

# Interpretive Breakpoints for Fluconazole and *Candida* Revisited: a Blueprint for the Future of Antifungal Susceptibility Testing

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## INTRODUCTION

Fluconazole is a triazole antifungal agent that has been available for the treatment of infections due to *Candida*, *Cryptococcus*, and other opportunistic yeasts since 1990 (43, 97). The drug is available as a tablet (50, 100, or 200 mg), as an oral suspension, and as an intravenous formulation (200 or 400 mg). When used in the treatment of invasive candidiasis (e.g., bloodstream infections [BSI], deep tissue sites, other normally sterile site infections), fluconazole is administered as an initial loading dose of 800 mg (oral or intravenous) followed by a daily maintenance dose of 400 mg (oral or intravenous). Higher daily doses of 800 mg or greater may be used in selected circumstances (24, 59, 87, 105).

There is now a very broad clinical experience of using fluconazole to treat both mucosal and invasive candidiasis, to the extent that it must be considered one of the first-line agents for the treatment of all forms of candidiasis (54). Clinical resistance of *Candida* spp. to fluconazole has been well documented in many settings (5, 6, 14, 15, 37, 38, 78, 80–82, 88, 100, 106); however, aside from infections with *Candida glabrata* and *C. krusei*, true antimicrobial resistance is rare among species of *Candida* commonly associated with invasive disease (5, 16, 18, 38, 64, 68, 82, 83, 96, 97, 100, 110, 112). The application of in vitro susceptibility testing and the use of molecular methods have served to detect potentially resistant strains of *Candida* and to characterize completely the various mechanisms of resistance to fluconazole and other azoles in clinical isolates of *Candida* spp. (12, 44, 52, 64, 68, 78, 96, 110).

The Clinical and Laboratory Standards Institute (CLSI) (formerly the National Committee for Clinical Laboratory Standards [NCCLS]) Subcommittee for Antifungal Testing has developed and standardized broth microdilution (BMD), broth macrodilution, and disk diffusion methods for in vitro susceptibility testing of *Candida* spp. (and other yeasts) against fluconazole (50, 51). In addition to standardized testing methods, the CLSI Subcommittee for Antifungal Testing has approved quality control limits for both MIC and disk diffusion methods with fluconazole (11, 50, 51). These methods have been applied worldwide to generate a very detailed and comprehensive understanding of the in vitro susceptibility profile of *Candida* spp. to fluconazole (8, 9, 16–18, 20, 21, 29, 32, 33, 38, 54, 64, 68–73, 75, 83, 100, 112).

In 1997, the CLSI Subcommittee for Antifungal Testing used accumulated clinical and microbiological data to propose interpretive breakpoints for MIC testing of fluconazole against *Candida* spp. (84). The breakpoints, which were subsequently incorporated into NCCLS document M27-A (48), were as follows: susceptible (S), MIC  $\leq$  8  $\mu$ g/ml; susceptible-dose dependent (SDD), MIC = 16 to 32  $\mu$ g/ml; resistant (R), MIC  $\geq$  64  $\mu$ g/ml. Despite the fact that the process and rationale for developing these breakpoints was described in great detail (84), and that the breakpoints were arrived at using the best available clinical and in vitro data (85, 86), concerns have been raised regarding the clinical utility of these breakpoints and of in vitro susceptibility testing of fluconazole in general (99). In particular, concern has been expressed that most (~80%) of the data supporting the breakpoints were derived from studies of mucosal candidiasis and that, in addition, there was little clinical outcome data for isolates for which the MICs were elevated (85). Thus, it was unclear how useful such testing would be in predicting the outcome of more serious invasive

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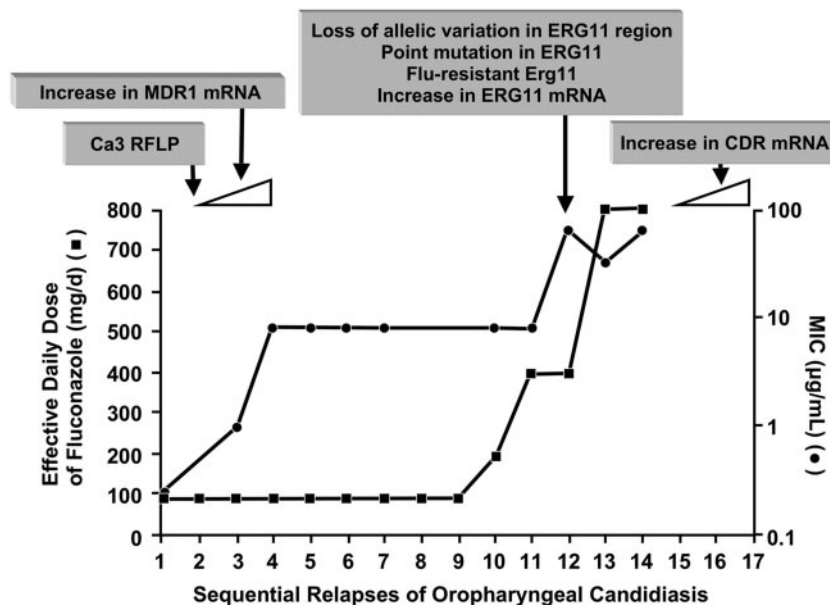


