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Multicenter Evaluation of a Broth Macrodilution Antifungal Susceptibility Test for Yeasts

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Thirteen laboratories collaborated to optimize interlaboratory agreement of results of a broth macrodilution procedure for testing three classes of antifungal drugs against pathogenic yeasts. The activities of amphotericin B, flucytosine, and ketoconazole were tested against 100 coded isolates of *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida lusitaniae*, *Torulopsis* (*Candida*) *glabrata*, and *Cryptococcus neoformans*. Two starting yeast inoculum sizes (5×10^4 and 2.5×10^3 cells per ml) were compared, and readings were taken after 24 and 48 h of incubation. All other test conditions were standardized. The resultant turbidities in all tubes were estimated visually on a scale from 0 to 4+ turbidity, and MIC-0, MIC-1, and MIC-2 were defined as the lowest drug concentrations that reduced growth to 0, 1+, or 2+ turbidity, respectively. For flucytosine, agreement among laboratories varied between 57 and 87% for different inocula, times of incubation, and end point criteria. Agreement was maximized (85%) when the lower inoculum was incubated for 2 days and the MICs were defined as 1+ turbidity or less. For amphotericin B, variations in test conditions produced much smaller differences in interlaboratory agreement. For ketoconazole, interlaboratory agreement was poorer by all end point criteria. However, MIC-2 endpoints distinguished *T. glabrata* as resistant compared with the other species. Overall, the studies indicated that readings from the lower inoculum obtained on the second day of reading result in the greatest interlaboratory agreement. In combination with data from previous multicenter studies (National Committee for Clinical Laboratory Standards, *Antifungal Susceptibility Testing: Committee Report*, Vol. 5, No. 17, 1988; M. A. Pfaller, L. Burmeister, M. S. Bartlett, and M. G. Rinaldi, J. Clin. Microbiol. 26:1437–1441, 1988; M. A. Pfaller, M. G. Rinaldi, J. N. Galgiani, M. S. Bartlett, B. A. Body, A. Espinel-Ingroff, R. A. Fromtling, G. S. Hall, C. E. Hughes, F. C. Odds, and A. M. Sugar, J. Clin. Microbiol. 34:1648–1654, 1990), these findings will be used by the National Committee for Clinical Laboratory Standards to develop a standardized method for in vitro antifungal susceptibility testing for yeasts.

Fungal infections have emerged as major causes of morbidity and mortality in compromised patient populations. During the last 15 years, the incidence of fungal infections has continued to increase in part because of improved diagnosis and the increasing number of patients with AIDS, organ transplants, malignancies, and other immunocompromising factors (4, 40). The frequency of fungal infections has greatly increased interest in testing new fungal isolates from patients by clinical laboratories, and efforts to develop standardized methodologies for such tests have been reported (18, 27, 33, 39). In addition, an increasing number of reports suggest that clinically significant resistance exists in some strains (13–15, 19, 22, 37, 38, 43). Standardized methods of testing isolates would permit the development of a more reliable data base from which to study this clinically relevant issue.

Since standardization is vital to meaningful communication between clinical laboratories and physicians and to the application of in vitro data to patient responses to therapy, the National Committee for Clinical Laboratory Standards formed a subcommittee on antifungal susceptibility testing in 1982. Four years later, the first subcommittee report was published in which the results of a questionnaire and small collaborative study were presented (5, 24). Among the findings were that most centers used a broth dilution meth-

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odology, only a few isolates were tested annually, *Candida albicans* or other species of yeasts were the most commonly tested species of fungi, and MIC data among the laboratories that participated in the collaborative study were unacceptably low. Two subsequent multicenter studies (30, 32) have focused on inoculum preparation, inoculum size, selection of synthetic media, temperature and duration of incubation, and end point definition. These studies established criteria for inoculum preparation by using a spectrophotometric method (30), selection of RPMI 1640 medium (32), and an incubation temperature of 35°C (32) for antifungal susceptibility testing of yeasts.

