconazole (C) for 100 yeasts tested by the NCCLS macrobroth and Etest methods.

The cumulative MIC percentage curves in Fig. 2A illustrate the close relationship between MICs determined by the Etest and reference methods at all drug concentrations. The MIC_{50} and MIC_{90} curves for both methods could be superimposed (Table 2). The details of the EA between the MICs determined by the two methods are given in Table 3. The overall EA was 80%, with agreement rates among the five species of yeasts tested covering a range from 53 to 96%. The distribution by species of the 20 disagreements in MIC results for the isolates tested is as follows: 7 *Candida tropicalis*, 7 *Candida parapsilosis*, 4 *Candida albicans*, 1 *T. glabrata*, and 1 *Cryptococcus neoformans* var. *neoformans*. Among these 20 disagreements, those for two isolates of *Candida tropicalis* were notable. The MICs for these isolates were >32 μg/ml by the reference method but only 0.25 μg/ml and 0.5 μg/ml by the Etest. It is important to note that those two isolates demonstrated very clear MIC end point readings by the Etest but heavy trailing by the macrobroth dilution test. With regard to the other 18 disagreements, 12 of them consisted of MIC discrepancies by both methods of between 0.125 and 2 μg/ml and 5 of them were discrepancies of from 1 to 4 μg/ml. The MICs for one *Cryptococcus neoformans* var. *neoformans* isolate were 2 μg/ml and 8 μg/ml by the macrobroth and Etest methods, respectively.

Ketoconazole MICs. The MICs as determined by both methods for the 100 isolates tested, as illustrated in Tables 1 and 2, cover a broad range, with the majority of them falling between 0.03 and 0.5 μg/ml. The cumulative MIC percentage curves for ketoconazole (Fig. 2B) show discrepancies between the results of the two methods, mainly for the two lower concentrations (0.03 and 0.06 μg/ml). It is clear from Fig. 2B that MICs determined by the Etest have a tendency to be lower than those determined by the macrobroth dilution method, primarily for concentrations <1 μg/ml. The MIC_{50}s and MIC_{90}s determined by the Etest (Table 2) were both lower than the MIC_{50}s and MIC_{90}s determined by the macrobroth test. The details of the EA between the ketoconazole MICs as determined by the two methods are given in Table 4. The overall EA was 71%, with agreement rates among the five species of yeasts tested covering a range from 45 to 88%. In addition, 28 of the 29 disagreements between the two methods were associated with MICs 2 or more dilutions lower by the Etest than those determined by the macrobroth test (Table 4). Among those 29 disagreements, 13 of them consisted of MIC discrepancies by both methods of between 0.03 and 0.25 μg/ml, 9 of them were discrepancies of from 0.06 to 0.5 μg/ml, and 5 were discrepancies of from 0.25 to 1 μg/ml. The two other disagreements were related to isolates of *Candida tropicalis*, the same ones that had

TABLE 2. In vitro susceptibilities of 100 yeasts to fluconazole, ketoconazole, and itraconazole as determined by the reference and Etest methods

Antifungal agent	Method	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Fluconazole	Reference[a]	0.125–>32	2	>32
	Etest	0.125–>32	2	>32
Ketoconazole	Reference	≤0.03–>32	0.125	1
	Etest	≤0.03–>32	0.06	0.5
Itraconazole	Reference	≤0.03–>32	0.125	0.5
	Etest	≤0.03–>32	0.125	1

[a] The reference method is the NCCLS macrobroth dilution method described in reference 14.

TABLE 3. Distribution of differences of Etest and reference method MICs of fluconazole for 100 yeast isolates and EA percentages

Species (no. of isolates tested)	No. of isolates with Etest MICs different from reference method[a] MICs by number of $\log_2$ dilutions							EA[b] (%)
	>−2	−2	−1	0	+1	+2	>+2	
Candida albicans (25)	0	0	3	14	4	4	0	84
Cryptococcus neoformans (25)	0	0	5	13	6	1	0	96
Torulopsis glabrata (20)	0	1	6	12	1	0	0	95
Candida tropicalis (15)	3	4	3	5	0	0	0	53
Candida parapsilosis (15)	0	7	6	2	0	0	0	53
Total	3	12	23	46	11	5	0	80

[a] The reference method is the NCCLS macrobroth dilution method described in reference 14.
[b] EA of MIC results of the two methods is defined as exact agreement or agreement within ±1 twofold dilution.

the highest fluconazole MIC disagreements. The MICs of ketoconazole for these isolates, as determined by the macrobroth and Etest methods, were 4 and 0.03 μg/ml, respectively.

