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In Vitro Susceptibility of Breakthrough *Candida* Bloodstream Isolates Correlates with Daily and Cumulative Doses of Fluconazole

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We determined MICs for 28 *Candida* isolates recovered from patients who developed breakthrough candidemia while receiving fluconazole. MICs correlated with daily and cumulative doses of fluconazole. Patients receiving ≥2,000 mg cumulatively or >200 mg daily were more likely to be infected with isolates that were not fluconazole susceptible.

Fluconazole prophylaxis is effective for the prevention of candidiasis in selected high-risk populations (3, 16). The practice is limited, however, by the potential for breakthrough candidiasis due to isolates that exhibit diminished susceptibilities to fluconazole (2, 4–9). This phenomenon of relative resistance to fluconazole has been well documented in particular cases (19, 20). In larger studies, the literature is conflicting. Some studies have shown that breakthrough candidemia is associated with the emergence of non-*Candida albicans* spp. and isolates of diminished fluconazole susceptibility (2, 4–9, 14), but others have not (10, 11, 15, 17). Studies have assessed different patient populations, associated risk factors, and fluconazole regimens. In most of the studies, patients received a fixed daily dose of fluconazole (3, 4, 11, 17). In others, doses differed (2, 14), but the relationship between dose and fluconazole susceptibility was not assessed.

We previously demonstrated that the fluconazole MIC and the fluconazole daily dose/MIC ratio correlated with therapeutic responses among patients with candidemia (1). As such, we predicted that MICs of breakthrough *Candida* bloodstream isolates would correlate with fluconazole doses used for prophylactic or empirical therapy. To test this hypothesis, we studied 28 isolates that caused consecutive episodes of breakthrough candidemia among unique patients. All of the patients received fluconazole daily for at least 3 days prior to the development of candidemia. Thirteen of the patients were enrolled in a prospective, multicenter, observational study of candidemia (1, 8). The remaining 15 were identified from 2004 to 2006 at the Shands Teaching Hospital at the University of Florida.

MICs were measured at 24 h using a standardized broth dilution method (1) (Table 1). Reference strains (*C. albicans* ATCC 90028 and ATCC 90029, *C. parapsilosis* ATCC 90018, and *C. glabrata* ATCC 90030) were incorporated into each set of experiments as quality controls. There was a correlation between the MIC and the daily fluconazole dose ($P = 0.03$, linear regression) (Fig. 1). Fifty-seven percent (13/23) of isolates recovered from patients who received ≤200 mg/day were

in vitro susceptible to fluconazole (13) compared to 0% (0/5) of isolates recovered from patients who received >200 mg/day ($P = 0.04$, Fisher's exact test) (Table 2). In our earlier study, a daily fluconazole dose/MIC ratio of ≤50 was associated with a significantly greater likelihood of therapeutic failure (1). In this study, 20 of the 28 isolates demonstrated such ratios (Table 1).

There was also a correlation between MIC and the cumulative fluconazole dose ($P = 0.02$, linear regression) (Fig. 2A). Eighty-nine percent (8/9) of isolates recovered from patients who received <2,000 mg were in vitro susceptible to fluconazole compared to 26% (5/19) of isolates recovered from patients who received ≥2,000 mg ($P = 0.004$, Fisher's exact test)

TABLE 1. MICs and doses for breakthrough *Candida* bloodstream isolates

Candida sp.	Daily fluconazole dose (mg)	Cumulative fluconazole dose (mg)	MIC (μg/ml)	Daily dose/ MIC ratio
C. albicans	50	250	1	50
C. parapsilosis	100	1,300	1	100
C. albicans	100	700	1	100
C. tropicalis	100	400	2	50
C. glabrata	100	1,200	2	50
C. glabrata	100	2,100	64	1.56
C. krusei	100	2,200	64	1.56
C. glabrata	100	2,400	64	1.56
C. parapsilosis	200	5,000	0.5	400
C. parapsilosis	200	2,200	1	200
C. albicans	200	1,400	1	200
C. albicans	200	2,400	1	200
C. parapsilosis	200	800	1	200
C. glabrata	200	1,800	2	100
C. tropicalis	200	13,200	4	50
C. parapsilosis	200	3,600	4	50
C. glabrata	200	2,400	16	12.5
C. lusitaniae	200	3,600	32	6.25
C. krusei	200	2,000	64	3.12
C. krusei	200	5,000	64	3.12
C. albicans	200	3,600	64	3.12
C. glabrata	200	600	64	3.12
C. tropicalis	200	2,800	128	1.56
C. glabrata	400	5,400	32	12.5
C. glabrata	400	2,400	32	12.5
C. krusei	400	4,400	64	6.25
C. albicans	400	7,200	128	3.12
C. glabrata	800	12,400	128	6.25

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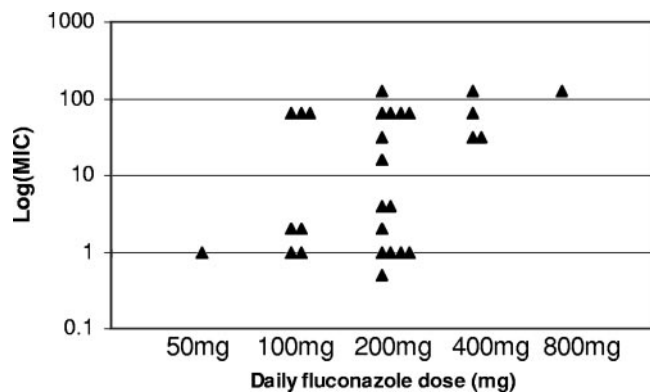
FIG. 1. Relationships between daily fluconazole dose and MIC. Each symbol represents an isolate recovered from a patient treated with the dose of fluconazole indicated on the *x* axis.