The present collaborative study was performed to evaluate the utility of RPMI 1640 medium buffered with morpholinepropanesulfonic acid (MOPS) and an incubation temperature of 35°C in a broth macrodilution susceptibility test method with a panel of 100 pathogenic yeast isolates and three classes of antifungal agents. The effects of inoculum size and incubation times of 24 and 48 h on the ability to determine the susceptibilities of isolates of *Candida* species, *Torulopsis* (*Candida*) *glabrata*, and *Cryptococcus neoformans* also were assessed. The data obtained in the present study, when combined with the information gained in the previous studies of the National Committee for Clinical Laboratory Standards (5, 24, 30, 32), have supported the development of a proposed standardized method for in vitro antifungal susceptibility testing for yeasts.

MATERIALS AND METHODS

Antifungal agents. The following three antifungal agents were used: amphotericin B (Bristol Myers-Squibb), flucytosine (5-FC; Hoffmann-La Roche Laboratories), and ketoconazole (Janssen Pharmaceutica). These agents were obtained from the manufacturers as standard powders, each from a single lot, and were distributed to the participating laboratories. Stock solutions of 16,000 μg/ml were prepared in 100% dimethyl sulfoxide (amphotericin B and ketoconazole) or sterile distilled water (5-FC) immediately prior to use in the study. Final dimethyl sulfoxide concentrations in the test drug preparations used were well below the levels shown to have antifungal effects.

Test organisms. A panel of 100 pathogenic yeasts was used in the study. Eighty-five strains of noncryptococcal yeasts were sequentially isolated from cultures of blood from patients treated at the University of Iowa. This panel consisted of 35 strains of *C. albicans*, 15 strains of *Candida tropicalis*, 15 strains of *Candida parapsilosis*, 10 strains of *Candida lusitaniae*, and 10 strains of *T. glabrata*. Biochemical and molecular analyses demonstrated that each of the strains possessed one or another unique characteristic (31, 34). In addition, 15 strains of *C. neoformans*, 8 of which were used in previous studies (9), were contributed by the Medical College of Virginia. This group was selected to include strains with a range of 5-FC susceptibilities. In addition, eight isolates were from patients with human immunodeficiency virus infections. *C. parapsilosis* (Medical College of Virginia culture collection number 52-493) was provided as a quality control isolate.

Test medium. Two lots of RPMI 1640 medium with L-glutamine, without sodium bicarbonate, and buffered with MOPS (0.165 M; 34.54 g/liter; Sigma) in final liquid form were generously provided to each laboratory by the manufacturers (BioWhittaker, Inc., Walkersville, Md., and ICN Biomedical, Inc., Costa Mesa, Calif.). Although it was intended that the media would be used at a pH of 7.0, during the study it was discovered that one lot had a pH of 7.4. Analysis stratified for this difference determined that the higher pH affected only the agreement of 5-FC results with *C. neoformans*. Therefore, results for the medium with the higher pH for this component of the study were excluded from this report.

Susceptibility testing procedure. Detailed instructions for performing inoculum preparation, drug dilutions, and end point determinations were provided to each participating laboratory. Broth macrodilution testing was performed with twofold drug dilutions. Stock solutions of antifungal agents were diluted by previously described methods recommended for minimizing systematic pipetting errors (25). Final drug concentrations were 0.03 to 16 μg/ml for amphotericin B and ketoconazole and 0.125 to 64 μg/ml for 5-FC.

Yeasts were grown on Sabouraud dextrose agar (Difco) for 24 h (*Candida* spp. and *T. glabrata*) or 48 h (*C. neoformans*) at 35°C. Yeast inoculum sizes were prepared by a previously described method (33). Briefly, five colonies, each at least 1 mm in diameter, were suspended in 5 ml of sterile 0.85% saline. The turbidity of the suspension was adjusted spectrophotometrically to match the transmittance of a 0.5 McFarland barium sulfate turbidity standard at 530 nm. The saline suspension was diluted 1:100 with RPMI 1640 medium to yield a final inoculum of approximately 5×10^4 cells per ml; a second inoculum size of approximately 2.5×10^3 cells per ml was prepared by an additional 1:20 dilution. Inocula were verified by enumeration of the CFU obtained by subculture on Sabouraud dextrose agar by two of the collaborating laboratories. These two inocula were used directly in the assay tubes to which drug was added.

