ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1993, p. 685–691
0066-4804/93/040685-07$02.00/0

Vol. 37, No. 4

Effects of Temperature on Anti-*Candida* Activities of Antifungal Antibiotics

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The relative growth (percentage of growth relative to control growth) of 767 *Candida* isolates representing five species was measured in microcultures at 25 and 37°C. In the presence of 10^{-4} M flucytosine, the distribution of relative yeast growth data indicated that Candida albicans isolates were less susceptible at 25°C than at 37°C, while the opposite was found with 4×10^{-5} M amorolfine for most of the isolates tested. Repetition of the experiments at four different temperatures with 99 *C. albicans* isolates and five antifungal agents confirmed a direct relationship between growth inhibition and increasing temperature from 25 to 40°C with amphotericin B, flucytosine, and terconazole; a strong inverse relationship between inhibition and temperature with amorolfine; and a weak inverse relationship with terbinafine. However, these relationships were not always noted with other *Candida* spp.: in particular, the growth of *C. glabrata* and *C. parapsilosis* isolates tended to be greater at 37°C than at 25°C in the presence of the azole-derivative antifungal agents itraconazole and terconazole. These findings stress the species-specific individuality of yeast susceptibility to azole antifungal agents. The results with *C. albicans* and amorolfine and terbinafine accord with their known in vivo efficacy in mycoses involving low-temperature superficial sites and poor activity against mycoses involving deep body sites. The data also reinforce the need for control of experimental variables such as temperature in the design of standardized yeast susceptibility tests.

Irreproducibility in antifungal susceptibility studies with pathogenic yeasts is a widely recognized problem (5–9, 15, 19, 23). Several environmental factors of which the most frequently cited are inoculum size, duration of incubation, and medium composition (2, 3, 5–9, 15, 19, 23, 25), are known to influence the outcome of susceptibility tests in vitro.

Incubation temperature is another variable that might influence antifungal MICs for yeasts in broth dilution tests, but the nature and extent of temperature effects differ for different authors. Most studies have shown minor or no differences in MICs of various antifungal agents tested against yeasts at temperatures ranging from 22 to 37°C (1, 3, 13, 16, 17). However, Block et al. (2) noted a considerable increase in the susceptibility of *Cryptococcus neoformans* to flucytosine at 37°C compared with that at 32°C, while precisely the opposite effect of temperature was noted by Johnson et al., working with nystatin (14), and Galgiani et al., working with flucytosine (11), both in tests with *Candida* spp. An incubation temperature of 35°C has emerged in some recent reports as preferable both to 37°C (5) and to 30°C (23, 25) for optimal reader agreement with visual MIC endpoints. Temperature was unequivocally a less significant factor influencing interlaboratory agreement between MICs than was the composition of the test medium (23).

In all of the reports cited, MIC was the susceptibility endpoint used to determine the effect of temperature, with one exception in which ion efflux in response to nystatin was measured with a K^+ electrode (14). However, it has often been stressed that azole-derivative antifungal agents frequently cause partial inhibition of yeast growth at concentrations well below the MIC (1, 8, 12, 19, 21). An MIC determination records only the point of complete growth inhibition and takes no account of the possible significance of measurable but incomplete inhibitory effects at lower drug concentrations. Several alternative susceptibility measurements that are based on non-MIC endpoints of an azole versus a yeast have been devised, for example, the 50% inhibitory concentration (10), the relative area under the dose-response curve (22), and relative yeast growth at a single azole concentration below the MIC (21), but these have not yet been used to examine the influence of temperature on yeast susceptibility in vitro. The present study was undertaken to examine the effects of temperature on relative yeast growth at single, sub-MIC antifungal concentrations; it was prompted by the serendipitous finding of some marked alterations in relative yeast growth associated with an incubator breakdown.

MATERIALS AND METHODS

The study comprised two main experimental elements: a retrospective analysis of data for inhibited yeast relative growth at two temperatures and a prospective study of the effects of four different incubation temperatures. In both experiments large numbers of yeast isolates were tested in batches of up to 50 isolates at a time. The tests involved cultures prepared in 96-well microdilution plates according to the protocol detailed elsewhere (20). Susceptibility of yeasts to antifungal agents was determined in terms of their relative growth in the presence of a single antifungal concentration lower than the characteristic anti-*Candida* MIC of each agent (21). Relative growth is the turbidity of a test culture expressed as a percentage of control growth: the higher the relative growth percentage for a given yeast isolate-antifungal agent combination is, the lower the susceptibility of the isolate to that agent is.