(Table 3; Fig. 2B). There was no correlation between the duration of fluconazole therapy prior to the development of candidemia and the MIC of the breakthrough isolate.

The breakthrough species were *C. glabrata* (32%, 9/28), *C. albicans* (21%, 6/28), *C. parapsilosis* (18%, 5/28), *C. krusei* (14%, 4/28), *C. tropicalis* (11%, 3/28), and *C. lusitaniae* (4%, 1/28) (Table 1). All *C. krusei* and *C. parapsilosis* isolates were fluconazole resistant and fluconazole susceptible, respectively; the breakthrough isolates of other *Candida* spp. were susceptible, susceptible and dose dependent, or resistant.

To our knowledge, this is the first study to demonstrate a relationship between the daily and cumulative doses of fluconazole and the MICs of breakthrough *Candida* bloodstream isolates. Our demonstration that MICs correlated directly with the fluconazole dose received prior to the development of breakthrough candidemia appears to be clinically relevant, since the percentage of isolates susceptible to fluconazole decreased as the dose increased. Our data set differed in important ways from those of earlier studies, which likely accounts for the success of this study. Most notably, we collected isolates from a relatively large number of patients who had received wide ranges of fluconazole doses; these isolates, in turn, exhibited diverse MICs, including a large percentage that was not susceptible to fluconazole.

The results are important because of their implications for the management of breakthrough candidemia. Indeed, we have incorporated our data into a management strategy at our institution. Prior to species identification and antifungal susceptibility testing, we assume that yeasts isolated from the blood of patients receiving prophylactic or empirical fluconazole are not susceptible to the drug. Patients, therefore, are switched to an amphotericin B formulation or echinocandin. If susceptibility testing is performed, these results can be used to guide subsequent therapy. If susceptibility testing is not available or not performed, then treatment decisions must be made based upon species identification. *C. glabrata* breakthrough isolates, like *C. krusei*, should be considered resistant to fluconazole. Since *C. parapsilosis* breakthrough isolates are generally fluconazole susceptible (12; our unpublished data), the agent is a therapeutic option, provided vascular catheters are removed. For *C. albicans* and *C. tropicalis* isolates in our study, the cumulative fluconazole dose better predicted the susceptibility pattern than the daily dose did. Isolates recovered from patients who received <2,000 mg remained susceptible to fluconazole (Fig. 2B). Therefore, once the patient is stable on antifungal therapy and species identification is available, we believe it is reasonable to consider switching to fluconazole to complete the course of therapy. For cumulative doses of ≥2,000 mg, *C. albicans* and *C. tropicalis* susceptibility patterns are difficult to predict; in such cases, we complete treatment with amphotericin B or an echinocandin.
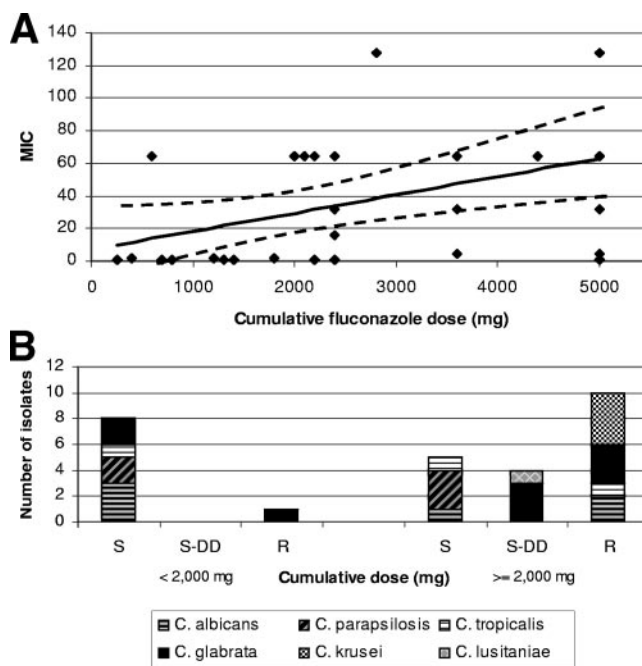

FIG. 2. Relationships between cumulative fluconazole dose, MICs, and susceptibility patterns. (A) The distribution of MICs stratified by cumulative fluconazole dose. (B) The association between cumulative doses and susceptibility patterns of the breakthrough *Candida* isolates.

TABLE 2. Fluconazole susceptibility patterns stratified by daily dose

Daily dose	% (No.) of isolates[a]			P value[b]
	S	S-DD	R	
50 to 200 mg	57 (13)	9 (2)	35 (8)	
>200 mg	0 (0)	40 (2)	60 (3)	0.04

[a] The total percentages for the 50-to-200-mg group exceed 100% due to rounding. S, susceptible; S-DD, susceptible and dose dependent; R, resistant.
[b] Three-by-two comparison; chi-square test.

TABLE 3. Fluconazole susceptibility patterns stratified by cumulative dose

Cumulative dose	% (No.) of isolates[a]			P value[b]
	S	S-DD	R	
<2,000 mg	89 (8)	0	11 (1)	
≥2,000 mg	26 (5)	21 (4)	53 (10)	0.008

[a] S, susceptible; S-DD, susceptible and dose dependent; R, resistant.
[b] Three-by-two comparison; chi-square test.

The design of this study does not allow us to draw any conclusions about the efficacy of empirical or preventive fluconazole therapy, appropriate doses for such therapy, or the potential role of fluconazole in selecting for non-*C. albicans* spp. It is also important to recognize that our data cannot be extrapolated to assess the use of the fluconazole dose/MIC ratio (or, alternatively, the area under the exposure curve/MIC ratio) as a pharmacodynamic tool to prevent the emergence of resistance (18). Carefully designed in vitro studies would be the first step toward addressing this important issue.

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