Yeast inocula (0.9 ml) were added to polystyrene plastic tubes (12 by 75 mm; Falcon 2054; Becton Dickinson, Lincoln Park, N.J.) containing dilutions of one of the antifungal agents (0.1 ml at a 10× final concentration) by using sterile, individual 1-ml pipettes. Each yeast isolate was tested at two inocula, and drug-free and yeast-free control tubes were included. The contents of the tubes were mixed, and racks of loosely capped tubes were incubated without agitation in air at 35°C.

Tubes were examined on the first and second days on which significant growth was evident in the drug-free control tube. For species other than *C. neoformans*, this was after 24 and 48 h of incubation. For nearly all isolates of *C. neoformans*, this was after 48 and 72 h of incubation. At the times of examination, each tube was flicked gently and the turbidity was estimated by using the following scale: 0, optically clear; 1+, slightly hazy; 2+, prominent reduction in turbidity compared with that of the drug-free control; 3+, slight reduction in turbidity compared with that of the drug-free control; 4+, no reduction in turbidity compared with that of the drug-free control. Investigators recorded the estimated turbidity for all tubes on standardized data sheets.

Study design and analysis of results. Each of the 13 participating laboratories received a protocol, antifungal drugs, media, coded yeast isolates, and standardized data sheets. When tests were completed, the data sheets were forwarded to one center where the results were entered into a microcomputer data base (Rbase; Microrim Inc., Redmond, Wash.), and the data were subsequently transferred to a statistical program (Systat; Systat Inc., Evanston, Ill.) for analysis. Listings of the entered data were returned to the reporting laboratory, where the accuracies of the entries were checked and errors were corrected.

The visual estimates of turbidity were used to calculate different end point results. MIC-0, MIC-1, and MIC-2 were

TABLE 1. Percent agreement of different yeast strains for amphotericin B results with low and high starting inoculum sizes and first and second day readings

Species (no. of tests)	% within twofold of mode for[a]:							
	MIC-0				MIC-1			
	Day 1		Day 2		Day 1		Day 2	
	Low	High	Low	High	Low	High	Low	High
C. albicans (1,761)	85 (0.98)	87 (0.98)	90 (0.99)	86 (0.99)	78 (0.96)	83 (0.97)	89 (0.99)	91 (0.99)
C. lusitaniae (520)	83 (0.94)	84 (0.96)	85 (0.99)	83 (0.99)	82 (0.91)	82 (0.94)	88 (0.97)	85 (0.98)
C. neoformans (597)	69 (0.92)	78 (0.95)	81 (1.0)	79 (1.0)	63 (0.76)	70 (0.80)	83 (0.91)	85 (0.93)
C. parapsilosis (768)	82 (0.95)	85 (0.95)	90 (0.99)	87 (0.98)	73 (0.93)	83 (0.92)	86 (0.99)	81 (0.98)
C. tropicalis (724)	85 (0.99)	89 (0.99)	92 (0.99)	91 (1.0)	90 (1.0)	90 (1.0)	92 (1.0)	92 (1.0)
T. glabrata (480)	90 (0.99)	93 (0.99)	95 (1.0)	87 (1.0)	88 (0.99)	95 (0.99)	97 (1.0)	94 (1.0)

[a] Values in parentheses are the proportions of results that were within the drug concentration range and included in the analysis. Low and high indicate inoculum sizes.

defined as the lowest drug concentration that produced 0, 1+, or 2+ turbidity, respectively. If inhibition was inconsistent, then the end point was taken as the higher drug concentration. Parallel analyses were conducted by using each end point.