All of the yeast isolates tested were originally obtained from clinical samples, and each was a unique isolate from any individual site in a patient. Thus, repeated isolations from the same site in a patient were excluded, but isolates from different sites in a single patient were included. In the retrospective analysis, a total of 767 yeast isolates representing five *Candida* spp. were tested. For each species, some isolates were obtained freshly from the original isolation plate within 6 weeks of its inoculation with clinical material while others had been maintained in culture or under distilled water for periods varying from 6 months to several

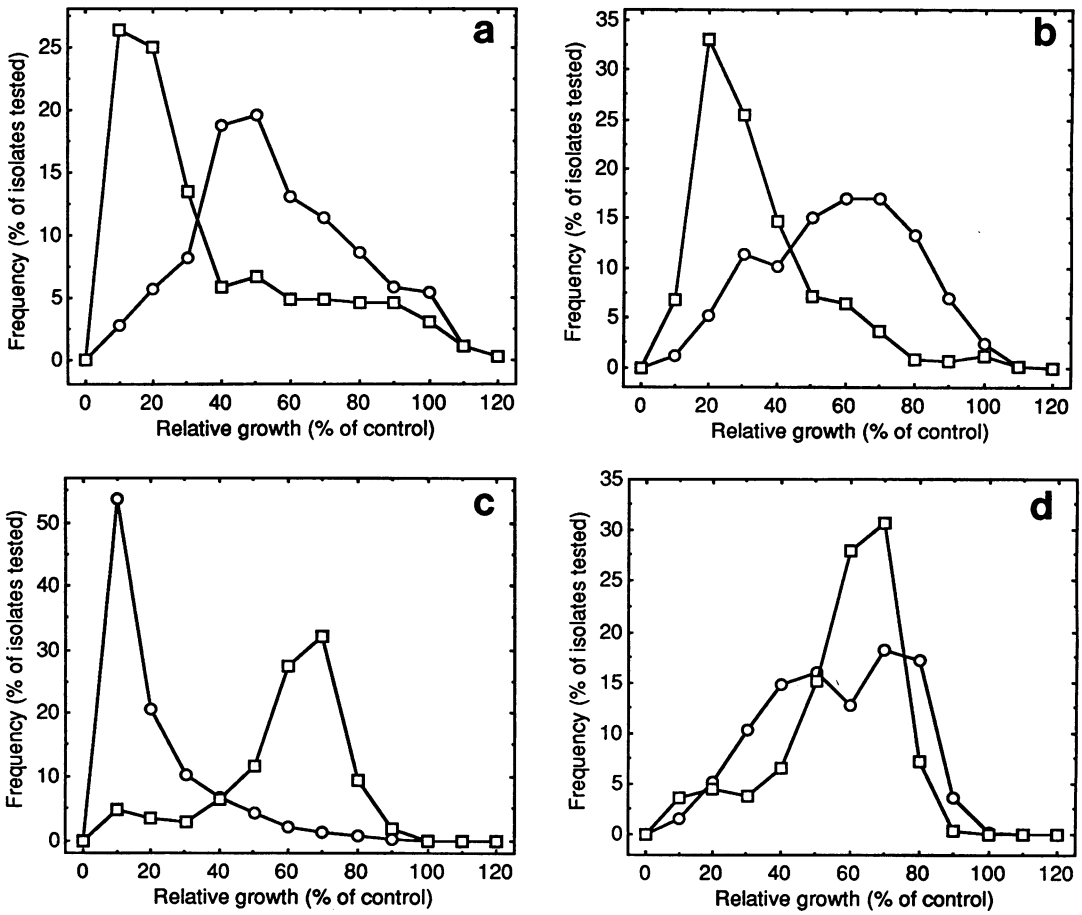

FIG. 1. Distribution curves for relative growth of 502 *C. albicans* isolates in cultures with 1×10^{-4} M flucytosine (a), 1×10^{-6} M terconazole (b), 4×10^{-5} M amorolfine (c), and 32 mM boric acid (d). ○, 25°C; □, 37°C.

