

Patterns of Amphotericin B Killing Kinetics against Seven *Candida* Species

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Received 28 November 2003/Returned for modification 1 February 2004/Accepted 1 April 2004

In a previous study tolerance to amphotericin B (AMB) was found among *Candida parapsilosis* and *C. dubliniensis* strains by seeding the whole volumes of wells used for MIC determinations, and minimum fungicidal concentrations (MFC) for non-*C. albicans* *Candida* strains were demonstrated to be above the levels safely achievable in serum. As an extension of that study, we performed time-kill assays with 26 blood culture isolates (6 *C. albicans*, 5 *C. parapsilosis*, 5 *C. krusei*, 4 *C. glabrata*, 3 *C. lusitaniae*, and 3 *C. tropicalis* isolates), 3 oropharyngeal *C. dubliniensis* isolates, 3 AMB-susceptible isolates (ATCC 90028, ATCC 22019, ATCC 6254), and 6 AMB-resistant isolates (ATCC 200955, ATCC 200956, ATCC 200950, ATCC 200951, ATCC 200952, ATCC 200953) using RPMI 1640 medium and 0.12 to 32 µg of AMB per ml and determined the numbers of CFU per milliliter at 0, 2, 4, 8, 12, 24, and 48 h. MFCs and time-kill patterns were species specific (MFCs, ≤1 µg/ml for all *C. dubliniensis* and *C. albicans* isolates except AMB-resistant strain ATCC 200955; MFCs, 2 to >16 µg/ml for the other isolates). The times required to reach the fungicidal endpoint (99.9% killing) at four times the MIC were 2 h for *C. albicans* and *C. dubliniensis*, 16 h for *C. glabrata*, 24 h for *C. parapsilosis* and *C. lusitaniae*, and ≥40 h for *C. tropicalis* and *C. krusei*. The killing rate increased as the AMB concentration was increased up to 2 µg/ml. The highest killing rates were achieved for *C. albicans*, *C. dubliniensis*, and *C. lusitaniae*, while viable *C. tropicalis*, *C. krusei*, and *C. parapsilosis* cells were present after 48 h (MICs, ≤2 µg/ml) when AMB was used at 2 µg/ml. Time-kill curves and MFCs can detect viable cells after 48 h when AMB is used at ≥2 µg/ml. The failure of AMB treatment could be due to its poor killing activity against some species at the concentrations reached in patients' serum.

Amphotericin B deoxycholate has been considered the “gold standard” for the treatment of invasive fungal infections since its introduction in the 1950s. Although the toxicity of this drug has somewhat limited its use, clinical experience has demonstrated that its less toxic lipid formulations can be used as suitable alternatives to the parental drug (18). Therefore, it is still important to obtain a clearer understanding of the killing patterns of amphotericin B. In general, the pharmacodynamic characteristics of amphotericin B have mainly been evaluated with *Candida albicans*, and scarce data are available for other *Candida* spp. In a prior study (4), by using an inoculum larger than that recommended in NCCLS document M27-A2 (16) and subculturing the total volume in the well used for MIC determination, we have shown that the difference between the fungistatic activities and the fungicidal activities could be species dependent among *Candida* spp. Tolerance to amphotericin B (by using the minimum fungicidal concentration [MFC] determination procedure described above) has been reported among *C. parapsilosis* and *C. dubliniensis* strains (4, 27), as have MFCs for non-*C. albicans* *Candida* isolates that are above the safe levels achievable in serum (4). Several investigators (17, 26, 32) have postulated that the MFCs and time-kill curve

study results could be more clinically relevant than the MICs of amphotericin B. Because time-kill curves provide a quantitative assessment of fungicidal activity as well as the rapidity of killing over time, they provide more information than MIC or MFC endpoints.

Our previous evaluation of the MICs versus the MFCs of amphotericin B deoxycholate suggested that the same MIC may correspond to different killing activities, depending on the species or the strain tested. The purpose of the present study was (i) to examine the killing patterns (by species and strains) of amphotericin B deoxycholate for seven *Candida* spp., (ii) to develop a mathematical model for the identification of the time (in hours) required for its fungicidal activity, and (iii) to examine the value of the MFC endpoint as an alternative means of detecting the fungicidal activity of amphotericin B deoxycholate.