For analysis, a median result for each yeast isolate-drug combination was determined. For example, if all 13 laboratories tested an isolate at both inocula and at both days of reading, there were 52 datum points from which to determine the median. If the median fell between two dilutions, it was assigned to the higher concentration. After transformation to $\log_2$, the median value was subtracted from each individual result obtained with the isolate. This procedure allowed data for isolates with different intrinsic susceptibilities to be grouped without altering the distribution of results. Results were further divided relative to inoculum size and time of reading period. Within each of the four subgroups, tests that were within 1 dilution of each subgroup's mode (in most instances, equivalent to the median) were considered to be in agreement and results were expressed as the percent agreement. If a median determined in this way was outside the drug's dilution range, then results with that isolate would artifactually improve interlaboratory agreement. To avoid this effect, such results were excluded from estimates of agreement. Differences in percent agreement between various sets of conditions were assessed by Pearson's chi-square tests. The test of significance was made at the 0.050 level.

RESULTS

A total of 14,914 test results (96% of the possible data set) were available. Results were obtained for 97.6% of *C. albicans*, 99.9% of *C. lusitaniae*, 99.0% of *C. parapsilosis*, 94.3% of *C. tropicalis*, and 94.6% of *T. glabrata* isolates. Of the thirteen laboratories, 12 contributed 93.9% of their total possible results for *C. neoformans*. In the present analysis, no attempt was made to correct for missing data.

Interlaboratory agreement for three antifungal drugs. Overall agreements between MIC-0 and MIC-1 end points for each test condition are given in Tables 1 and 2 for amphotericin B and 5-FC, respectively. Results for all three end points with ketoconazole are given in Table 3.

Amphotericin B generally showed the best overall agreement with either MIC-0 or MIC-1 end points. Results ranged from 63 to 97% agreement among the different test conditions and species. Overall, low starting inoculum sizes read on the second day produced optimal agreement, and this was superior to the condition with the next closest percent agreement (Table 4). With these test conditions, there was no difference in the percent agreement between MIC-0 and MIC-1.

5-FC displayed a wide range of agreement with different test conditions, ranging from 55 to 90% for different species. As with amphotericin B, MIC-0 readings of the lower starting inoculum size on the second day showed significantly better agreement than the next best test condition. However, for MIC-1 readings, second day low-inoculum and first day high-inoculum readings were not significantly different, and both afforded greater agreement than did any MIC-0 end point (Table 4). MIC-2 results were equivalent to those obtained with MIC-1 end points.

Ketoconazole displayed poorer agreement than either of the other two drugs with all end points analyzed. For some

TABLE 2. Percent agreement of different yeast strains for 5-FC results with low and high starting inoculum sizes and first and second day readings

Species (no. of tests)	% within twofold of mode for[a]:							
	MIC-0				MIC-1			
	Day 1		Day 2		Day 1		Day 2	
	Low	High	Low	High	Low	High	Low	High
C. albicans (1,761)	76 (0.70)	69 (0.77)	76 (0.81)	56 (0.72)	61 (0.44)	85 (0.72)	86 (0.78)	81 (0.80)
C. lusitaniae (520)	87 (0.12)	75 (0.21)	78 (0.21)	72 (0.38)	79 (0.11)	57 (0.10)	65 (0.13)	70 (0.22)
C. neoformans (484)	55 (0.85)	62 (0.86)	75 (0.76)	62 (0.83)	57 (0.71)	74 (0.86)	79 (0.76)	84 (0.83)
C. parapsilosis (768)	75 (0.51)	63 (0.22)	79 (0.84)	64 (0.84)	72 (0.19)	78 (0.66)	90 (0.72)	78 (0.83)
C. tropicalis (723)	79 (0.58)	81 (0.80)	81 (0.86)	66 (0.81)	80 (0.36)	85 (0.65)	86 (0.81)	76 (0.83)
T. glabrata (480)	63 (0.81)	79 (0.66)	76 (0.44)	73 (0.69)	69 (0.11)	72 (0.23)	83 (0.30)	79 (0.60)

[a] Values in parentheses are the proportion of results that were within the drug concentration range and included in the analysis. Low and high indicate inoculum sizes.