years. Details of the isolates studied were as follows: *Candida albicans*, 502 isolates, including 338 fresh isolates; *Candida glabrata*, 84 isolates (36 fresh); *Candida krusei*, 52 isolates (6 fresh); *Candida parapsilosis*, 90 isolates (29 fresh); and *Candida tropicalis*, 39 isolates (2 fresh). In the prospective, four-temperature experiment, the test panel comprised 99 *C. albicans* isolates, all tested within 3 weeks to 3 months of their original recovery from clinical material. All isolates were maintained in subculture on buffered casein hydrolysate-yeast extract-glucose (CYG) agar (21).

The antifungal agents tested were flucytosine and boric acid (both from Janssen Chimica, Geel, Belgium); amphotericin B (Fungizone; Bristol Myers-Squibb, Princeton, N.J.), itraconazole, ketoconazole, miconazole, and terconazole (Janssen Pharmaceutica, Beerse, Belgium); amorolfine (Hoffmann-La Roche, Basel, Switzerland); and terbinafine (Sandoz Forschungsinstitut, Basel, Switzerland). The single test concentrations chosen for amorolfine, flucytosine, terbinafine, and terconazole were those shown in previous experiments to reveal variation in the extent of relative growth inhibition between isolates (20, 21). Amphotericin B was tested at three concentrations that were chosen to cover the likely area of points of inflection in dose-response curves.

Amphotericin B was reconstituted to 5 mg of active compound per ml in sterile water; flucytosine was dissolved to 1.375 mg/ml in water and filter sterilized, and the remaining compounds were prepared as 1,100-fold concentrated solutions in dimethyl sulfoxide. These stock solutions were stored at −20°C for up to 3 months. The test medium was CYG (20, 21). The medium was filter sterilized, and then stock solutions of test compounds were diluted 100-fold in portions of the medium to yield 11 times the chosen final concentration of the antifungal agent. The test media were added to microdilution plate wells in 20-μl volumes, and the plates were stored for up to 2 months at −20°C. Rows of wells containing different test substances also contained duplicate or triplicate positive growth control wells to which no test substance was added. In the prospective study dimethyl sulfoxide was added (1 ml/100 ml) to positive control media.

Yeast inocula were grown overnight at 30°C with rotation at 20 rpm in either YNBg, a glucose-limiting yeast nitrogen base medium (20), or CYGi, a 10-fold dilution of CYG (21), to yield suspensions containing 4×10^7 (± 0.5 $\log_{10}$) yeast cells per ml (20, 21). For inoculation of microdilution plates, 25 μl of YNBg culture was diluted in 10 ml of sterile water and 200-μl lots of this suspension were added to the microculture wells. The plates were sealed with stickers and incubated for 72 h at 25, 30, 37, or 40°C, each ±1°C. The incubation time was previously shown to be optimal for microculture relative growth experiments (21). The stickers were then removed from the incubated plates, and the turbidity of the yeast growth in each well was measured by

TABLE 1. Inhibitory effects of five antifungal compounds at four temperatures against 99 isolates of *C. albicans* as determined by percentage of growth relative to control growth

Test compound (concn)	Temp (°C)	Relative growth (%)		% of isolates growing to:	
		Geometric mean	Mode	≤20% of control	>50% of control
Amorolfine (4×10^{-5} M)	25	23.5	6	41.4	38.4
	30	54.9	80	7.1	85.9
	37	61.1	70	3.0	88.9
	40	47.9	69	8.1	65.6
Amphotericin B (0.3 μg/ml)	25	70.2	96	8.1	87.9
	30	23.3	5	45.5	49.5
	37	20.0	1	48.5	46.5
	40	17.7	6	53.5	39.4
Flucytosine (1.0×10^{-4} M)	25	35.2	38	10.1	34.3
	30	20.8	25	32.3	20.2
	37	11.8	9	69.7	16.2
	40	9.0	5	78.8	8.1
Terbinafine (16 μg/ml)	25	20.0	17	47.4	9.1
	30	35.6	35	12.1	15.1
	37	34.9	35	9.1	15.1
	40	28.8	35	17.1	7.1
Terconazole (1×10^{-6} M)	25	52.1	71	4.0	62.6
	30	44.2	75	5.1	53.5
	37	30.2	42	22.2	22.2
	40	17.3	11	60.6	1.0