MATERIALS AND METHODS

Drugs. Amphotericin B deoxycholate (Squibb Industria Farmacéutica, S.A. Grupo Bristol-Myers Squibb, Madrid, Spain) was dissolved in dimethyl sulfoxide to obtain a stock solution of 1,600 µg/ml. Further dilutions were made in standard RPMI 1640 medium (RPMI; Sigma Aldrich, Madrid, Spain), as recommended in NCCLS document M27-A2 (16).

Test isolates. A total of 38 strains were selected for testing. These strains comprised 26 isolates recovered from blood cultures (6 *C. albicans*, 5 *C. parapsilosis*, 5 *C. krusei*, and 4 *C. glabrata* isolates and 3 strains each of *C. lusitaniae* and *C. tropicalis*) and 3 *C. dubliniensis* isolates recovered from patients with oropharyngeal infections. In addition, three amphotericin B-susceptible isolates (*C.*

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albicans ATCC 90028, *C. parapsilosis* ATCC 22019, and *C. krusei* ATCC 6254) and 6 amphotericin B-resistant isolates (*C. albicans* ATCC 200955; *C. tropicalis* ATCC 200956; and *C. lusitaniae* ATCC 200950, ATCC 200951, ATCC 200952, and ATCC 200953) were evaluated (25).

MIC and MFC determinations. MICs and MFCs were determined as described previously (4). Briefly, MICs were evaluated by the NCCLS M27-A2 broth microdilution method by using two final inocula (2.5×10^3 and 2.5×10^4 CFU/ml). MFCs were determined by seeding the entire volumes from all clear MIC wells with the highest inoculum onto Sabouraud dextrose agar (SDA) plates. The MFC was the lowest drug concentration that killed 99.9% (with less than five colonies remaining) of the final inoculum.

Time-kill curve studies. Before the time-kill curve studies were performed, the antifungal carryover effect was determined by the method described by Klepser et al. (12). Additionally, the aliquots were deposited as a spot onto the SDA plate and allowed to soak. After the plate had dried, streaking was performed as described by Moore et al. (15); no carryover antifungal effect was detected. Time-kill curve studies were performed in standard RPMI (16) by the method described by Klepser et al. (12, 13). Before the tests were performed, the isolates were subcultured at least twice and grown for 24 h at 35°C on SDA plates. The inoculum was adjusted spectrophotometrically to the density of a 0.5 McFarland turbidity standard at 530 nm. The adjusted inoculum suspension was diluted 1:20 in RPMI containing the appropriate concentrations of amphotericin B, and tubes with the test solution were incubated at 35°C without agitation; the final volume was 5 ml. This procedure yielded an initial inoculum ranging from 2×10^5 to 8×10^5 CFU/ml and amphotericin B concentrations of 0.12, 0.5, 2, 8, and 32 µg/ml. At predetermined time points (0, 2, 4, 6, 12, 24, and 48 h), a 0.1-ml aliquot was removed from both the control tube (drug free) and each tube with a test solution and serially diluted in sterile water. Volumes of 0.1 ml, 30 µl, or 10 µl (depending on the dilution and the concentration of the drug) were spread onto SDA plates and incubated at 35°C for 24 to 48 h to determine the numbers of CFU per milliliter. When the colony counts were expected to be less than 1,000 CFU/ml, samples of 30 or 10 µl were taken directly from the test solution and plated without dilution. The lower limit of accurate and reproducible detectable colony counts is 100 CFU/ml. All time-kill curve studies were conducted in duplicate and on two separate occasions.