TABLE 3. Percent agreement of different yeast strains for ketoconazole results with low and high starting inoculum sizes and first and second day readings

Species (no. of tests)	% within twofold of mode for[a]:											
	MIC^{-0}				MIC^{-1}				MIC^{-2}			
	Day 1		Day 2		Day 1		Day 2		Day 1		Day 2	
	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
C. albicans (1,762)	39 (0.23)	37 (0.22)	45 (0.25)	35 (0.13)	28 (0.36)	19 (0.39)	22 (0.38)	25 (0.36)	83 (0.16)	71 (0.27)	63 (0.36)	66 (0.42)
C. lusitaniae (519)	55 (0.72)	65 (0.72)	74 (0.82)	59 (0.70)	64 (0.62)	73 (0.68)	78 (0.75)	75 (0.77)	68 (0.44)	73 (0.51)	83 (0.62)	56 (0.68)
C. neoformans (597)	49 (0.80)	48 (0.70)	60 (0.85)	37 (0.64)	53 (0.61)	57 (0.67)	66 (0.79)	61 (0.76)	70 (0.72)	37 (0.76)	73 (0.83)	69 (0.82)
C. parapsilosis (768)	69 (0.80)	63 (0.77)	79 (0.90)	58 (0.79)	73 (0.60)	57 (0.78)	66 (0.91)	61 (0.92)	57 (0.70)	73 (0.70)	74 (0.84)	81 (0.83)
C. tropicalis (724)	35 (0.68)	30 (0.51)	31 (0.51)	49 (0.32)	46 (0.54)	29 (0.62)	28 (0.73)	35 (0.61)	92 (0.21)	80 (0.33)	78 (0.46)	59 (0.58)
T. glabrata (480)	50 (0.85)	45 (0.54)	55 (0.73)	60 (0.33)	65 (0.86)	58 (0.83)	64 (0.91)	43 (0.67)	72 (0.90)	72 (0.86)	73 (0.90)	57 (0.92)

[a] Values in parentheses are the proportion of results that were within the drug concentration range and included in the analysis. Low and high indicate inoculum sizes.

species and test condition combinations, agreement was as low as 19%. Overall, MIC-2 end points were better than either MIC-0 or MIC-1 end points, and day 2 low-inoculum results displayed significantly better agreement than those obtained with other test conditions (Table 4).

Distribution of susceptibility results by different end points. Since optimal conditions for broth macrodilution testing appeared to include low inoculum size and readings on day 2, modal results for those conditions for each strain were used to construct susceptibility distributions for each drug and species tested. For amphotericin B, the MIC-0 end points, which were equivalent to MIC-1 results, are shown in Fig. 1. There was remarkable homogeneity of results for all species, and the MIC-0 for all strains ranged from 0.25 to 1.0 μg/ml. A more resistant cluster was not detected in this group of isolates.

For 5-FC, MIC-1 results, which showed better agreement than MIC-0 results, are shown in Fig. 2. The predominant distribution of results ranged from ≤0.125 to 8 μg/ml. However, in four of the species tested, for one or more isolates MIC-1 results were >64 μg/ml. This result was from 16- to 1,024-fold greater than those for other isolates of the same species, thus suggesting a more resistant grouping above that for the norm.

For ketoconazole, all three endpoints are shown in Fig. 3 and 4, and the modal distributions demonstrated sharply different patterns. MIC-0 results were uniformly higher, most notably for *C. albicans*, *C. tropicalis*, and *T. glabrata*. With MIC-1 and MIC-2 results, end points shifted dramatically lower for *C. albicans* and *C. tropicalis*, whereas *T. glabrata* showed much less of a shift. As a result, by using the MIC-2 end point, for 70% of the *T. glabrata* isolates the MIC-2 was ≥2.0 μg/ml, whereas for all strains of other species, the MIC-2 was ≤1.0 μg/ml.

