Antifungal Susceptibility Testing of Fluconazole by Flow Cytometry Correlates with Clinical Outcome

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Susceptibility testing of fungi by flow cytometry (also called fluorescence-activated cell sorting [FACS]) using vital staining with FUN-1 showed a good correlation with the standard M27-A procedure for assessing MICs. In this study we determined MICs for blood culture isolates from patients with candidemia by NCCLS M27-A and FACS methods and correlated the clinical outcome of these patients with in vitro antifungal resistance test results. A total of 24 patients with candidemia for whom one or more blood cultures were positive for a *Candida* sp. were included. Susceptibility testing was performed by NCCLS M27-A and FACS methods. The correlation of MICs (NCCLS M27-A and FACS) and clinical outcome was calculated. In 83% of the cases, the MICs of fluconazole determined by FACS were within 1 dilution of the MICs determined by the NCCLS M27-A method. For proposed susceptibility breakpoints, there was 100% agreement between the M27-A and FACS methods. In the FACS assay, a fluconazole MIC of <1 μg/ml was associated with cure ($P < 0.001$) whereas an MIC of ≥ 1 μg/ml was associated with death ($P < 0.001$). The M27-A-derived fluconazole MICs did not correlate with outcome ($P = 1$ and $P = 0.133$).

The number of patients predisposed to infections with opportunistic microorganisms such as *Candida* species has increased significantly over the last decade (3). The importance of opportunistic fungal infections in neutropenic patients is well recognized, and systemic candidiasis may also constitute a problem for severely ill patients in intensive care units (4, 8). Amphotericin B and fluconazole are mainstays of therapy for infections caused by *Candida* species (7, 26). Two large randomized studies have demonstrated that amphotericin B and fluconazole are similarly effective as therapy in patients without neutropenia (16, 21). Nevertheless, the emergence of fungal resistance and the increasing isolation of intrinsically resistant *Candida* species has led to interest in clinically relevant methods for antifungal susceptibility testing (14, 28). It was demonstrated that MIC results could vary as much as 50,000-fold when a variety of disparate methods were compared (5, 6). Therefore the National Committee for Clinical Laboratory Standards (NCCLS) developed standardized antifungal susceptibility testing procedures, the *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts* (NCCLS document M27-A) (15). The rationale for the fluconazole breakpoints is based on analysis of fluconazole MIC and outcome data by the Subcommittee on Antifungal Susceptibility Testing of NCCLS (24). Since MICs or minimum lethal concentrations obtained by the NCCLS M27-A method or methods similar to it have generally correlated well with outcome in various animal models of infection, it was expected that M27-A would prove useful in prediction of the likelihood of response to a

given antifungal agent (1, 25). However, only three studies have carefully related in vitro test results based on the M27-A document to clinically antifungal efficacy in humans. One study showed an inverse correlation of fluconazole MIC with outcome in invasive *Candida* infection: lower MICs correlated with failure rather than success (23). Whereas in that study high MICs did not predict failure, in another study amphotericin B resistance correlated with failure or death (18, 23). A third study found that the clinical correlation between the M27-A antifungal susceptibility test results and outcome is high for patients with hematogenous and deep-seated *Candida* infections (11).

A number of investigators have used flow cytometry (fluorescence-activated cell sorting [FACS]) methods to obtain rapid susceptibility results for *Candida* spp. (9, 10, 17, 19, 20). Susceptibility testing of fungi by FACS using vital staining with FUN-1 showed a good correlation with the standard M27-A procedure for assessing MICs (27).

In this study we determined antifungal MICs for blood culture isolates from patients with candidemia by the NCCLS M27-A and FACS methods and correlated the clinical outcome of these patients with in vitro antifungal resistance test results.

MATERIALS AND METHODS

Patients. Between May 1998 and May 1999, 24 patients with candidemia were included if one or more blood cultures were positive for a *Candida* sp. The baseline clinical data are shown in Table 1. Patients were observed for clinical and microbiological cure. At the end of the antifungal treatment, clinical cure was defined as resolution of signs and symptoms related to candidemia. Microbiological efficacy of treatment was defined as the finding of at least two negative blood cultures.