Mathematical model for fungicidal activity. The killing kinetics of the fungicidal activity were analyzed by fitting the mean data at each time point to an exponential equation: $N_t = N_0 \times e^{-Kt}$, where N_t expresses the number of viable yeasts at time t , N_0 is the number of viable yeasts at the beginning of the experiment, K is the killing (or lethality) rate, and t is the incubation time. The exponential equation was transformed into a line by applying natural logarithms ($\log N_t = \log N_0 + Kt$). The goodness of fit of the data was determined from the correlation coefficient (r^2 ; range, 0.8 to 0.98). The fungicidal activities were compared by use of the K values, positive values of which indicate growth and negative values of which indicate killing. Thus, the seven time points on each killing curve were reduced to one value, K . The following parameters were derived from the killing equation: the mean times to achieve reductions in the proportions of viable cells of 50% ($t_{50} = 0.30103/K$), 90% ($t_{90} = 1/K$), and 99% ($t_{99} = 2/K$) and the time to reach the fungicidal endpoint ($t_{99.9} = 3/K$) for each amphotericin B concentration and each strain (3).

RESULTS

Antifungal susceptibility results. The MICs and MFCs of amphotericin B for the test isolates are shown in Table 1. Except for amphotericin B-resistant strain *C. albicans* ATCC 200955, MFCs were below 1 µg/ml for the *C. albicans* and *C. dubliniensis* isolates. For the strains belonging to other species, the MFCs were in the range of 2 to >16 µg/ml for all except two isolates: one *C. parapsilosis* isolate (MIC = 1 µg/ml) and *C. krusei* ATCC 6258 (MFC = 0.5 µg/ml). The MICs for the strains previously defined as resistant to amphotericin B were 1 to 8 µg/ml and the MFCs were 2 to >16 µg/ml, while for the other strains, the MICs were 0.06 to 2 µg/ml and the MFCs were 0.25 to >16 µg/ml.

Killing curve studies. The killing activity of amphotericin B for each species (mean and standard deviation) is represented in Fig. 1. The fungicidal activity of amphotericin B was very fast (2 to 4 h at concentrations equal to two times the MIC) against

TABLE 1. MICs and MFCs of amphotericin B for test isolates

Species (no. of isolates)	MIC range (µg/ml)	MFC range (µg/ml)
<i>C. albicans</i> (6)	0.06–0.25	0.25
<i>C. parapsilosis</i> (5)	0.25–1	1–8
<i>C. lusitaniae</i> (3)	0.06–0.5	2
<i>C. tropicalis</i> (3)	1	2
<i>C. krusei</i> (5)	1–2	2–>16
<i>C. dubliniensis</i> (3)	0.06–0.5	0.5
<i>C. glabrata</i> (4)	0.5	2
<i>C. albicans</i> ATCC 90028	0.12	0.25
<i>C. albicans</i> ATCC 200955 ^a	2	8
<i>C. parapsilosis</i> ATCC 22019	0.12	2
<i>C. lusitaniae</i> ATCC 200950 ^a	1	32
<i>C. lusitaniae</i> ATCC 200951 ^a	1	8
<i>C. lusitaniae</i> ATCC 200952 ^a	2	2
<i>C. lusitaniae</i> ATCC 200953 ^a	1	2
<i>C. tropicalis</i> ATCC 200956 ^a	8	>32
<i>C. krusei</i> ATCC 6258	0.5	0.5

^a Amphotericin B-resistant strains.

C. albicans and *C. dubliniensis*; the reduction in the number of CFU per milliliter was greater than 3 log units (99.9%). On the contrary, for *C. parapsilosis* the fungicidal endpoint was reached after 48 h of incubation at four times the MIC, and for *C. glabrata* it was reached after either 16 to 32 h at the MIC or 24 h at four times the MIC; the effect was strain dependent for the latter species. The killing activity of amphotericin B against *C. lusitaniae* was also strain dependent (either very fast or slow); 4 or 12 h was required for amphotericin B at eight times the MIC to be fungicidal against the three clinical strains assayed. There was a rapid decrease in the numbers of CFU per milliliter in the first 12 h, with little killing after that point for *C. tropicalis*. The results were similar for *C. krusei*; 48 h was required to reach the fungicidal endpoint for both species.

The relationship between amphotericin B concentrations and the lethality rate (K value) for the seven species of *Candida* is represented in Fig. 2. The highest K values were obtained for *C. dubliniensis*, *C. albicans*, and *C. lusitaniae* (−2.7, −1.4, and −1.4 CFU/ml/h, respectively, with amphotericin B concentrations of ≤0.5, 2, and 32 µg/ml, respectively). The lowest K value was obtained for *C. tropicalis* and *C. krusei* (−0.05 CFU/ml/h at an amphotericin B concentration of ≥2 µg/ml). With the exception of *C. dubliniensis* (K value, ≤0.5 µg/ml) and *C. lusitaniae* and *C. parapsilosis* (K values, 32 µg/ml), the K values increased as the amphotericin B concentration increased to 2 µg/ml.