FIG. 1. Relationship between MIC, dose of fluconazole, and emergence/expression of specific resistance mechanisms in oropharyngeal candidiasis. ●, MIC of fluconazole for the clinical isolate; ■, effective daily dose of fluconazole. MICs are represented on the secondary y axis, in logarithmic scale. Boxes above the graph represent genetic changes identified at each stage. Based on data from Redding et al. (78), White (108, 109), and White et al. (110). (Reprinted from reference 23 with permission.)

infections treated with fluconazole and for those involving strains for which the fluconazole MICs were elevated (86).

In this review we readdress the issue of fluconazole breakpoints for *Candida* spp. by using the available published microbiological and clinical data to provide further validation of the breakpoints proposed by CLSI in 1997. The analytical model employed was that used previously (84) and as outlined for all types of antimicrobial testing in CLSI document M23-A2 (49). We considered the data relating the MICs to known resistance mechanisms, the MIC (and disk zone diameter) distribution profiles, pharmacokinetic (PK) and pharmacodynamic (PD) parameters, and the relationship between in vitro activity (MIC) and clinical outcome, as determined by the investigators in a total of 12 (7 mucosal and 5 invasive) published clinical efficacy studies. These analyses are summarized below.

#### MECHANISMS OF RESISTANCE TO FLUCONAZOLE IN *CANDIDA* SPP.

Fluconazole, like all azoles, acts by inhibiting the fungal cytochrome P-450-dependent enzyme lanosterol 14- $\alpha$ -demethylase. This enzyme functions to convert lanosterol to ergosterol, and its inhibition disrupts membrane synthesis in the fungal cell (97).

The mechanism of azole resistance in *Candida* has been well worked out for fluconazole and *C. albicans* (61, 95, 110). Resistance can arise from a modification in the quality or quantity of the target enzyme, reduced access of the drug to the target, or some combination of these mechanisms (23, 110). In the first instance, point mutations in the gene (*ERG11*) encoding the target enzyme, 14- $\alpha$ -demethylase, lead to an altered target with decreased affinity for azoles. Overexpression of *ERG11*

results in the production of high concentrations of the target enzyme, creating the need for higher intracellular fluconazole concentrations to inhibit all of the enzyme molecules in the cell. Loss of allelic variation in the *ERG11* promoter may result in a resistant strain that is homozygous for the mutated gene (Fig. 1) (109).

The second major mechanism involves active efflux of fluconazole out of the cell through the activation of two types of multidrug efflux transporters: the major facilitators (encoded by *MDR* genes) and those of the ATP-binding cassette superfamily (encoded by *CDR* genes) (23, 61, 95, 110). Upregulation of the *MDR1* gene leads to elevated fluconazole MICs (Fig. 1), whereas upregulation of *CDR* genes leads to resistance to multiple azoles (12, 90–93, 96, 110). Evidence that these mechanisms may act individually, sequentially, and in concert has been derived by studying serial isolates of *C. albicans* from AIDS patients with oropharyngeal candidiasis (39, 40, 78, 108) as well as from patients with invasive disease (44, 52). An example of the evolution of fluconazole resistance in a single patient is shown graphically in Fig. 1 (78, 108, 110). This example clearly shows the relationship between the fluconazole MIC, the dose of fluconazole, and the emergence/expression of specific resistance mechanisms. It provides excellent support for the CLSI MIC breakpoints.

It is also now well established that the mechanism of resistance to fluconazole, and other azoles, in *C. glabrata* involves upregulation of the *CDR1* and *CDR2* genes, resulting in resistance to multiple azoles (12, 94, 96). Thus, exposure of *C. glabrata* to subtherapeutic doses (i.e., <400 mg/day) of fluconazole may result in resistance not only to fluconazole but to other azoles (i.e., itraconazole and voriconazole) as well (12, 64, 71). Fluconazole resistance in *C. krusei* appears to be me-

TABLE 1. Susceptibility of *Candida* BSI isolates to fluconazole by MIC: Global Antifungal Surveillance Program, 1992–2004<sup>a</sup>