Itraconazole MICs. The MICs as determined by both methods for the 100 isolates tested, as illustrated in Tables 1 and 2, cover a broad range, with the majority of them falling between 0.03 and 0.5 μg/ml. The cumulative MIC percentage curves (Fig. 2C) for itraconazole show the close relationship between the MICs determined by both methods at all drug concentrations. The MIC_{50}s were the same by both methods, but the MIC_{90}s by the Etest were 1 dilution higher than those by the reference method. The details of the EA between itraconazole MICs determined by the two methods are given in Table 5. The overall EA was 84%, with agreement rates among the five species of yeasts tested covering a range from 33 to 100%. All 16 disagreements related to itraconazole MICs were associated with *Candida albicans* (6 isolates) and *Candida tropicalis* (10 isolates). Among those 16 disagreements, 9 of them consisted of MIC discrepancies by both methods of between 0.03 and 0.125 μg/ml and 4 of them were discrepancies of from 0.06 to 0.5 μg/ml. The three other disagreements were seen for isolates of *Candida albicans*, which demonstrated MIC discrepancies of between 0.25 and 2 μg/ml, with MICs determined by the Etest being higher than those determined by the macrobroth method.

DISCUSSION

The use of Etest strips for antimicrobial susceptibility testing is a new and promising method with broad applications in microbiology. The Etest retains the principle of the agar diffusion method while overcoming some of the limitations of disk diffusion. Since these strips contain a predefined continuous gradient of a drug, it is possible to obtain reproducible, quantitative MIC readings (22).

There have been several studies evaluating the performance of Etest antibacterial strips for susceptibility testing. The results of these studies have demonstrated that this method produces results that are in excellent agreement with those produced by NCCLS standardized methods (2, 9, 22). There is, however, a paucity of data regarding the performance of the Etest for susceptibility testing of fungi. Bolmström et al. conducted some elegant in-house studies which indicated that the Etest may provide a useful alternative for the antifungal susceptibility testing of yeasts (6, 7). Prior to this study, there was no literature that reported data obtained by an independent laboratory and that compared the Etest method for susceptibility testing of yeasts with any broth dilution method.

The present study was designed to evaluate the potential of the Etest as a tool to obtain the MICs of antifungal agents for different species of yeasts. The effects of inoculum size, media and buffer, pH, temperature, and time of incubation were all previously investigated by Bolmström (7). In this study, the Etest was performed according to the manufacturer's defined optimum conditions so that reproducible MIC results and a good correlation with broth methods could be obtained. The reference method was performed according to the NCCLS standardized method for macrobroth dilution (14), and the test conditions for this method were defined after several well-conducted collaborative studies (11, 12, 17, 19).

In general, our data confirm the previous conclusions of Bolmström that there is a good correlation between the MICs obtained by the Etest and broth dilution methods. If only the MIC results at ±2 dilutions for both methods are considered, the agreement rates would be 97% for all three azoles tested. However, discrepancies were noted with certain organism-drug combinations. The determination of the end points for the azoles is a significant factor in the variability of MIC results for these drugs. The usual partial growth inhibition (trailing) ob-

TABLE 4. Distribution of differences of Etest and reference method MICs of ketoconazole for 100 yeast isolates and EA percentages

Species (no. of isolates tested)	No. of isolates with Etest MICs different from reference method[a] MICs by number of $\log_2$ dilutions							EA[b] (%)
	>−2	−2	−1	0	+1	+2	>+2	
Candida albicans (25)	0	3	11	9	2	0	0	88
Cryptococcus neoformans (25)	0	9	10	4	2	0	0	64
Torulopsis glabrata (20)	0	10	7	1	1	1	0	45
Candida tropicalis (15)	3	1	6	5	0	0	0	73
Candida parapsilosis (15)	0	2	9	4	0	0	0	87
Total	3	25	43	23	5	1	0	71

[a] The reference method is the NCCLS macrobroth dilution method described in reference 14.
[b] EA of MIC results of the two methods is defined as exact agreement or agreement within ±1 twofold dilution.