Culture and inoculum suspension. A total of 24 clinical isolates and the resistant strain *Candida albicans* (ATCC 26278) subcultured on Sabouraud's dextrose agar were used for the study. Briefly, positive blood cultures were

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TABLE 1. Baseline clinical data for 24 patients with candidemia

Patient no.	Age (yr)	Sex	Wt (kg)	Underlying conditions	Organ involvement	Mechanical ventilation	Renal support	Central venous catheter[a]	Urinary catheter
1	71	M	80	Coronary artery bypass graft, subclavian vein thrombosis	None	No	No	Yes	Yes
2	35	M	60	Ulcerative colitis, ileus	None	Yes	Yes	Yes	Yes
3	55	M	55	Ischemic enterocolitis	None	No	No	No	No
4	26	M	50	Intravenous drug abuse, hepatitis C	Mitral valve endocarditis, cerebral abscess	Yes	Yes	Yes	Yes
5	74	M	99	Coronary heart disease, coronary artery bypass graft	Sternum osteomyelitis, mediastinitis	Yes	Yes	Yes	Yes
6	50	F	60	Cardiopulmonary reanimation, multiorgan failure	Pneumonia[b]	Yes	Yes	Yes	Yes
7	68	F	78	MALT[d] lymphoma	None	No	No	No	Yes
8	71	F	54	Pneumonia, multiorgan failure	None	Yes	Yes	Yes	Yes
9	53	F	62	Uremia, Tenckhoff catheter	Peritonitis	No	Yes	Yes	No
10	45	F	60	Breast cancer, chemotherapy-related neutropenia	None	Yes	Yes	Yes	Yes
11	56	M	75	Pancreatitis, multiorgan failure	None	Yes	Yes	Yes	Yes
12	43	M	60	AIDS	Stomatitis	No	No	No	No
13	82	F	60	Pituitary adenoma, aspiration	Pneumonia[b]	No	No	No	No
14	81	F	50	Colon carcinoma	None	Yes	No	Yes	No
15	0**	F	0.635	Premature rupture of membranes (wk 23),[c] congenital ureaplasma infection	None	Yes	No	Yes	Yes
16	48	F	62	Diabetes mellitus, hemodialysis	None	No	Yes	Yes	No
17	53	F	58	Uremia, Tenckhoff catheter	Peritonitis	No	No	No	No
18	61	M	87	Porth-a-Cath sepsis	None	No	No	No	Yes
19	47	M	72	Pneumonia (*Legionella*)	None	Yes	Yes	Yes	Yes
20	85	M	86	Pneumonia, arterial ulcer left foot	Pneumonia[b]	Yes	Yes	Yes	Yes
21	72	M	74	Rectal carcinoma	None	No	No	No	Yes
22	18	F	53	Small bowel volvulus	None	No	No	Yes	No
23	46	M	60	Pancreas carcinoma	None	No	No	Yes	No
24	34	M	59	Ewing's sarcoma	None	No	No	Yes	No

[a] Central venous catheters were changed when candidemia was documented.
[b] Presumed based on radiology and the respiratory culture.
[c] 3 months, born 30 November 1998 at 23 weeks of gestation.
[d] MALT, mucosa-associated lymphoid tissue.

detected using Vital, which indicates bacteria or fungi in blood culture bottles by using a fluorescent indicator to measure a decrease in pH (bioMerieux, Mary L'Etoile, France). Yeasts were cultured on Sabouraud's dextrose agar (Difco Laboratories, Detroit, Mich.), and inoculum suspensions were prepared for each experiment from 24-h-old subcultures grown at 35°C. Yeast cells were grown to the late log phase (usually 10^7 to 10^8 cells/ml in yeast peptone dextroxe [Difco, Detroit, Mich.] medium). Then 1 ml of the yeast culture was added to 20 ml of sterile, 0.2-μm-pore-size-filtered water containing 2% D-(+)-glucose and 10 mM sodium HEPES (pH 7.2) in a cell culture tube. The yeast cells were centrifuged for 5 min at $10,000 \times g$ at 25°C. The supernatant was removed, and the pellet was resuspended in sterile 0.2-μm-filtered water containing 2% D-(+)-glucose and 10 mM sodium HEPES (pH 7.2) test medium (TM broth). The turbidities of the resulting yeast suspensions were measured with a spectrophotometer at 530 nm and were adjusted to the percent transmission that matched the 0.5 McFarland standard. Final yeast concentrations from 10^5/ml to 10^8/ml were tested.