Figure 3 summarizes the killing patterns of amphotericin B at 2 µg/ml for each species. Although the maximum K values for most of the species were obtained at this concentration, viable *C. parapsilosis*, *C. krusei*, and *C. tropicalis* cells were observed after 48 h of incubation with amphotericin B at concentrations of >8 µg/ml (corresponding MICs, ≤2 µg/ml). Table 2 summarizes the times required to kill 50, 90, 99, and 99.9% of the initial inoculum for all strains tested (including American Type Culture Collection [ATCC] isolates) with 2 µg of the drug per ml. The mean times required to kill 50% of the cells ranged from 6 and 10 min for *C. dubliniensis* and *C. albicans*, respectively, to 5.8 h for *C. tropicalis*. However, the killing of 99.9% of *C. parapsilosis*, *C. krusei*, and *C. tropicalis*

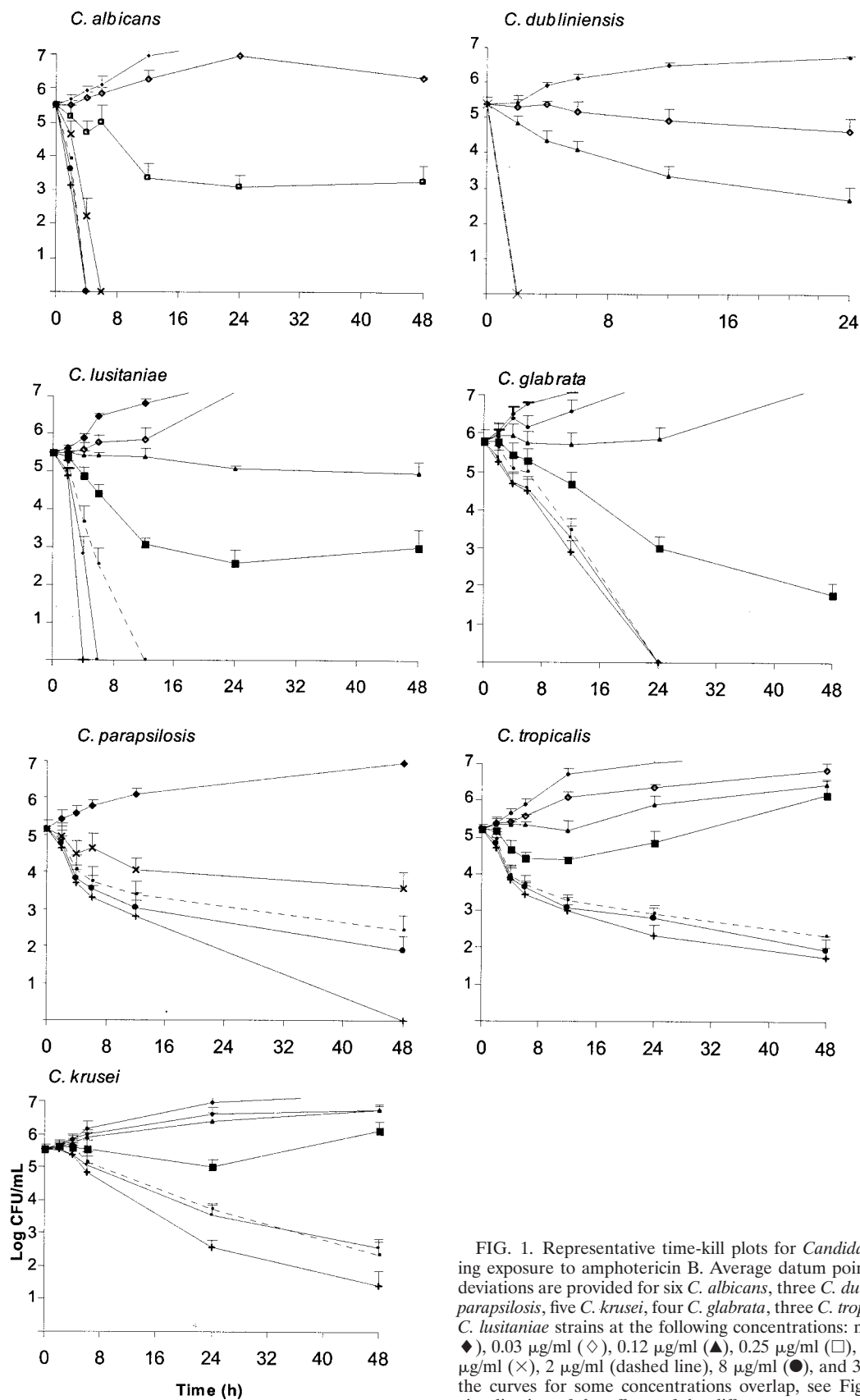


FIG. 1. Representative time-kill plots for *Candida* species following exposure to amphotericin B. Average datum points and standard deviations are provided for six *C. albicans*, three *C. dubliniensis*, five *C. parapsilosis*, five *C. krusei*, four *C. glabrata*, three *C. tropicalis*, and three *C. lusitanae* strains at the following concentrations: no drug (control; \blacklozenge), 0.03 $\mu\text{g/ml}$ (\diamond), 0.12 $\mu\text{g/ml}$ (\blacktriangle), 0.25 $\mu\text{g/ml}$ (\square), 0.5 $\mu\text{g/ml}$ (\blacksquare), 1 $\mu\text{g/ml}$ (\times), 2 $\mu\text{g/ml}$ (dashed line), 8 $\mu\text{g/ml}$ (\bullet), and 32 $\mu\text{g/ml}$ ($+$). As the curves for some concentrations overlap, see Fig. 2 for a better visualization of the effects of the different concentrations.

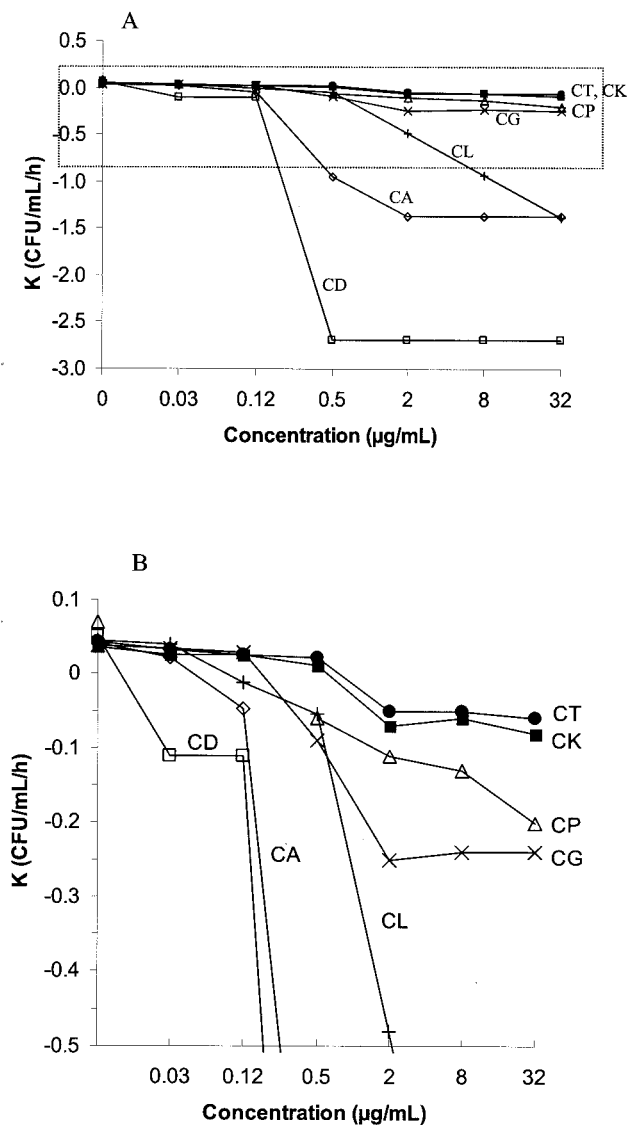


FIG. 2. (A) Relationship of amphotericin B concentrations and K values calculated from the regression line of survival times for *C. dubliniensis* (CD), *C. albicans* (CA), *C. lusitanae* (CL), *C. parapsilosis* (CP), *C. glabrata* (CG), *C. krusei* (CK), and *C. tropicalis* (CT). (B) Amplification of the area marked with dotted lines in panel A.

cells required longer incubation times (24 to >40 h) than those required for the other species (1 to 12 h).