TABLE 5. Distribution of differences of Etest and reference method MICs of itraconazole for 100 yeast isolates and EA percentages

Species (no. of isolates tested)	No. of isolates with Etest MICs different from the reference method[a] MICs by number of $\log_2$ dilutions							EA[b] (%)
	>−2	−2	−1	0	+1	+2	>+2	
Candida albicans (25)	0	1	2	7	10	5	0	76
Cryptococcus neoformans (25)	0	0	9	11	5	0	0	100
Torulopsis glabrata (20)	0	0	4	11	5	0	0	100
Candida tropicalis (15)	3	7	2	2	1	0	0	33
Candida parapsilosis (15)	0	0	5	5	5	0	0	87
Total	3	8	22	36	26	5	0	84

[a] The reference method is the NCCLS macrobroth dilution method described in reference 14.
[b] EA of MIC results of the two methods is defined as exact agreement or agreement within ±1 twofold dilution.

served with azoles makes it difficult to determine MIC accurately (21). As expected, this was a problem with a significant number of isolates by both the Etest and the reference method. It is interesting to note that most of the problems associated with MIC end point definition by the Etest were associated with two of the five species tested. The growth of microcolonies inside all (or almost all) of the inhibition zone around the Etest strips was judged in this study to be the most difficult reading pattern. Bolmström has shown that the medium used has a significant impact on the trailing phenomenon and Etest end point determinations (7). According to our data, this particular reading pattern was present with 10% of *T. glabrata*, 44% of *Candida albicans*, and 60% of *Candida tropicalis* isolates tested. The azole MIC definitions by the macrobroth method for *Candida albicans* and *Candida tropicalis* were also more difficult than those for the other three species tested. Consequently, 19 of 25 *Candida albicans* isolates and 13 of 16 *Candida tropicalis* isolates were tested by both methods at least twice to ensure reproducible MIC results.

An analysis of the distribution of MIC disagreements among the five species tested indicated that *Candida tropicalis* isolates represented the major problem. The study by both methods of 15 isolates of *Candida tropicalis* generated 45 pairs of MIC results, and 21 (47%) of them were in disagreement. The other four species tested had disagreements among their pairs of MIC results of 9 to 20%.

It is important to note that the agreement between the methods varied according to the species of the isolates tested. Our study did not attempt to elucidate why there were such differences in the Etest results for different isolates. However, as in antibacterial testing, antifungal susceptibility testing of yeasts may require different test conditions to accommodate different drug-organism combinations.

In general, the EA rates for fluconazole and itraconazole were higher than those for ketoconazole. It is important to note that there were no significant changes in the EA rates of the three azoles when the MICs determined by the Etest were read for a second time 24 h later (data not shown). There was a consistent tendency for ketoconazole MICs determined by the Etest to be lower than those determined by the reference method. This observation is illustrated by the fact that 28 (96.5%) of the 29 disagreements in MIC results involved Etest MICs 2 or more dilutions lower than reference method MICs. Whatever the susceptibility testing approach, ketoconazole seems to be a difficult azole to test. Espinel-Ingroff et al. reported that ketoconazole MICs had a lower percentage of agreement than fluconazole MICs when both azoles were tested by macro- and microdilution broth methods (11).

With regard to itraconazole and fluconazole, 72% of the MIC disagreements were associated with Etest MICs of 2 or more dilutions lower than reference method MICs. In fact, other authors have reported that absolute azole MICs generated by agar-based techniques tend to be lower than those produced by broth assays (8, 15). In contrast, when fluconazole and itraconazole were tested against *Candida albicans*, they gave results higher than those seen by the reference method. This could be partially explained by the difficulty of determining the end point by both methods.

As there is no consensus as to susceptibility breakpoints for the azoles, it is difficult to clearly define the impact of these 65 MIC disagreements in the susceptibility patterns of the organisms tested by both methods. However, if we arbitrarily consider a drug concentration equal to its MIC_{90} as the susceptibility breakpoint, then only 7 of the 65 MIC disagreements (associated with the five yeasts) would result in changes in the susceptibility patterns of the isolates involved. The majority of MIC disagreements were associated with low MIC results by both methods.

In conclusion, the Etest has potential utility as an alternative method to the NCCLS-proposed macrobroth dilution method. It is less labor intensive, and its results showed a good correlation with those of the reference method. Modifications in the Etest medium to produce sharper reading end points might further improve the performance of this test.

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