In Vitro Activities of 5-Fluorocytosine against 8,803 Clinical Isolates of *Candida* spp.: Global Assessment of Primary Resistance Using National Committee for Clinical Laboratory Standards Susceptibility Testing Methods

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We determined the in vitro activity of flucytosine (5-fluorocytosine [5FC]) against 8,803 clinical isolates of *Candida* spp. (18 species) obtained from more than 200 medical centers worldwide between 1992 and 2001. The MICs were determined by broth microdilution tests performed according to NCCLS guidelines by using RPMI 1640 as the test medium and the following interpretive breakpoints: susceptible (S), ≤ 4 µg/ml; intermediate (I), 8 to 16 µg/ml; resistant (R), ≥ 32 µg/ml. 5FC was very active against the 8,803 *Candida* isolates (MIC_{90}, 1 µg/ml), 95% S. A total of 99 to 100% of *C. glabrata* (MIC_{90}, 0.12 µg/ml), *C. parapsilosis* (MIC_{90}, 0.25 µg/ml), *C. dubliniensis* (MIC_{90}, 0.12 µg/ml), *C. guilliermondii* (MIC_{90}, 0.5 µg/ml), and *C. kefyr* (MIC_{90}, 1 µg/ml) were susceptible to 5FC at the NCCLS breakpoint. *C. albicans* (MIC_{90}, 1 µg/ml; 97% S) and *C. tropicalis* (MIC_{90}, 1 µg/ml; 92% S) were only slightly less susceptible. In contrast, *C. krusei* was the least susceptible species: 5% S; MIC_{90}, 32 µg/ml. Primary resistance to 5FC is very uncommon among *Candida* spp. (95% S, 2% I, and 3% R), with the exception of *C. krusei* (5% S, 67% I, and 28% R). The in vitro activity of 5FC, combined with previous data demonstrating a prolonged post-antifungal effect (2.5 to 4 h) and concentration-independent activity (optimized at 4× MIC), suggest that 5FC could be used in lower doses to reduce host toxicity while maintaining antifungal efficacy.

The recent development of several new systemically active agents for the treatment of invasive candidiasis promises to offer improved coverage of this important infectious disease (10, 12, 32). Although new anti-infective agents are always met with great enthusiasm, currently available agents should not be ignored (2, 15). New approaches that take into account the in vitro susceptibility of contemporary isolates determined by standardized methods (24, 26, 30), coupled with rational pharmacodynamic dosing that optimizes efficacies and limits toxicities of currently licensed agents, may provide effective, low-cost therapy with more immediate impact (2, 15).

Flucytosine (5-fluorocytosine [5FC]) has been used to treat candidiasis and other invasive mycoses since 1968 (36). Although not used as monotherapy, 5FC may be a useful adjunct to amphotericin B or azoles in the treatment of hematogenous candidiasis (11, 29, 38). Despite a general consensus regarding the clinical efficacy of 5FC when used in combination with amphotericin B (11), clinicians are often hesitant to use 5FC due to concerns about toxicity (13, 39) and either primary or secondary resistance (8, 22, 34, 35, 39). Although primary resistance to 5FC is stated to occur among 10 to 15% of *Candida albicans* isolates and even higher among other *Candida* species (31), there is very little recent data explicitly addressing this issue by using the standardized National Committee for Clinical Laboratory Standards (NCCLS) antifungal susceptibility testing method (24) against a large geographically diverse collection of contemporary clinical isolates of *Candida* spp. (6, 9).

Over the past 9 years (1992 to 2001) we have determined the in vitro activity of 5FC by using NCCLS broth microdilution methods against more than 8,000 incident *Candida* isolates obtained from blood, normally sterile body fluids, or other invasive sites and contributed by more than 200 hospitals worldwide. We report this experience and emphasize the relationship between the MIC distributions of the various species and the recently documented pharmacodynamic characteristics of 5FC with respect to *Candida* spp. (2, 21). We present here the largest experience with 5FC tested by NCCLS reference methods.

MATERIALS AND METHODS

Organisms. We tested 8,803 clinical *Candida* isolates obtained from more than 200 medical centers worldwide. The collection included 5,208 *C. albicans*, 1,267 *C. glabrata*, 1,047 *C. parapsilosis*, 759 *C. tropicalis*, 184 *C. krusei*, 90 *C. dubliniensis*, 100 *C. guilliermondii*, 82 *C. lusitaniae*, 17 *C. famata*, and 15 *C. kefyr* strains, as well as 34 other *Candida* spp., including *C. lipolytica* (8 isolates), *C. rugosa* (8 isolates), *C. pelliculosa* (6 isolates), *C. lambica* (2 isolates), *C. inconspicua* (3 isolates), *C. sake* (2 isolates), *C. norvegensis* (1 isolate), *C. zeylanoides* (1 isolate), and *Candida* spp. not further identified (2 isolates). These were all consecutive incident (first isolate from each patient episode) clinical isolates collected between 1992 and 2001, and more than 80% of them were from blood or normally sterile body fluids. The *C. dubliniensis* isolates were from mucosal sites. Isolates were identified by standard methods (40) and stored as water suspensions until they were used. Prior to testing each isolate was passaged on potato dextrose agar (Remel, Lenexa, Kans.) and CHROMagar (Hardy Laboratories, Santa Monica, Calif.) to ensure purity and viability.