monitoring its A_{405} with an automated microplate reader. The readings were corrected for blank absorbance by subtraction of 0.115 (average background reading calculated from preliminary tests). For each test isolate, the mean absorbance and coefficient of variation for triplicate positive growth control cultures were calculated. All isolates included in this study gave a mean A_{405} greater than 0.5 and a coefficient of variation of less than 20%. In practice fewer than 2.5% of all isolates gave a coefficient of variation greater than 15%. For each physiologic test or antifungal dilution, the A_{405} (corrected for blank absorbance) was expressed as a percentage of positive control growth.

RESULTS

Effects of 25 and 37°C incubation on relative yeast growth in the presence of antifungal agents. The relative growth phenotypes of yeasts incubated with antifungal agents at two temperatures were determined by retrospective analysis of data from *Candida* typing studies. Tests done with 502 *C. albicans* isolates showed that the distribution of growth turbidities in flucytosine or terconazole (expressed as a percentage of control turbidity) indicated more relative growth at 25°C than at 37°C (Fig. 1a and b) whereas those in cultures containing amorolfine showed the opposite effect, i.e., relative growth was generally less at 25°C than at 37°C (Fig. 1c), and cultures containing boric acid were less notably affected by incubation temperature (Fig. 1d). In the presence of 10^{-4} M flucytosine at 25°C only 8.4% of isolates were inhibited to ≤20% of control turbidity, compared with 51.2% of isolates at 37°C (Fig. 1a). Similarly, in cultures containing 10^{-6} M terconazole (Fig. 1b), only 6.4% were inhibited to ≤20% of control turbidity at 25°C, compared with 29.8% at 37°C. However, with 4×10^{-5} M amorolfine (Fig. 1c), 74.5% of *C. albicans* isolates were inhibited to ≤20% of the control level at 25°C, compared with only 8.2% at 37°C. The differences seen were unlikely to be related to major differences in amounts of control, drug-free growth at the two temperatures: the mean ± standard deviation for control A_{405} was 1.041 ± 0.082 at 25°C and 0.947 ± 0.087 at 37°C.

Effects of four temperatures on the antifungal susceptibility of *C. albicans* relative growth. To extend the observations of temperature effects on anticandidal behavior, a prospective study was set up involving 99 *C. albicans* isolates, each grown in microcultures at four different temperatures in the presence of five antifungal compounds, each of which represented a different chemical class of antifungal agent. Table 1 presents relative growth data that summarize the distributions of yeast relative growth with each agent and temperature. The results confirmed those of the two-temperature experiment with amorolfine, flucytosine, and terconazole.

For amorolfine the statistics indicated a distinct rise in relative yeast growth, and therefore a fall in susceptibility, between 25 and 30°C. Only minor differences in relative growth susceptibility were evident between 30 and 40°C. Terbinafine similarly showed a slightly higher inhibitory potency for *C. albicans* at 25°C compared with that at higher temperatures, although the data for terbinafine showed essentially no statistical variation in relative growth of *C. albicans* between 30 and 40°C. For amphotericin B, flucytosine, and terconazole, the relative growth data indicated a negative correlation with temperature (relative growth in the presence of these agents decreased with increasing incubation temperature), implying a positive correlation between susceptibility and temperature. The temperature association with relative yeast growth was virtually linear for flucytosine and terconazole (Table 1). Cultures with amphotericin B showed the most dramatic response to differences in temperature—only 8% of the isolates were inhibited to ≤20% of control growth at 25°C, compared with 54% at 40°C, and the modal (most frequent) relative growth datum fell from 96% at 25°C to 5% at 30°C and higher. However, amphotericin B was tested at 1 and 0.1 μg/ml in addition to the intermediate 0.3 μg/ml shown in Table 1. At these other concentrations no major temperature effect was discernible: most of the yeasts grew to below 20% of the control level at 1 μg/ml and grew to above 50% of the control level at 0.1 μg/ml, regardless of