The mean generation time determined from the growth control curves for each strain and species was 3.1 ± 0.5 h (range, 2.3 to 3.8 h), and the logarithmic phase lasted 12 h.

DISCUSSION

Plasma amphotericin B deoxycholate concentrations above 2 µg/ml have been associated with toxic effects and drug discontinuation, but such concentrations can be more safely achieved by using the less toxic lipid-associated formulations at increased dosages. The mean maximum concentrations of amphotericin B deoxycholate in serum (C_{max} s) reported after the

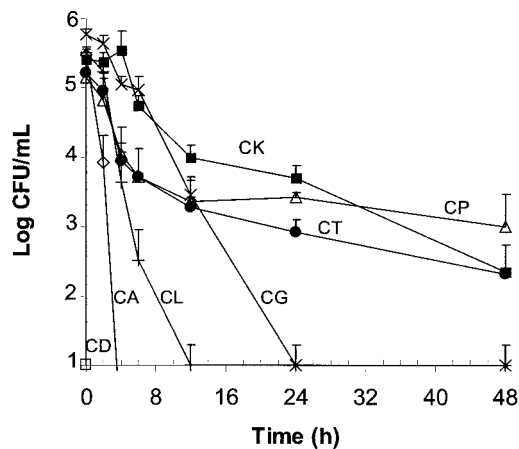


FIG. 3. Representative plots of mean \pm standard deviation time-kill curves for several *Candida* species with 2 µg of amphotericin B per ml: *C. dubliniensis* (CD; □), *C. albicans* (CA; ◇), *C. lusitanae* (CL; +), *C. parapsilosis* (CP; △), *C. glabrata* (CG; ×), *C. krusei* (CK; ■), and *C. tropicalis* (CT; ●).

administration of doses of 0.6 to 3 mg/kg of body weight/day are 1.1 to 3.6 µg/ml; for liposomal amphotericin B, however, C_{max} s are 17.2 to 83 µg/ml (after the administration of 3 to 5 mg/kg/day) and the C_{max} s of the lipid complex amphotericin B formulation are 22.9 µg/ml (after the administration of 2 mg/kg/day) (1, 7, 9, 28, 31). Furthermore, in the presence of serum, amphotericin B loses its killing activity (about 2 orders of magnitude); but the presence of serum does not affect its fungistatic activity (MIC for *C. albicans*), and it could have the same effect on other species (34). The method described in NCCLS guidelines is not reliable for the detection of resistance to amphotericin B in vitro, which has precluded the establishment of breakpoints. Although the Etest is used to determine the MICs of amphotericin B, the clinical value of Etest results needs to be determined.

Unlike earlier studies of fungicidal activity that only examined the 24-h killing effect of amphotericin B (8, 12, 13, 26), our investigation has identified the time required for the actual fungicidal activity (t_{50} to $t_{99.9}$) for each species and/or strain (Table 2 and Fig. 1) and has included a larger range of amphotericin B concentrations (0.03 to 32 µg/ml) than those previously studied with 3 *C. albicans* isolates (0.5 to 16 µg/ml [12] and 0.03 to 4 µg/ml [2]) and 11 *C. lusitanae* isolates (2 µg/ml [8]). Furthermore, we have included amphotericin B-resistant and -susceptible strains (ATCC strains) for which well-documented data from in vivo and in vitro studies are available (25).