Organism	No. tested	Cumulative % at MIC ( $\mu\text{g/ml}$ ) of <sup>b</sup> :									%R <sup>c</sup>
		0.12	0.25	0.5	1	2	4	8	16	32	
<i>C. albicans</i>	7,725	27	84	94	97	98	98	99	99	>99	0.06
<i>C. glabrata</i>	1,966	<1	<1	<1	1	10	34	62	87	91	9
<i>C. parapsilosis</i>	1,623	<1	10	49	79	92	96	97	99	>99	0.5
<i>C. tropicalis</i>	1,253	2	21	52	78	94	97	98	98	98	2
<i>C. krusei</i>	312						<1	3	13	60	40
<i>C. lusitaniae</i>	134	4	37	69	85	93	96	97	98	99	1
<i>C. dubliniensis</i>	103	45	83	86	87	87	87	91	93	97	3
<i>C. guilliermondii</i>	92		1	2	5	40	74	90	96	97	3
<i>C. pelliculosa</i>	34					18	79	100			0
<i>C. kefyr</i>	33	12	61	94	100						0
<i>C. famata</i>	19		5	21	37	42	63	74	100		0
<i>C. rugosa</i>	19	68	79	79	79	79	100				0
All <i>Candida</i> spp.	13,338	17	53	67	75	81	86	91	95	97	3

<sup>a</sup> Data compiled from references 22, 23, 68, and 70; BSI isolates from >200 institutions worldwide.

<sup>b</sup> Broth microdilution MICs determined in accordance with CLSI M27-A2 (50).

<sup>c</sup> %R, percent resistant at a MIC of  $\geq 64 \mu\text{g/ml}$ .

diated by reduced sensitivity of the target enzyme to inhibition by the agent (53).

#### PK AND PD CONSIDERATIONS

Fluconazole is a bistriazole antifungal with a half-life of approximately 30 h in adults and 15 h in children. Protein binding is low (11% to 12%), and it is distributed to virtually all organs and tissues, including the central nervous system (97). Fluconazole is renally excreted, with approximately 80% recovered in the urine as active, unchanged drug. Currently used dosages span the range of 50 mg/day to as high as 2 g/day; however, the standard dose in adults is 400 to 800 mg/day and in children is 6 to 12 mg/kg (of body weight)/day (1, 26). Dosages of 100 mg/day (1.5 mg/kg/day) produce peak serum drug levels of  $\sim 6.7 \mu\text{g/ml}$ , 400 mg/day (6 mg/kg/day) produces peak levels of 20 to 30  $\mu\text{g/ml}$ , and the linear pharmacokinetics of fluconazole would predict peak serum levels of 40 to 60  $\mu\text{g/ml}$  at a dosage of 800 mg/day (12 mg/kg/day) (25, 84). With daily dosing, trough levels would be approximately half the peak level. Thus, a daily dose of 400 mg/day would provide serum fluconazole levels in excess of 10  $\mu\text{g/ml}$  throughout the dosing interval (2, 3, 43).

PD investigations of fluconazole and *Candida* have been undertaken, and both in vitro and in vivo models have demonstrated a correlation between drug dose, organism MIC, and outcome (2–4, 36, 42). Fluconazole exhibits time-dependent, concentration-independent fungistatic activity against *Candida* (2–4, 13, 36). This concentration-independent activity, coupled with a prolonged in vivo post-antifungal effect, increases the importance of the total amount of drug administered (2, 3). The area under the serum concentration curve (AUC) represents the total amount of drug exposure, and the AUC/MIC ratio is the predictive PD parameter (2, 3). In vivo fluconazole dose-ranging studies with *C. albicans* strains for which the MICs varied 64-fold found that an AUC/MIC magnitude near 25 predicted efficacy (4). Subsequent studies have included *C. albicans* strains for which MICs covered a range of more than 500-fold and have confirmed treatment success with a fluconazole AUC/MIC ratio near 25 (42, 89, 103).

