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Fluconazole Susceptibilities of *Candida* Species and Distribution of Species Recovered from Blood Cultures over a 5-Year Period

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The distribution and fluconazole susceptibilities of *Candida* species isolated over a 5-year period were investigated. Susceptibilities were determined by using a new microtiter procedure and the National Committee for Clinical Laboratory Standards (NCCLS) proposed standard. The new method correlated well with the NCCLS proposed standard and gave very clear end points. Results indicate that there are species-related differences in MICs as reflected in the MICs for 90% of species tested. *Candida albicans* is most susceptible to fluconazole, while *Candida glabrata* is among the least susceptible. These findings coincided with the observation of a shift in distribution of yeast species recovered from blood cultures from 1987 to 1992. *C. albicans* was the predominant species (87%) in the pre- or early fluconazole years but decreased to only 31% of the isolates in 1992. Thus, *Candida* species for which MICs of fluconazole were higher have become more prominent in recent years. Significantly, throughout this period, MICs for each species did not change appreciably.

The incidence of serious fungal infections continues to increase. While amphotericin B was the only drug available for therapy in patients with serious fungal infections, susceptibility testing was considered unnecessary (3, 4). With the recent availability of the newer azoles, however, this situation is likely to change (7). To this end, collaborative efforts directed towards the standardization of in vitro antifungal susceptibility testing have produced a proposed National Committee for Clinical Laboratory Standards (NCCLS) standard for susceptibility testing of yeasts by broth macrodilution (5). However, clinical laboratories more familiar with broth microdilution technology may find the proposed standard difficult to embrace.

The aims of this study were to evaluate a modified broth microdilution method (1) for determining fluconazole susceptibilities of clinical isolates of *Candida* species. In addition, the incidence and distribution of these organisms in blood cultures collected from 1987 to 1992, a period coinciding with increasing fluconazole use, were determined.

Susceptibility testing was carried out on clinical isolates from a variety of sources (blood, sputum, and urine). These consisted of *Candida albicans* (157 isolates), *Candida glabrata* (66 isolates), *Candida tropicalis* (48 isolates), *Candida parapsilosis* (40 isolates), and *Candida krusei* (18 isolates) and included a total of 329 strains. Eighty-two isolates were obtained from the period 1987 to 1989, which was prior to the introduction of fluconazole, while 247 were recovered in 1991 and 1992, which was after fluconazole release (see Table 1). The procedure used was a modification of that described by Anaissie et al. (1). A major modification involved the growth medium used prior to testing. Columbia blood agar (for all *Candida* species but *C. glabrata*) or Schaedler's agar with vitamin K and 5% sheep blood (for *C. glabrata*) was used for this purpose (BBL Microbiology Systems, Cockeysville, Md.). Colonies from 18- to 24-h cultures were suspended in sterile saline to an optical density of 1.0 McFarland turbidity standard. Immediately, 10 μl of this saline suspension was transferred to 10 ml of sterile saline. After the suspension was mixed, it was poured into an inoculation tray. By use of a 12-channel pipette and sterile tips, 10 μl of the inoculum was added to wells 1 to 11 (wells 1 to 10 contained 0.125 to 64 μg of fluconazole per ml; well 11 was the growth control; well 12 was the sterility control or blank) in RPMI medium-MOPS (morpholinepropanesulfonic acid) buffer (pH 7) (1). This inoculum is equivalent to 2×10^3 to 5×10^3/ml. The microtiter plate was shaken on a plate shaker (Dynatech Laboratories, Inc., Chantilly, Va.) for 15 to 30 s to

TABLE 1. Fluconazole susceptibility of *Candida* species

Species	Yr collected	No.	Concn (μg/ml)		
			MIC_{50}	MIC_{90}	Range
C. albicans	1987–1989	36	≤0.125	0.25	≤0.125–1
	1991–1992	121	≤0.125	0.25	≤0.125–1
	1987–1992	157	≤0.125	0.25	≤0.125–1
C. tropicalis	1987–1989	9	0.5	2	≤0.125–2
	1991–1992	39	0.5	1	≤0.125–4
	1987–1992	48	0.5	1	≤0.125–4
C. parapsilopsis	1987–1989	20	1	1	0.25–2
	1991–1992	20	0.5	1	≤0.125–4
	1987–1992	40	0.5	1	≤0.125–4
C. glabrata	1987–1989	16	1	4	0.25–4
	1991–1992	50	2	8	0.25–16
	1987–1992	66	2	8	0.25–16
C. krusei	1987–1989	1		16	
	1991–1992	17	16	32	16–32
	1987–1992	18	16	32	16–32