FACS susceptibility tests. A 300-μl volume of the yeast suspension was added to each of the Falcon tubes (no. 2054; Becton Dickinson Immunocytometry Systems, Mountain View, Calif.). Stock solutions of amphotericin B (Bristol Myers Squibb, Munich, Germany) and fluconazole (Pfizer, Vienna, Austria) were provided in solution by the manufacturers. Serial twofold dilutions of the antifungal agents (128 to 0.0625 μg/ml) were prepared in TM broth in Falcon tubes (Becton Dickinson). For assay, 25-μl dilutions of antifungal compounds was added to 300 μl of fungi in TM broth. For controls, organisms were diluted in TM broth alone or suspended in 70% ethanol. The tubes were incubated at 35°C with continuous shaking for 30 min. Thereafter, FUN-1 (Molecular Probes, Eugene, Oreg.) was added at a final concentration of 5 μM. This concentration was determined in experiments using 5 to 20 μM FUN-1 to stain the yeast suspensions at concentrations ranging from 10^4 to 10^7/ml. Concentrations of 10 and 20 μM FUN-1 yielded a higher background fluorescence, and optimal staining was seen with 5 μM FUN-1. A constant mean fluorescent emission was seen at 5 μM FUN-1 with a yeast suspension ranging from 10^5 to 10^7/ml. Lower concentrations not only extensively prolonged the acquisition time but also increased the background fluorescence. With FUN-1 (5 μM), the yeast cell

suspension (± antifungal agents) was incubated for an additional 30 min at 35°C in a shaking water bath.

FACS data acquisition. The cells were analyzed with a single argon laser tuned at 488 nm (FACScan Flowcytometer; Becton Dickinson Immunocytometry Systems) and FACScan Lysis II software for data acquisition and analysis. Initially, cells were detected and differentiated from background fluorescence using dot blot analysis of foreward and side scatter. A total of 10,000 fungal cells were acquired for analysis.

FACS statistical analysis. Samples were analyzed by histogram profiles of FUN-1 fluorescence as previously shown (27). The mean channel fluorescence (intensity of fluorescence-labeled fungi) of control and drug-treated suspensions was compared in FL 2 (red fluorescence detection at 560 ± 15 nm). The values were obtained as part of the FACS statistical analysis provided with FACScan Lysis II software. When drug-treated cells were analyzed, impairment of cell metabolism and respiration was evidenced by decreased FUN-1 staining. Damaged fungi, such as those incubated in 70% ethanol, moved to the left on the *x* axis. The MIC was defined as the lowest drug concentration in the dilution series that produced a reduction in FUN-1 fluorescence (at least a 20% reduction of the mean fluorescence channel in FL 2). All samples were tested twice.

Conventional measurement of susceptibility by testing of fungi. MICs of the antifungal agents were determined by a microtiter modification of the M27-A procedure using a value that correlates approximately with an 80% drop in turbity compared to controls (MIC-2) (15). Fluconazole was tested using RPMI-1640 (Sigma, Poole, United Kingdom) plus 2% glucose, buffered with morpholinopropanesulfonic acid (MOPS; Sigma) and adjusted to pH 7.0. Antibiotic medium 3 with 2% glucose (pH 7.0; Difco, West Molesey, United Kingdom) was used to test amphotericin B (22). *C. albicans* Y01-09 strain (kindly provided by Pfizer UK) and *Candida kefyr* San Antonio strain (kindly donated by D. A. Stevens) were tested to ensure quality control.

Statistical analysis. Differences between groups were calculated using the Fisher exact test (two-tailed probability of a 2×2 contingency table; Jandel SigmaStat Version 2.0). Differences with a *P* value less than 0.05 were considered significant.