DISCUSSION

In the present study, results produced with the lower inoculum and interpreted on the second day of readings proved to be significantly more reproducible among laboratories than were found with either the higher inoculum or first day readings. Under optimal conditions, 90% of amphotericin B results for each strain from different laboratories agreed within a fourfold range regardless of the end point

TABLE 4. Overall percent agreement of MIC-0, MIC-1, and MIC-2 end points for low and high starting inoculum sizes read on days 1 and 2[a]

Drug	End point[b]	% Agreement on day 1[c]		Significance (*P*)[d]	% Agreement on day 2[c]	
		Low	High		Low	High
Amphotericin B	MIC-0 (4,253)	85	87	←0.03→	90	87
	MIC-1 (4,253)	81	86	←0.002→	90	89
5-FC	MIC-0 (3,257)	66	68	←0.001→	77	65
	MIC-1 (2,574)	57	83	NS[e]	85	79
Ketoconazole	MIC-0 (2,573)	43	48	NS	55	45
	MIC-1 (3,800)	46	42	NS	44	39
	MIC-2 (2,656)	52	69	←0.02→	75	65

[a] By a chi-square test of significance of differences among the four sets of conditions for each drug and end point, *P* values were all <0.004.
[b] Values in parentheses are the number of results (results for *C. neoformans* and offscale results were deleted).
[c] Significance of differences between day 1 high-inoculum and day 2 low-inoculum results are provided, but a Bonferroni correction was not applied.
[d] Low and high indicate inoculum sizes.
[e] NS, not significant.

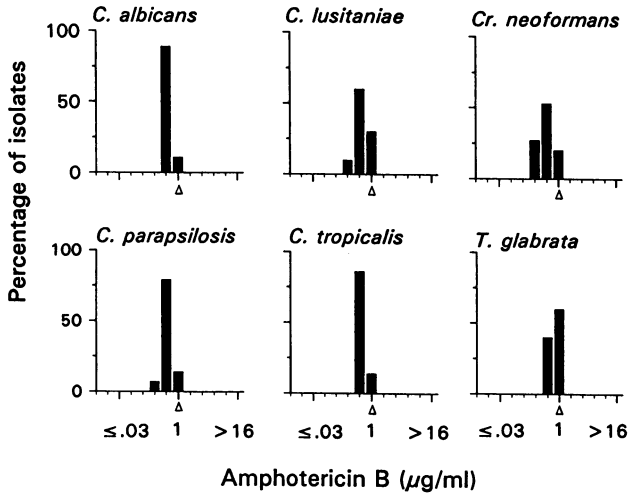

FIG. 1. Percent distribution of modal MIC-0 results for amphotericin B against *C. albicans* (35 strains), *C. lusitaniae* (10 strains), *C. neoformans* (15 strains), *C. parapsilosis* (15 strains), *C. tropicalis* (15 strains), and *T. glabrata* (10 strains) when tested with the lower starting inoculum and readings were taken on the second day. △, proposed upper limits of the susceptible range.

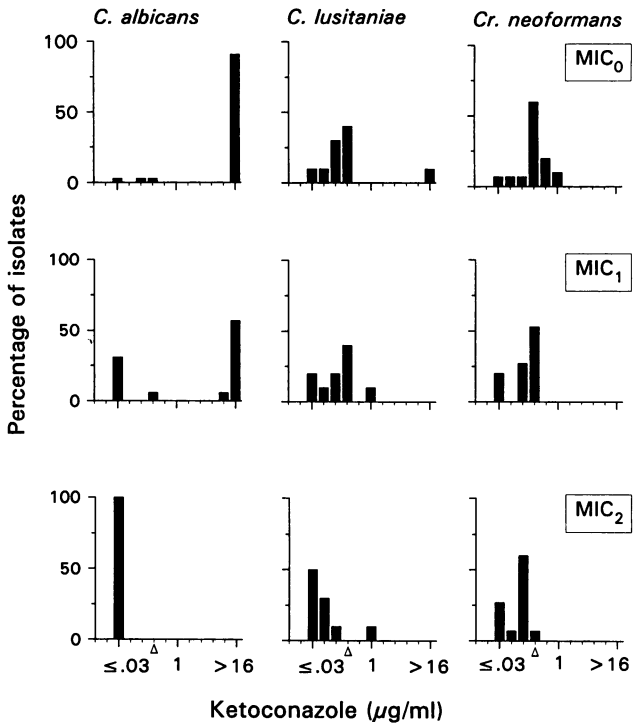

FIG. 3. Percent distribution of modal MIC-0, MIC-1, and MIC-2 results for ketoconazole against *C. albicans* (35 strains), *C. lusitaniae* (10 strains), and *C. neoformans* (15 strains). Test conditions are as indicated in the Fig. 1 legend. △, proposed upper limits of the susceptible range.