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TABLE 2. Comparison of microtiter and NCCLS methods for susceptibility testing of *Candida* species

Species (*n*)	MIC by Microtiter method (μg/ml)	Total no. of strains	No. of strains with MIC by NCCLS method of[a]:			
			Same	+1	−1	>+1
Candida spp. (41)[b]	≤0.125	9	3	6	0	0
	0.25	10	5	4	0	1
	0.5	5	3	1	1	0
	1	2	0	0	2	0
	2	1	1	0	0	0
	4	2	0	1	0	1
	16	9	1	6	0	2
	32	2	1	1	0	0
	64	1	1	0	0	0
Total		41	15	20	3	4
C. glabrata (16)	≤0.125–16	16	1	1	1	13

[a] The MIC resulting from the NCCLS method was either equal to (Same), one dilution tube greater than (+1), one dilution tube less than (−1), or more than one dilution tube greater than (>+1) the MIC of the microtiter method.

[b] The *Candida* spp. included *C. albicans* (20 isolates), *C. tropicalis* (11 isolates), and *C. krusei* (10 isolates).

TABLE 3. Comparison of MICs of microtiter and NCCLS methods at 24 and 48 h

Candida species	Strain no.	MIC (μg/ml) at:			
		24 h		48 h	
		Microtiter[a]	NCCLS	Microtiter	NCCLS[a]
C. glabrata	1	2	4	8	16
	2	4	4	16	16
	3	2	4	8	16
	4	2	4	8	16
	5	0.5	1	4	2
	6	2	4	8	64
	7	4	4	8	16
	8	4	4	8	16
C. krusei	1	32	16	64	32
	2	16	16	32	32
	3	16	16	32	32
	4	16	16	64	64
	5	16	16	32	32
	6	16	16	32	32
	7	16	16	32	32

[a] Time used as MIC end point.

ensure even distribution of the inoculum. After incubation at 35°C for 24 h, the microtiter plate was removed from the incubator and shaken for 5 min (setting 50; Dynatech plate shaker), and the optical density of each well was read at 530 nm (model MR400 Dynatech plate reader). The MIC was considered to be that concentration of drug which allowed 25% or less growth than that of the control (1, 8). *C. albicans* ATCC 14053 was used to test each new batch of microtiter plates and was included initially in every series of MICs tested. In all cases, the MIC for this strain was ≤0.125 or 0.25 μg/ml, predominantly 0.25 μg/ml. For future quality control purposes, the use of a *Candida* strain for which the MIC is higher (0.25 to 0.5 μg/ml) should ensure growth at the end point. The growth on blood agar (or Schaedler's agar) prior to susceptibility testing effectively resolved the tailing problem seen previously with azoles (4, 8). When it occurred, growth decreased dramatically to give a clear end point rather than gradually declining with a tailing end point through a number of dilution wells, as seen previously. This effect may, at least in part, be related to the growth phase of these microorganisms when they are exposed to azole (2), an important factor in antibacterial susceptibility testing. *Candida* species grow well on blood agar; however, *C. glabrata* has special growth requirements (6). All strains of this fungus grew well on Schaedler's agar. To confirm that prior growth on this medium, which contains yeast extract, did not interfere with end point determination, four strains of *C. glabrata* were grown on both Schaedler's agar and in RPMI medium prior to susceptibility testing by the microtiter method. In three cases, the MICs for the strains were identical, and in one case, there was a single dilution difference. Any carryover of yeast extract would likely result in a much greater discrepancy between the two groups.

The MICs for 50 and 90% of the strains (MIC_{50} and MIC_{90}, respectively) obtained before and after the institutional use of fluconazole are shown in Table 1. The MIC_{50} and MIC_{90} of fluconazole for *C. albicans* appear to be less than those of other species.

A total of 57 *Candida* strains were also tested by the NCCLS-recommended method. The results are shown in Table 2. For most *Candida* species tested, the NCCLS method gave an MIC equal to or one tube greater than that of the microtiter method. These strains, including five *C. albicans* strains that are susceptible to different concentrations of drugs (0.25 to 64 μg/ml for fluconazole), ranked similarly by both methods. For *C. glabrata*, there was a greater discrepancy of end points between the two methods. End points for this microorganism are more affected than those for other *Candida* species by the difference in incubation period between the microtiter (24 h) and macrodilution (48 h) methods. This was established by determining the MICs for eight *C. glabrata* and seven *C. krusei* strains by both methods at 24 and 48 h (Table 3). These species were selected because the MICs determined by the NCCLS method for them were high but those determined by the microtiter method were significantly different.