TABLE 2. Outcome data for 24 patients with candidemia

Patient no.	*Candida* sp.	Therapy	Daily dose (mg)	Duration of therapy (days)	Clinical outcome	Microbiological outcome	Fluconazole MIC (μg/ml) by:		Amphotericin B MIC (μg/ml) by:	
							NCCLS M27-A	FACS assay	NCCLS M27-A	FACS assay
1	*C. albicans*	Fluconazole	400	25	Cure	Eradication	0.5	0.5	0.125	0.06
2	*C. glabrata*	Fluconazole	600	14	Death	Persistence	64	128	0.125	0.06
3	*C. glabrata*	Amphotericin	50	14	Cure	Eradication	16	8	0.06	0.03
4	*C. albicans*	Fluconazole	1,200	28	Death	Eradication	0.5	1	0.125	0.125
5	*C. albicans*	Fluconazole	1,000	28	Cure	Eradication	0.25	0.25	0.125	0.25
6	*C. albicans*	Fluconazole	600	3	Death	Persistence	1	2	0.125	0.125
7	*C. albicans*	Fluconazole	400	21	Cure	Eradication	0.25	0.25	0.125	0.25
8	*C. albicans*	Fluconazole	400	12	Death	NA[a]	0.5	2	0.125	0.125
9	*C. pelliculosa*	Fluconazole	600	14	Cure	Eradication	4	4	0.03	0.06
10	*C. humicola*	Fluconazole	800	10	Death	Persistence	>128	>128	0.03	0.03
11	*C. albicans*	Fluconazole	400	14	Death	NA	0.5	8	0.06	0.06
12	*C. albicans*	Amphotericin	50	24	Death	Persistence	32	64	0.25	0.125
13	*C. albicans*	Fluconazole	400	10	Death	Persistence	0.5	4	0.125	0.25
14	*C. albicans*	Fluconazole	400	14	Cure	Eradication	0.5	0.25	0.06	0.03
15	*C. albicans*	Fluconazole	12	10	Cure	Eradication	0.5	0.5	0.125	0.125
16	*C. albicans*	Fluconazole	400	16	Cure	Eradication	0.5	0.25	0.125	0.03
17	*C. albicans*	Amphotericin	60	12	Cure	Eradication	0.25	0.5	0.125	0.03
18	*C. albicans*	Fluconazole	600	21	Cure	Eradication	0.5	0.25	0.125	0.125
19	*C. albicans*	Fluconazole	600	14	Cure	NA	1	0.5	0.125	0.125
20	*C. albicans*	Fluconazole	800	18	Death	Persistence	0.5	8	0.125	0.25
21	*C. albicans*	Fluconazole	400	24	Cure	Eradiaction	1	0.5	0.125	0.25
22	*C. albicans*	Fluconazole	400	14	Cure	Eradication	0.5	0.5	0.125	0.125
23	*C. albicans*	Fluconazole	600	14	Cure	Eradication	0.5	0.5	0.125	0.125
24	*C. albicans*	Fluconazole	200	14	Cure	Eradication	2	1	0.125	0.25

[a] NA, not applicable.

RESULTS

A total of 24 patients (11 female and 13 male patients; age range, 0 to 85 years) were included in this study. The weights and underlying conditions of the patients are given in Table 1. Of the 24 patients, 16 had undergone previous antibacterial treatment (for any infection). *C. albicans* was isolated in 20 patients, *Candida glabrata* was isolated in 2 patients, and *Candida humicola* and *Candida pelliculosa* were isolated in 1 patient each. A total of 21 patients were treated with fluconazole (200 to 1,000 mg daily for 3 to 28 days), and 3 patients received amphotericin B (12 to 60 mg daily for 12 to 24 days). Of the 24 patients, 17 had central venous catheters, 11 were on renal support, 11 were on mechanical ventilation, and 14 had urinary catheters. Vascular catheters that were infected or presumed to be infected were removed promptly. Nine patients died despite antifungal treatment (eight of the patients were being given fluconazole therapy, and one was being given amphotericin therapy). The microbiological outcome was eradication in 15 patients (14 patients on fluconazole therapy and 1 on amphotericin therapy) and persistence in 6 patients (5 patients on fluconazole therapy and 1 on amphotericin therapy). In three patients, the microbiological outcome could not be assessed because subsequent blood cultures were not collected (Table 2).

In vitro susceptibility results based on current recommended breakpoints and clinical and microbiological outcome are shown in Table 2. Two fluconazole-resistant isolates (*C. glabrata* isolate 2, and *C. humicola* isolate 10), for which the MICs were 64 μg/ml (M27-A) and 128 μg/ml (FACS) (isolate 2) and ≥128 μg/ml in both tests (isolate 10), were obtained from patients treated with fluconazole. One amphotericin B-suscep-

tible but fluconazole-resistant isolate (*C. albicans* isolate 12) was obtained from one patient who died despite amphotericin B therapy. In this patient the isolate persisted. Six fluconazole-susceptible isolates were obtained from patients who were treated with fluconazole but died. In three of these patients, fluconazole-susceptible isolates persisted despite fluconazole therapy. In four instances, fluconazole MICs were 0.5 μg/ml by the M27-A method and at least four times higher by FACS; in all cases the patients died despite fluconazole therapy, and persistent candidemia was documented in two of these patients.