TABLE 2. Inhibitory effects of 4×10^{-5} M amorolfine at two incubation temperatures on the relative growth (compared with drug-free control growth) of five *Candida* species

Species (*n*)	Temp (°C)	Relative growth (%)		% of isolates growing to:	
		Geometric mean	Mode	≤20% of control	>50% of control
C. albicans (502)	25	10.3	6	74.5	4.2
	37	47.3	65	8.2	70.7
C. glabrata (84)	25	23.8	2	31.0	39.3
	37	22.0	25	33.3	14.3
C. krusei (52)	25	5.2	1	98.1	1.9
	37	4.3	2	100.0	0.0
C. parapsilosis (90)	25	4.9	5	97.7	0.0
	37	12.9	15	73.3	8.8
C. tropicalis (39)	25	14.6	15	51.3	20.5
	37	27.7	5	20.5	20.5

the incubation temperature. The data for terconazole suggested a particularly large increase in yeast susceptibility to this compound at 40°C compared with that at 37°C.

Growth in control cultures showed a modest tendency towards higher turbidities at lower temperatures. The mean ± standard deviation for control A_{405} at each of the four temperatures tested was 1.008 ± 0.066 (25°C), 0.986 ± 0.059 (30°C), 0.956 ± 0.065 (37°C), and 0.875 ± 0.069 (40°C).

Temperature effects on relative growth of different *Candida* spp. To determine whether the influence of incubation temperature on relative growth inhibition of *C. albicans* by various antifungal agents extended to other *Candida* species, the distributions of relative growth for four other species were compared with those for *C. albicans* in the presence of five different antifungal agents at 25 and 37°C. The marked increase in relative growth seen with amorolfine and *C. albicans* was not evident with *C. glabrata*, *C. krusei*, and *C. parapsilosis* (Table 2). Data indicating a decrease in amorolfine susceptibility at 37°C compared with that at 25°C were obtained only for isolates of *C. tropicalis*. The data for *C. krusei* and *C. parapsilosis* indicated that these species were generally more susceptible than *C. albicans* to amorolfine inhibition at both incubation temperatures.

For all five *Candida* spp. tested, flucytosine showed a greater inhibitory potency (lower relative growth) at 37°C than at 25°C (Table 3). The relative growth data were broadly similar for *C. albicans*, *C. glabrata*, and *C. parapsilosis* in the presence of flucytosine, but the figures for *C. krusei* and *C. tropicalis* suggested a generally lower susceptibility of these species to flucytosine.

With terconazole, the relative growth distribution statistics showed susceptibility properties that were species specific in terms of both temperature effects and overall susceptibility (Table 4). Isolates of *C. albicans* and *C. tropicalis* were both more strongly inhibited at 37°C than at 25°C. However, relative growth of the other three species in the presence of terconazole showed little variation between the two temperatures and even a suggestion of a small negative temperature effect on inhibition (i.e., a positive effect on relative growth) at the higher temperature. The data indicated an overall greater susceptibility of *C. krusei* and *C. parapsilosis* to terconazole than the other three species tested.

Itraconazole, like terconazole a triazole-derivative antifungal agent, also showed tendencies to species-specific effects in terms of both overall susceptibility and temperature influences (Table 5). With itraconazole the influence of temperature on relative growth distributions of *C. glabrata* and *C. parapsilosis* was clearly the inverse of that seen with *C. albicans*. The first two species grew to higher relative yields at 37°C than at 25°C. The results for *C. krusei* and *C. tropicalis*, by contrast, suggested little or no influence of the two temperatures on the distributions of their relative growth in the presence of itraconazole. Among the five species tested, the relative growth data overall suggested that itraconazole was more inhibitory for the non-*C. albicans Candida* spp. tested.