Since in vitro testing with amphotericin B lipid-associated formulations can be influenced by the differences in drug release related to the lipid carrier, testing was performed with amphotericin B deoxycholate. The inclusion of a wide range of amphotericin B concentrations in the present study yielded a large number of curves, which precluded an easy comparison of the results. However, by mathematical calculation of the K value, the time-kill curve achieved with each concentration was reduced to one point, thus making it easier to visualize the killing pattern for each species (Fig. 2).

In our study, the killing pattern of amphotericin B was spe-

TABLE 2. Times to achieve 50, 90, 99, and 99.9% reductions in growth from starting inoculum with 2 µg of amphotericin B per ml

Isolate (no. of isolates)	t_{50} (h)		t_{90} (h)		t_{99} (h)		$t_{99.9}$ (h)	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
<i>C. albicans</i> (6)	0.10–0.22	0.16 ± 0.06	0.36–0.74	0.55 ± 0.19	0.72–1.47	1.09 ± 0.39	1.08–2.20	1.64 ± 0.58
<i>C. dubliniensis</i> (3)	0.10–0.11	0.11 ± 0.005	0.36–0.39	0.122 ± 0.43	0.72–0.78	0.75 ± 0.03	1.08–1.17	1.13 ± 0.05
<i>C. glabrata</i> (4)	1.23–0.11	0.56 ± 0.47	0.36–4.10	1.85 ± 1.57	0.72–8.21	3.74 ± 3.12	1.07–12.32	5.61 ± 4.68
<i>C. krusei</i> (5)	2.55–5.47	4.27 ± 1.45	8.49–18.18	14.18 ± 4.84	16.99–36	28.37 ± 9.69	25.48–54.55	42.55 ± 14.53
<i>C. lusitaniae</i> (3)	0.21–0.61	0.35 ± 0.23	0.72–2.04	1.16 ± 0.76	1.44–4.08	2.32 ± 1.51	2.16–6.11	3.48 ± 2.27
<i>C. parapsilosis</i> (5)	0.81–4.83	2.71 ± 1.53	2.71–16.07	8.99 ± 5.09	5.43–32.13	17.99 ± 10.19	8.15–48.20	26.99 ± 15.29
<i>C. tropicalis</i> (3)	5.28–6.15	5.82 ± 0.47	17.85–20.46	19.33 ± 1.56	35.10–40.92	38.67 ± 3.12	52.65–61.38	58.01 ± 4.68
<i>C. lusitaniae</i> ATCC 200950 ^a		5.04		16.7		33.5		>48
<i>C. lusitaniae</i> ATCC 200951 ^a		4.42		14.7		29.4		>48
<i>C. lusitaniae</i> ATCC 200952 ^a		3.78		12.38		24.4		>48
<i>C. lusitaniae</i> ATCC 200953 ^a		0.33		1.10		22		>48
<i>C. albicans</i> ATCC 200955 ^a		4.99		16.6		33.21		>48
<i>C. tropicalis</i> ATCC 200956 ^a		>48		>48		>48		>48
<i>C. albicans</i> ATCC 90028		0.65		2.17		4.34		6.5
<i>C. krusei</i> ATCC 6254		1.3		4.34		8.69		13.1
<i>C. parapsilosis</i> ATCC 22019		0.7		1.36		4.72		7.08

^a Amphotericin B-resistant strains.

cies and strain dependent; the fastest killing rate activity was achieved against *C. dubliniensis* and *C. albicans*, even in the presence of subinhibitory concentrations (one-half the MIC). Cells were killed by amphotericin B at concentrations below 1 µg/ml, as demonstrated by the absence of colonies and the absence of turbidity in the tube(s) used for the killing curve study. These results are in agreement with the results of 24-h killing curve studies reported previously (2, 12, 13).

Traditionally, *C. lusitaniae* has been considered intrinsically resistant to amphotericin B or prone to the development of resistance to this drug during the course of treatment (11, 14, 19, 21). Our data suggest that the rate of killing of *C. lusitaniae* by amphotericin B increases as the drug concentration increases (up to 32 µg/ml) and that the killing is strain dependent. Three of our recent clinical isolates were killed by 2 µg of amphotericin B per ml at 2 to 12 h, in agreement with the MFC (2 µg/ml). Other investigators have reported similar killing rates for strains of this species with 2 µg of amphotericin B per ml (8).