Antifungal agents. A standard antifungal powder of 5FC was obtained from Sigma (St. Louis, Mo.). A stock solution (1,000 µg/ml) was prepared in water,

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TABLE 1. In vitro susceptibilities of 8,803 clinical isolates of *Candida* spp. to 5FC[a]

Organism (no. of strains tested)	% S at MIC (μg/ml) of:										
	0.12	0.25	0.5	1	2	4	8	16	32	64	128
C. albicans (5,208)	49	69	77	92	96	97[b]	97	97	98	98	99
C. glabrata (1,267)	95	98	98	99	99	99[b]	99	99	99	99	99
C. parapsilosis (1,047)	73	96	98	99	99	99[b]	99	99	99	99	99
C. tropicalis (759)	36	72	87	91	91	92[b]	92	93	93	95	99
C. krusei (184)	2	2	3	3	4	5[b]	13	72	98	99	99
C. lusitaniae (82)	87	89	90	93	93	93[b]	94	95	95	95	99
C. dubliniensis (90)	100										
C. guilliermondii (100)	51	89	97	98	99	100[b]					
C. famata (17)	53	88	93	93	93	93[b]	94		94	94	100
C. kefyr (15)	67	67	73	100							
Other *Candida* spp. (34)	24	35	38	50	53	56[b]	62	79	91	97	97
All *Candida* species (8,803)	57	76	83	92	94	95[b]	95	97	98	98	99

[a] Broth microdilution testing was done according to NCCLS guideline M27-A (24).
[b] The percentage of isolates susceptible to 5FC at the NCCLS breakpoint of $\leq$4 μg/ml.

and serial twofold dilutions were prepared exactly as outlined in NCCLS document M27-A (24). The test medium was RPMI 1640 medium (Sigma) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid buffer (Sigma). Aliquots (100 μg) of each 5FC dilution at 2$\times$ final concentrations were dispensed into the wells of plastic microdilution trays by suing a Quick Spense II System (Dynatech Laboratories, Chantilly, Va.). The trays were sealed and frozen at -70°C until they were used.

Antifungal susceptibility studies. Broth microdilution testing was performed in accordance with NCCLS guidelines (24). The inoculum suspension was prepared by the spectrophotometric method of inoculum preparation and with a final inoculum of $(1.5 \pm 1.0) \times 10^3$ cells per ml. A 100-μl yeast inoculum was added to each well of the microdilution trays. The final concentrations of 5FC ranged from 0.12 to 128 μg/ml. The trays were incubated at 35°C, and the MIC endpoints were read after 48 h of incubation. Drug- and yeast-free controls were included.

After incubation, the MICs of 5FC were read at the lowest concentration at which a prominent decrease (ca. 50%) in turbidity relative to the growth control well was observed (24). Quality control was ensured by testing the NCCLS-recommended strains *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 (7, 24).

The interpretive criteria for susceptibility to 5FC were those published by Rex et al. (28) and the NCCLS (24): susceptible (S), $\leq$4 μg/ml; intermediate (I), 8 to 16 μg/ml; resistant (R), $\geq$32 μg/ml.

RESULTS AND DISCUSSION

Table 1 summarizes the in vitro susceptibility of 8,803 *Candida* isolates to 5FC. The results are presented by species in a continuous fashion as cumulative percentages of susceptible organisms at each concentration of 5FC throughout the full dilution series. This manner of presentation facilitates comparison of MIC distributions among species between various studies that used the same methodology and makes the data more generalizable since continuous data are not restricted by interpretive criteria that may vary by nation or by international consensus groups (19, 23).

Overall, 5FC was very active in vitro (MIC$_{90}$, 1 μg/ml; 95% S, 2% I, and 3% R) against the 8,803 isolates. 5FC was most active against *C. glabrata* (MIC$_{90}$, 0.12 μg/ml; 99% S), *C. parapsilosis* (MIC$_{90}$, 0.25 μg/ml; 99% S), *C. dubliniensis* (MIC$_{90}$, 0.12 μg/ml; 100% S), *C. guilliermondii* (MIC$_{90}$, 0.5 μg/ml; 100% S), and *C. kefyr* (MIC$_{90}$, 1 μg/ml; 100% S). *C. albicans* (MIC$_{90}$, 1 μg/ml; 97% S) and *C. tropicalis* (MIC$_{90}$, 1 μg/ml; 92% S) were slightly less susceptible to 5FC at the NCCLS breakpoint of 4 μg/ml. Notably, only 3% of 5,208 isolates of *C. albicans* and 1% of 1,267 isolates of *C. glabrata*

exhibited primary resistance to 5FC (Table 1). In contrast, *C. krusei* was the least susceptible species (MIC$_{90}$, 32 μg/ml; 5% S, 67% I, and 28% R). The 5FC MIC distributions stratified by year and by geographic location were virtually identical to the aggregate data presented in Table 1. There were no differences in the MIC distribution observed over time or among the different geographic regions (North America, Latin America, Europe, and Asia-Pacific) represented (data not shown).