Only *C. tropicalis*, among the species studied, gave relative growth distributions suggestive of temperature influences on the action of terbinafine under the experimental conditions used (Table 6). The data distributions for *C. tropicalis* grown in the presence of terbinafine indicated a

TABLE 3. Inhibitory effects of 10^{-4} M flucytosine at two incubation temperatures on the relative growth (compared with drug-free control growth) of five *Candida* species

Species (*n*)	Temp (°C)	Relative growth (%)		% of isolates growing to:	
		Geometric mean	Mode	≤20% of control	>50% of control
C. albicans (502)	25	43.9	45	8.4	45.2
	37	19.7	7	51.2	22.9
C. glabrata (84)	25	33.4	44	14.3	28.6
	37	18.0	6	51.2	16.7
C. krusei (52)	25	73.1	71	0.0	92.3
	37	49.1	65	3.8	63.5
C. parapsilosis (90)	25	52.7	74	5.5	66.7
	37	35.2	9	24.4	43.3
C. tropicalis (39)	25	70.8	74	0.0	94.9
	37	46.3	42	10.1	51.3

TABLE 4. Inhibitory effects of 10^{-6} M terconazole at two incubation temperatures on the relative growth (compared with drug-free control growth) of five *Candida* species

Species (*n*)	Temp (°C)	Relative growth (%)		% of isolates growing to:	
		Geometric mean	Mode	≤20% of control	>50% of control
C. albicans (502)	25	47.1	66	6.4	56.8
	37	22.9	17	39.8	12.8
C. glabrata (84)	25	25.1	76	26.2	52.4
	37	39.9	68	14.3	50.0
C. krusei (52)	25	15.6	2	59.6	28.8
	37	18.3	3	38.5	26.9
C. parapsilosis (90)	25	4.1	3	93.3	2.2
	37	12.1	17	75.6	2.2
C. tropicalis (39)	25	30.2	62	23.0	25.6
	37	26.0	13	33.3	10.3

lower susceptibility at 37°C than at 25°C. Isolates of *C. parapsilosis* reached markedly lower relative growth yields overall in the presence of terbinafine, suggesting a particular susceptibility of this species to this agent.

Additional evidence concerning temperature effects on anti-*Candida* activity of antifungal agents. To determine whether the temperature effects described above for terconazole and itraconazole were specific to these compounds or were similar for other azole-derivative antifungal agents, a short study was undertaken at 25 and 37°C with ketoconazole and miconazole at final concentrations of 1×10^{-6} and 3×10^{-7} M, respectively. Among 80 fresh *C. albicans* isolates tested with these azoles, there were, respectively, 7.5 and 12.5% of isolates inhibited to 20% or less of control growth by ketoconazole and miconazole at 25°C, compared with 50.0 and 58.8% at 37°C. These data confirm that the increased susceptibility of *C. albicans* at the higher incubation temperature was similar for all four azole antifungal agents tested in this study.

DISCUSSION

The nature of the in vitro susceptibility measurement used in this study is unorthodox, and it has not yet been subjected to scrutiny of its relevance to the outcome of therapy in vivo. Nevertheless, relative growth of a yeast in the presence of a single inhibitor concentration is a valid comparative measure of the inherent susceptibility of yeast strains and species at inhibitor concentrations below the MIC, and it may be therefore indicate more subtle potential differences in the response of isolates to low drug concentrations than can be detected or predicted with MIC endpoints. This might explain why much more substantial effects of temperature on yeast susceptibility were found in this study than in MIC-based studies that found little or no influence of temperature on susceptibility in vitro (1, 3, 13, 16, 17).

The limitation of relative growth as a susceptibility measurement can be seen in the experiments done with amphotericin B: over a 1-log change in concentration from 1.0 to 0.1 μg/ml, most of the *C. albicans* isolates tested changed from a low to a high relative growth percentage; only at the (logarithmic) midpoint between these two concentrations (0.3 μg/ml) was there a broad distribution of relative growth between 0 and 100% of the control level for the 148 yeast isolates, and only at this concentration was a temperature effect evident. Yet the extent of the effect for amphotericin B was dramatic at the intermediate concentration.

It is well known that the slope of inflection between full growth and full inhibition of yeasts is very sharp for an amphotericin B dose-response curve (see, e.g., reference 18), whereas partial inhibitory effects with flucytosine and azole-derivative compounds lead to much more extended inflection slopes with these agents (18, 21) and a consequently greater potential relevance of differential susceptibility measurement within the inflection concentration region. The present study indicates that the susceptibility of some yeasts to sub-MIC levels of flucytosine and the azole compounds increases with rising incubation temperature in vitro: this can only be interpreted as a positive finding in the context of treatment of patients in whom fever is a virtually universal symptom of deep-seated yeast infection. It also conforms with the findings of two MIC-based studies, one with flucytosine and *Cryptococcus* isolates (2) and one with