The MICs for each isolate are shown in Table 2. In 81% of the cases, the FACS MICs (fluconazole and amphotericin B) were within 1 dilution of the M27-A MICs. In all four instances in which the fluconazole MIC differed by more than 1 dilution between the methods, the FACS value was higher. For proposed susceptibility breakpoints (15), there was 100% agreement between the M27-A and FACS methods (Table 2).

Correlations of fluconazole MICs (M27-A and FACS methods) and clinical outcome are shown in Table 3. In the FACS assay, a fluconazole MIC of <1 μg/ml was associated with cure ($P < 0.001$) whereas an MIC of ≥1 μg/ml was associated with death ($P < 0.001$). The M27-A fluconazole MICs did not correlate with outcome ($P = 1$ and 0.133) (Table 3). Correlation of amphotericin B MICs and clinical outcome was not assessed because the numbers tested were too small.

DISCUSSION

The aim of antimicrobial susceptibility testing is to provide the treating clinician with MIC endpoints which are reproducible and predictive of therapeutic success or failure. They are

TABLE 3. Correlation of fluconazole MICs (by the NCCLS M27-A and FACS methods) and clinical outcome in patients treated with fluconazole

MIC (µg/ml)	NCCLS M27-A			FACS		
	No. cured	No. who died	P	No. cured	No. who died	P
<1	9	5	1	11	0	<0.001
≥1	4	3	1	2	8	<0.001
≤8	14	5	0.100	13	6	0.133
>8	0	2	0.100	0	2	0.133
<64	13	6	0.133	13	6	0.133
≥64	0	2	0.133	0	2	0.133

only useful clinically if the results are determined after a short incubation period. In addition, MIC results should serve as predictors of drug efficacy or in vivo response. FACS provides MICs which correlate with MICs obtained by the standard M27-A procedure (19, 27). The results are rapidly generated, which has definite advantages in the clinical setting. However, susceptibility testing by FACS may have some limitations. Undertaking multiple assays of different concentrations of the drug may be time-consuming even though the result for each assay in our study was quick. Therefore, a breakpoint type of analysis, such as one that can be derived from our results (more than or equal to 1 µg/ml) would be feasible by FACS in routine microbiological practice, whereas a complete dilution series probably is not.

In our study, the FACS method produced an acceptable level of agreement with M27-A. The overall agreement for the MICs between the two methods was 83% (for both amphotericin and fluconazole). Particularly, for fluconazole the agreement between the FACS method and the M27-A protocol is 83%, which is similar to the agreement between the microdilution method and the M27-A protocol (80% at 24 h) (23). Moreover, the current breakpoint agreement between FACS and M27-A was 100% in our study.

Since MICs obtained by M27-A have generally correlated well with outcome in various animal models of infection, it was expected that MICs will prove useful in prediction of the likelihood of response to antifungal agents in human infections (23, 25). A NCCLS subcommittee analyzed data correlating susceptibility in vitro with outcome in vivo to define interpretive breakpoints for fluconazole. The data package contained 420 isolates from patients with oropharyngeal candidiasis and 108 isolates from patients with candidemia or visceral *Candida* infection. The authors indicated that the "conclusions are strongest for patients with oropharyngeal candidiasis and *C. albicans* infection and that the available data for correlating MIC with outcome are much more limited for non-albicans *Candida* infections and for invasive *Candida* infections" (24). In the area of invasive *Candida* infections, two studies has systematically investigated the fluconazole MICs with the outcome (11, 23). In one study the MICs obtained by macrodilution (M27-A) and microdilution methods did not correlate with outcome; e.g., high MICs did not predict failure. Moreover, an inverse correlation between MICs and outcome was found (23). The authors suggested that factors other than intrinsic antifungal susceptibility were operative in their patients and that artificial test conditions are remote from those that

exist in vivo, so that the susceptibility tests can be expected to offer only a crude estimate of the likely outcome of treatment (23). Recently, other predictors of the response of experimental invasive candidiasis to fluconazole have been investigated (2, 12). Data from these studies suggested that AUC (area under the concentration-time curve) to MIC ratios are good predictors of outcome. These predictors have been investigated in patients with oropharyngeal candidiasis, and an AUC/MIC ratio of 25 or higher with a 48-h M27-A MIC predicted success (J. H. Rex, M. A. Pfaller, T. J. Walsh, V. Chaturvedi, A. Espinel-Ingroff, M. A. Ghannoum, L. L. Gosey, F. C. Odds, M. G. Rinaldi, D. J. Sheehan, and D. W. Warnock, Program Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., poster, 2000).