criterion used. However, with 5-FC and ketoconazole, agreement was improved with MIC-1 and MIC-2, respectively.

The reason that the less stringent end points improved agreement for these two drugs is likely related to the trailing nature of the drug effect documented in past studies; this also would be expected to be evident with other azole congeners such as miconazole and fluconazole (11, 16, 26, 28, 36). The titration curve for the effect of amphotericin B is much sharper than exists with the other drugs studied. In addition, the onset of the effect of 5-FC and azoles is delayed, allowing growth to proceed for some period before inhibition is initiated (17, 23). In antibacterial susceptibility testing, an analogous problem has been encountered for sulfa drugs tested by an agar diffusion test; in that circumstance an end point criterion of 80% inhibition has been adopted (2, 3).

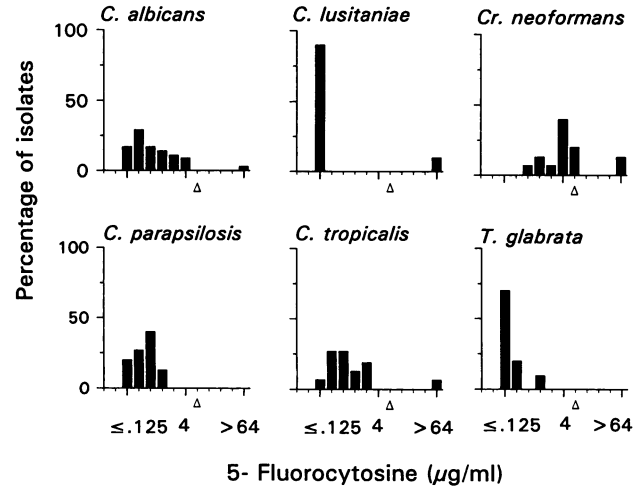

FIG. 2. Percent distribution of modal MIC-1 results for 5-FC against *C. albicans* (35 strains), *C. lusitaniae* (10 strains), *C. neoformans* (15 strains), *C. parapsilosis* (15 strains), *C. tropicalis* (15 strains), and *T. glabrata* (10 strains). Test conditions are as indicated in the Fig. 1 legend. △, proposed upper limits of the susceptible range.

Although not specifically examined in the present study, recent parallel studies reported elsewhere (10) have determined that the estimates of turbidity described here as 1+ and 2+ can be approximated by 1:10 and 1:5 dilutions of the drug-free control, respectively. This approach provides a convenient and direct method of establishing a turbidity end point for a specific isolate that precisely reflects 90 and 80% inhibition, respectively, and further reduces the likelihood of interobserver error. The need for disregarding slight amounts of turbidity for some antifungal agents is related to the person making the reading as well as to other factors, including incomplete drug solubility.

In addition to interlaboratory agreement, an important consideration is the effect that different test conditions have on identifying potentially resistant strains. The isolates of *C. neoformans* were selected with this in mind for 5-FC; the putatively resistant isolates could be distinguished as resistant under the optimized procedure. However, the susceptibility of *C. neoformans* to other drugs and the susceptibilities of the other species to any of the drugs tested were not used as criteria to select isolates. On the basis of the assumption that isolates of most *Candida* species are susceptible to amphotericin B, 5-FC, and azoles, it would be expected that most isolates would constitute a susceptible distribution. On the basis of this assumption, we propose that the upper limits of susceptibility test results by the