The frequency of *C. glabrata* as the cause of severe fungal infections has increased worldwide. *C. glabrata* is the *Candida* species that is the second most frequently associated with candidemia in the United States (5, 22). For this species, the fungicidal endpoint is reached after 16 h at four times the MIC. High Etest MICs (>1 µg/ml; 53% of strains) (22, 23) and MFCs >2 µg/ml (MFC for 90% of strains tested, 16 µg/ml) have been reported for this species (4).

C. parapsilosis is the *Candida* species that is the second most frequently recovered from children (5, 10, 20, 29) as well as a frequent cause of endocarditis among parenteral drug abusers (33). Although the mortality rate for infections caused by *C. parapsilosis* is lower than that caused by other non-*C. albicans* *Candida* species, the rate of mortality from *C. parapsilosis* endocarditis is comparable to that from invasive *C. albicans* infections (33). In the present study, the killing rate for *C. parapsilosis* was slower and increased slightly with the drug concentration. At least ≥2 µg of the drug per ml is required to reach the fungicidal endpoint within 24 h when viable cells are present, a phenomenon previously reported by Vazquez et al. (30).

Amphotericin B had the lowest level of fungicidal activity

against *C. krusei* and *C. tropicalis* (endpoints at four times the MIC after 48 and 40 h, respectively). The maximum rate of killing of these species was reached with the drug at 2 µg/ml and hardly increased at higher concentrations. As reported by Vazquez et al. (30), viable cells are observed after 24 h of exposure to 2 µg of amphotericin B per ml; those investigators concluded that a fungicidal endpoint could perhaps have been achieved if the time of incubation had been extended beyond 24 h. However, in our study viable cells were still observed after an extended incubation time (48 h). The slow killing rate and the presence of viable cells after exposure to 2 µg of amphotericin B per ml could explain the lack of clinical response to amphotericin B treatment for certain infections caused by these two species (6, 24; J. Pemán, E. Cantón, A. Espinel-Ingroff, I. Jarque, M. Salvat, A. Querol, R. De Llanos, and M. Gobernado, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1025, 2003). High MICs by Etest (>2 µg/ml) have also been reported for *C. krusei* (93% of strains) and *C. tropicalis* (43% of strains) (23). Interestingly, the killing activity against the *C. krusei* reference strain (ATCC 6258) was faster (fungicidal endpoint after 13 h at four times the MIC) than that against other isolates of this species (48 h).

The killing activity of amphotericin B against the previously defined amphotericin B-resistant species varied by strain. Amphotericin B showed killing activity at 0.5 µg/ml (90% killing) only against *C. lusitaniae* ATCC 200953, and the maximum killing rate was reached with 2 µg/ml. For the other amphotericin B-resistant *C. lusitaniae* and *C. albicans* strains, the killing activity began at 2 µg/ml and increased with the concentration, with 22 to 33.5 h required to kill 99% of the initial inoculum at this concentration. Viable *C. lusitaniae* ATCC 200950 cells were still present after 48 h of incubation with 32 µg of amphotericin B per ml, in accordance with the MFCs. No killing activity against *C. tropicalis* ATCC 200956 was observed at any concentration assayed.

In summary, both amphotericin B time-kill curves and determination of the MFCs for *Candida* spp., as described in the present study, can detect viable cells after 48 h of incubation with ≥2 µg of amphotericin B per ml. This effect is strain

dependent. The time required for the fungicidal activity (99.9% killing) of amphotericin B was also species and concentration dependent. The speed at which amphotericin B killed *C. albicans* (*K* values) was faster than that at which it killed the other species tested (3, 6, 13, 21, and 30 times more rapid killing compared with that for *C. lusitaniae*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. tropicalis*, respectively). Nevertheless, the killing rate for *C. dubliniensis* was twice as fast as that for *C. albicans*. Therefore, since the performance of time-kill curve studies is cumbersome for routine use in the laboratory, the more practical determination of MFCs could become an alternative choice for the detection of the fungicidal activity of amphotericin B against those deep fungal infections for which determination of fungicidal activity could be clinically relevant. Clinical studies to determine the correlation between the in vivo response and the in vitro MFCs of amphotericin B are under way.

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