We did not find that MICs below the breakpoint were associated with cure when using the fluconazole breakpoints suggested by NCCLS in the M27-A protocol (resistance, MIC ≥ 64 µg/ml) (Table 3). In contrast, six fluconazole-treated patients died despite harboring *Candida* isolates whose fluconazole MICs were <64 µg/ml by the M27-A method. As mentioned above, an AUC/MIC ratio of ≥25 predicted success in patients with oropharyngeal candidiasis. The total daily fluconazole dose closely approximates $AUC_{0-24 \ h}$ in humans with generally normal habitus and renal function (13; Rex et al., 40th ICAAC). A daily dose/MIC ratio of ≥25 is achieved with 400/16 (daily dose/MIC in micrograms per milliliter). This suggests that the clinically relevant M27-A MIC breakpoint for 400 mg of fluconazole is probably lower (Rex et al., 40th ICAAC). However, using fluconazole MICs (obtained by FACS and M27-A) for our *Candida* bloodstream isolates, we did not find that a total daily dose/MIC ratio of ≥25 predicted success. In contrast, 6 of 16 patients with a fluconazole daily dose/MIC ratio of ≥25 died (Table 2). Although a daily dose/MIC ratio of ≥25 predicted success in patients with oropharyngeal candidiasis and experimental invasive candidiasis (12; Rex et al., 40th ICAAC), our data indicate that this is not the case in candidemic patients.

Recent dose fractionation studies by Louie et al. (12) have suggested that AUC is the parameter most closely linked to outcome in an immunocompetent murine candidiasis model. In a neutropenic murine disseminated candidiasis model, AUC/MIC ratios of 12 to 24 achieved a microbiological effect of 50% of the maximal effect (2). The calculation of these parameters had application to the NCCLS in vitro susceptibility breakpoint guidelines in the therapy of invasive *Candida* disease (15). However, the authors of the animal studies used fungal densities in the kidneys and not survival as the study end point. In addition, Rex at al. (24) noted that therapeutic failures were seen even with isolates for which the MICs were very low while success was obtained at times even when the MICs were very high. The higher overall failure rate was attributed to the increasing clinical severity of illness in these patients. Although the proposed FACS breakpoint of 1 µg/ml could be related to the small patient number, we are confident that response to fluconazole is a function of both the MIC and drug dosage.

In our study we found a correlation between MICs obtained by the FACS method and clinical outcome. As shown in Table 3, a FACS MIC of <1 µg/ml was associated with cure and a FACS MIC of ≥1 µg/ml was associated with death. One rea-

son why the MICs obtained by the M27A protocol did not show a correlation with outcome may be the small number of patients included (21 fluconazole-treated patients). However, in one study the success rate of fluconazole treatment of hematogenous or deep-seated *Candida* infections in 24 patients infected with *Candida* strains for which the fluconazole MICs were ≤8 μg/ml (interpreted as susceptible according to the M27-A method) was 79% (11). Using the same MIC interpretation in our 19 patients infected with susceptible *Candida* spp. (≤8 μg/ml according to the M27-A method), we found a comparable success rate of 74%, well below the success rate of 100% using MICs obtained by FACS and suggested FACS breakpoints. However, the M27-A data show that the success rate in patients infected with susceptible strains is higher than that in patients infected with resistant strains (74 and 0%, respectively). Nevertheless, MICs obtained by FACS correlated with the MICs obtained by the M27-A protocol.

The main advantage of the FACS method lies in the rapid availability of the results (within a few hours). However, there are a number of significant disadvantages of using an expensive instrument such as FACS for susceptibility testing. Critical parameters such as the model and design of the instrument, instrument settings, effects of antifungals per se on fluorescence, and types of fluorescent dyes all require standardization.

In summary, susceptibility testing of blood culture isolates by a FACS method yielded results comparable to those obtained by the standard M27-A procedure. Our data suggest that FACS MICs correlate with clinical outcome: an MIC of <1 μg/ml is associated with cure, whereas an elevated MIC of ≥1 μg/ml predicts treatment failure. However, despite the limited number of isolates investigated, these results warrant further studies on the consistency, reproducibility, and clinical correlation of antifungal susceptibility testing by a FACS method.

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