Much of the data concerning primary resistance to 5FC among *Candida* spp. has been derived from studies conducted in the 1970s and 1980s (3, 5, 8, 31, 34) prior to the standardization of in vitro testing methods. Primary resistance among *C. albicans* has been reported to range from 6.5% in Europe to 33% in the United States, with an overall prevalence of 7 to 9% (3, 31). *C. albicans* serotype B, found mainly in Africans and among human immunodeficiency virus (HIV)-infected patients with thrush (18, 37), is reported to be significantly more resistant (49%) than serotype A strains (11%) (5). These data were derived by nonstandardized testing methods that have been shown to vary by as much as 10,000-fold when used to determine 5FC MICs for *Candida* spp. in different laboratories (14). Furthermore, the isolates tested were often a mix of strains representing both superficial mucosal and invasive isolates.

The data generated by NCCLS-based methods for determining the MICs for *Candida* spp. are markedly different than those cited above. Studies from Canada (17, 33), the United States (20), Italy (6), and Spain (9) in recent years estimated resistance to 5FC to be 0 to 0.6% for *C. albicans* and 0.6 to 6% for all *Candida* species combined. The Canadian and U.S. data were derived with strains isolated from blood cultures, whereas the Spanish and Italian data represented oral isolates from HIV-infected patients, as well as invasive isolates.

Our data markedly expand the database of 5FC MICs determined by NCCLS methods for clinically important invasive (blood and normally sterile sites) isolates of *Candida* spp. Moreover, this collection spans the past decade and includes isolates from medical centers throughout the world.

Like the findings of the studies by Hoban et al. (17), Kao et al. (20), Barchiesi et al. (6), and Cuenca-Estrella et al. (9), these data document a very low level of primary resistance

among virtually all *Candida* spp. with the exception of *C. krusei*. Furthermore, the MIC$_{90}$ for the four most common species—*C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*—ranges from 0.12 µg/ml (*C. glabrata*) to 1 µg/ml (*C. albicans* and *C. tropicalis*). Thus, the most common species causing hematogenous candidiasis (25) are exquisitely susceptible to 5FC.

Animal infection models have documented the efficacy of 5FC alone and in combination with other systemically active antifungal agents against various *Candida* spp. (4, 16, 27), and several studies have demonstrated concordance between in vitro susceptibility and in vivo endpoints (1, 30, 35). Recent in vitro pharmacodynamic studies have determined that 5FC exhibits concentration-independent fungistatic activity against *Candida* spp. that is optimized at concentrations of 4× MIC (21). These findings have been confirmed in vivo by Andes and van Ogtrop (2), who showed that time above the MIC was the pharmacodynamic parameter that most strongly correlated with outcome in 5FC treatment of murine candidiasis and that maximum efficacy was observed when 5FC blood levels exceeded the MIC for only 20 to 25% of the dosing interval. Both in vitro and in vivo studies have documented a significant post-antifungal effect for 5FC and *Candida* spp. occurring at from 2.5 to 4 h (2, 21). This post-antifungal effect probably accounts for the relatively brief time that levels must exceed the MIC to demonstrate efficacy in the murine model (2).

The excellent spectrum and potency of 5FC versus *Candida* spp., along with the favorable pharmacokinetic and pharmacodynamic parameters noted above, suggest that 5FC might be used with dosing regimens that produce lower concentrations in serum than those currently employed (25 mg/kg q 6 h; 40 to 60 µg/ml). Lower dosing could reduce the risk of toxicity to the host while maintaining the overall efficacy of 5FC when it is coupled with amphotericin B or an azole in the treatment of invasive candidiasis (2, 13, 21, 30).

In summary, the present study provides an extensive database of 5FC MICs for *Candida* spp. from defined clinically important (blood and normally sterile sites) sites of infection. The MIC distribution for each species, determined by a standardized reference method, presented in a continuous fashion will ensure the broadest utility of the data (19, 23). The favorable pharmacokinetic and pharmacodynamic properties of 5FC, coupled with its spectrum and potency, could lead to improved dosing practices and a "rebirth" of this agent as a useful adjunct in the treatment of serious *Candida* infection.

ACKNOWLEDGMENTS

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