TABLE 5. Inhibitory effects of 10^{-6} M itraconazole at two incubation temperatures on the relative growth (compared with drug-free control growth) of five *Candida* species

Species (*n*)	Temp (°C)	Relative growth (%)		% of isolates growing to:	
		Geometric mean	Mode	≤20% of control	>50% of control
C. albicans (344)	25	41.3	52	10.5	48.5
	37	22.9	14	37.5	18.4
C. glabrata (54)	25	10.6	4	68.4	16.7
	37	25.4	14	35.2	16.4
C. krusei (52)	25	1.2	1	96.2	3.8
	37	5.3	2	94.2	1.9
C. parapsilosis (90)	25	5.8	2	94.4	2.2
	37	11.6	5	74.4	3.3
C. tropicalis (39)	25	23.7	25	38.5	23.1
	37	25.4	18	35.9	10.3

TABLE 6. Inhibitory effects of terbinafine (16 μg/ml) at two incubation temperatures on the relative growth (compared with drug-free control growth) of five *Candida* species

Species (*n*)	Temp (°C)	Relative growth (%)		% of isolates growing to:	
		Geometric mean	Mode	≤20% of control	>50% of control
C. albicans (344)	25	57.0	71	5.8	79.9
	37	60.1	63	2.3	86.6
C. glabrata (54)	25	88.3	88	0.0	100.0
	37	84.7	87	1.9	92.6
C. krusei (52)	25	63.1	82	7.7	88.5
	37	70.0	87	7.7	92.3
C. parapsilosis (90)	25	8.3	2	87.8	3.3
	37	10.6	4	75.6	16.5
C. tropicalis (39)	25	56.9	64	7.7	69.2
	37	80.3	95	0.0	89.7

azoles and dermatophytes (27), in which a similar marked increase in susceptibility was noted at higher incubation temperatures.

However, the influences of temperature on relative growth were not noted universally among the five *Candida* species studied, except in the case of cultures with flucytosine. Indeed, for *C. glabrata* and *C. parapsilosis* the relative susceptibility of the test isolates to itraconazole was lower at 37°C than at 25°C and to terconazole was possibly similarly lower at the higher temperature, even though the overall relative growth distributions for these species at both temperatures suggested an overall susceptibility equal to or greater than that of *C. albicans* to both agents. *C. parapsilosis* showed a much lower relative growth in the presence of terbinafine than the other four species, regardless of incubation temperature: this observation correlates with previous MIC data suggesting an exquisite susceptibility of this species to this agent (4). The results presented in this study emphasize the individuality of susceptibility responses of different *Candida* species to different antifungal agents.

Neither amorolfine nor boric acid, the two agents that were found in this study to have a reduced yeast inhibitory potency at or above 30°C, has proved to be effective for the treatment of yeast infections involving deep organs where a body temperature of 37°C or higher is the norm, though both have some efficacy for topical treatment of yeast infections of superficial sites (24, 26) where the temperature is lower. Thus, their temperature and activity spectrum in vivo reflects the susceptibility spectrum found in vitro in the present study.

Notwithstanding possible extrapolations of relative growth data in vitro to performance of antifungal agents in vivo, the temperature effects described in the present study reinforce the need for careful standardization of all experimental variables in the design of standard antifungal susceptibility test protocols. Widely different incubation temperatures are currently favored by different laboratories, and such differences are known to have an impact on the poor interlaboratory reproducibility of MIC data (23) even though temperature appears less significant than other variables in influencing MIC. The current trend is towards 35°C as a standard average incubation temperature for susceptibility testing with antifungal agents, and indeed 35°C is to be recommended as optimal for yeast susceptibility testing by the National Committee for Clinical and Laboratory Standards (7a). However, the results of this study suggest that the quality of correlation between in vitro and in vivo susceptibilities may in fact require different incubation standards for different susceptibility situations: a temperature lower than 35°C may be more appropriate for susceptibility determinations in the context of superficial-site infections, while a temperature of 37°C or above may reflect better the real situation in a deep-organ mycosis.

ACKNOWLEDGMENT

The skilled technical assistance of Gery Dams is gratefully acknowledged.

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