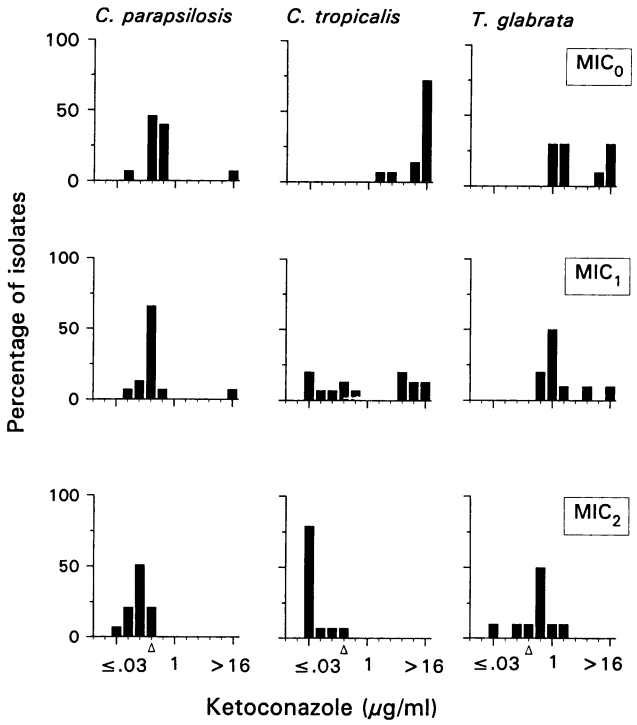

FIG. 4. Percent distribution of modal MIC-0, MIC-1, and MIC-2 results for ketoconazole against *C. parapsilosis* (15 strains), *C. tropicalis* (15 strains), and *T. glabrata* (10 strains). Test conditions are as indicated in the Fig. 1 legend. △, proposed upper limits of the susceptible range.

optimized method be 1.0, 8.0, and 0.25 μg/ml for amphotericin B, 5-FC, and ketoconazole, respectively. In the absence of clinical data, determination of breakpoint values could be proposed by using both pharmacokinetic and population distribution data. This approach will require a study with a greater number of strains. Ultimately, however, clinical correlation will be essential for validating any breakpoint for this method.

By using the MIC-2 criterion for ketoconazole, the distribution of modal susceptibilities for *T. glabrata* indicated that it was notably more resistant than other species. This is in keeping with results of previous in vitro studies and several clinical observations with ketoconazole and other azoles (20, 21, 41, 42). In contrast, the MIC-0 criterion did not distinguish *T. glabrata* from other species. Not tested in this study but of additional interest would be the MICs for isolates of *Candida krusei*, which has also been associated with azole resistance (1, 6, 8, 12, 29, 35, 44). In a future study, it will be important to determine whether the resistance of this species can be identified by the MIC-2 criterion as well.

The amphotericin B susceptibility of *C. lusitaniae* noted in our results also is of special interest, since a few isolates of this species from some institutions have been reported to be resistant to amphotericin B (7). All of the strains of this species tested in the present study appeared to have susceptibilities identical to those of the other species tested, and this raises the possibility that in attaining optimal interlaboratory agreement, the procedure has become insensitive to potentially important amphotericin B resistance. This important issue needs to be addressed in future studies.

This and previous collaborative studies (24, 30, 32) done in coordination with the National Committee for Clinical Laboratory Standards have resulted in demonstrable progress in standardizing antifungal susceptibility testing of yeasts. Although excellent multicenter studies have been done by others (18, 27, 33, 39), none of them have been developed into a widely used standardized procedure for a range of chemical classes of antifungal drugs. The data obtained in the present study, in combination with data from previous multicenter studies (24, 30, 32), have been used by the National Committee for Clinical Laboratory Standards in the development of a standardized method for in vitro antifungal susceptibility testing for yeasts (25a). It is hoped that the procedure will provide a tool for determining the clinical utility of testing for the clinician. In addition to drug susceptibility, other factors, such as anatomy, immunologic competence, or drug distribution and metabolism, are critical for estimating clinical responses in patients. Future studies are needed to define the significance of in vitro test results in relation to these other factors and how in vitro tests should be used to guide therapy.

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