A distribution of *Candida* species recovered from blood cultures was obtained by review of the St. Luke's Episcopal Hospital clinical microbiology laboratory records of all blood cultures from 1987 to 1992 (Table 4). Blood cultures were performed by either broth amplification (BACTEC 660; Becton Dickinson, Cockeysville, Md.), lysis centrifugation (ISOSTAT; Wampole Laboratories, Cranbury, N.Y.), or both, depending on the physician's request. The annual proportion of blood cultures obtained by lysis centrifugation was approximately 10%. Between 1987 and 1992, the volume of blood cultures submitted to the laboratory showed an increase of 24%, but the proportion of total positive blood cultures as well as the proportion of cultures growing yeast species remained relatively constant. Results, however, showed a marked shift in the distribution of yeast species over the 5-year period (Table 3). In 1987, *C. albicans* was the most commonly isolated yeast, making up 87% of the total. This number declined to a low of 31% in 1992 with concomitant increases in *C. glabrata* (2 to 26%), *C. tropicalis* (2 to 24%), and *C. parapsilosis* (9 to 20%) over the same 5-year period. These results do not take into account patterns of individual patient use of fluconazole but rather reflect overall changes in distribution of *Candida* species isolated in the clinical laboratory. Coincidentally, fluconazole use in St. Luke's Episcopal Hospital rose from 0 in 1987 to around 7,000 patient doses per year in 1992. As suggested by the fluconazole susceptibility data on clinical yeast isolates, it is interesting to speculate that the increased use of fluconazole may have contributed to a shift in the incidence of fungemia

TABLE 4. Distribution of *Candida* species in blood cultures collected from 1987 to 1992

Yr	Blood cultures		Total no. (%) positive cultures for:				
	Total no.	No. (%) positive	*Candida* spp.	*C. albicans*	*C. glabrata*	*C. parapsilosis*	*C. tropicalis*
1987	11,684	1,327 (11)	54 (4)	47 (87)	1 (2)	5 (9)	1 (2)
1988	11,412	1,226 (11)	60 (5)	41 (75)	9 (15)	5 (8)	5 (8)
1989	10,126	1,028 (10)	46 (4.5)	39 (85)	5 (11)	2 (4)	0 (0)
1991	15,175	1,730 (11)	112 (6.5)	51 (46)	30 (27)	14 (13)	17 (15)
1992	14,540	1,673 (12)	102 (6)	32 (31)	26 (26)	20 (20)	24 (24)

due to yeasts other than *C. albicans*, which may be less responsive to fluconazole therapy.

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REFERENCES

1. **Anaissie, E., V. Paetznick, and G. P. Bodey.** 1991. Fluconazole susceptibility testing of *Candida albicans*: microtiter method that is independent of inoculum size, temperature, and time of reading. Antimicrob. Agents Chemother. **35:**1641–1646.
2. **Beggs, W. H.** 1984. Growth phase in relation to ketoconazole and miconazole susceptibilities of *Candida albicans*. Antimicrob. Agents Chemother. **25:**316–318.
3. **Galgiani, J.** 1989. Progress in standardizing antifungal susceptibility tests. Clin. Lab. Med. **9:**269–277.
4. **LaRocco, M.** 1991. Recent developments in antifungal susceptibility testing. Clin. Microbiol. Newsl. **13:**81–85.
5. **National Committee for Clinical Laboratory Standards.** 1992. Reference method for broth dilution antifungal susceptibility testing of yeasts. Proposed guideline M27-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
6. **Odds, F. C.** 1988. Biological aspects of pathogenic *Candida* species, p. 7–15. *In Candida* and candidosis: a review and bibliography, 2nd ed. Saunders, London.
7. **Pfaller, M. A., B. Dupont, G. S. Kobayashi, J. Muller, M. G. Rinaldi, A. Espinel-Ingroff, S. Shadomy, P. F. Troke, T. J. Walsh, and D. W. Warnock.** 1992. Standardized susceptibility testing of fluconazole: an international collaborative study. Antimicrob. Agents Chemother. **36:**1805–1809.
8. **Shawar, R., V. Paetznick, Z. Witte, L. G. Ensign, E. Anaissie, and M. LaRocco.** 1992. Collaborative investigation of broth microdilution and semisolid agar dilution for in vitro susceptibility testing of *Candida albicans*. J. Clin. Microbiol. **30:**1976–1981.