#### FLUCONAZOLE MIC DISTRIBUTION PROFILE FOR *CANDIDA* SPECIES

The fluconazole MIC profile for each of 12 different species of *Candida* (13,338 isolates) is shown in Table 1. These results were all determined in a single reference laboratory (University of Iowa) by CLSI-recommended BMD methods (50). This large data set represents recent (1992 to 2004), clinically important (blood and normally sterile-site) isolates from more than 200 different medical centers throughout the world (22, 23, 64, 67–70, 73, 75). The overall MIC at which 90% of the isolates were inhibited ( $\text{MIC}_{90}$ ) for fluconazole was 8  $\mu\text{g/ml}$ ; 91% of the 13,338 isolates tested were encompassed by the CLSI susceptible (S) category ( $\text{MIC} \leq 8 \mu\text{g/ml}$ ), and 3% were resistant (R) ( $\text{MIC} \geq 64 \mu\text{g/ml}$ ). The  $\text{MIC}_{90}$  was highest for *C. krusei* ( $\text{MIC} \geq 64 \mu\text{g/ml}$ ) and *C. glabrata* ( $\text{MIC}$ , 32  $\mu\text{g/ml}$ ) and was  $\leq 2 \mu\text{g/ml}$  for *C. albicans* (0.5  $\mu\text{g/ml}$ ), *C. parapsilosis* (2  $\mu\text{g/ml}$ ), *C. tropicalis* (2  $\mu\text{g/ml}$ ), *C. lusitaniae* (2  $\mu\text{g/ml}$ ), and *C. kefyr* (0.5  $\mu\text{g/ml}$ ).

Resistance to fluconazole, designated by an MIC of  $\geq 64 \mu\text{g/ml}$ , was  $\leq 3\%$  for all species of *Candida* with the exception of *C. glabrata* (9%) and *C. krusei* (40%). These data, including the species distribution rank order, are highly representative of that published in numerous in vitro surveys of *Candida* BSI isolates (Table 2) (8, 16–21, 29, 32, 33, 54, 63, 64, 66, 83, 100). It is notable that fluconazole resistance among BSI isolates of *C. albicans*, *C. tropicalis*, and *C. parapsilosis*, as determined by CLSI (or EUCAST [European Committee for Antimicrobial Susceptibility Testing]) BMD methods, has remained very infrequent (usually  $\leq 3\%$ ) worldwide from 1990 to the present (Table 2). However, important variation has been seen among isolates of *C. glabrata* over the same period (Table 2). Although the frequency of fluconazole resistance among BSI isolates of *C. glabrata* was  $<10\%$  in many different surveys conducted in various countries, the MICs for this species were always higher than those seen with other species (Table 1), and higher-than-standard doses of fluconazole (e.g.,  $\geq 800 \text{ mg/day}$ ) are recommended if fluconazole is used to treat infections with this species (59). Although fluconazole resistance has remained low among *Candida* spp. over the 14-year period in

TABLE 2. Fluconazole resistance among *Candida* BSI isolates as determined by different surveillance programs<sup>a</sup>

Surveillance program <sup>b</sup>	Yr	Reference	% Resistant by species (no. tested) <sup>c</sup>			
			<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. parapsilosis</i>	<i>C. tropicalis</i>
Iceland	1980–1999	8	0 (67)	0 (12)	0 (11)	0 (5)
CDC	1992–1993	35	1 (183)	14 (59)	0 (83)	2 (59)
CDC	1998–2000	30	1 (423)	7 (226)	0 (123)	6 (118)
Sweden	1994–1998	17	0 (123)	40 (52)	15 (33)	0 (11)
Quebec	1996–1998	98	1 (240)	9 (67)	0 (53)	0 (41)
Taiwan	1994–1995	16	0 (59)	0 (17)	0 (24)	0 (33)
Taiwan	1999–2000	16	0 (62)	0 (39)	0 (43)	0 (47)
Taiwan	2003	34	0 (207)	14 (59)	0 (41)	0 (67)
Argentina	1996–1999	20	9 <sup>d</sup> (94)	50 <sup>d</sup> (6)	1 <sup>d</sup> (70)	6 <sup>d</sup> (47)
Spain	1996–1999	20	2 <sup>d</sup> (155)	43 <sup>d</sup> (49)	0 <sup>d</sup> (201)	0 <sup>d</sup> (55)
Spain	2002–2003	21	0 <sup>d</sup> (178)	19 <sup>d</sup> (31)	1 <sup>d</sup> (81)	3 <sup>d</sup> (36)
Italy	1997–1999	102	NA <sup>e</sup>	4 (80)	0 (49)	5 (44)
SENTRY	1997–2000	66	1 (1,114)	7 (334)	0 (301)	1 (209)
EIEIO	1998–2001	22	0 (147)	10 (51)	0 (18)	0 (28)
ARTEMIS	2001–2002	69	1 (2,359)	9 (607)	1 (439)	1 (319)

<sup>a</sup> Adapted from Pfaller (62).

<sup>b</sup> All multicenter studies used CLSI or EUCAST broth microdilution methods. CDC, Centers for Disease Control and Prevention; EIEIO, Emerging Infections and the Epidemiology of Iowa Organisms.

<sup>c</sup> Resistant isolates were those for which the MIC was  $\geq 64$   $\mu\text{g/ml}$ .

<sup>d</sup> Includes both SDD (MIC = 16 to 32  $\mu\text{g/ml}$ ) and R (MIC  $\geq 64$   $\mu\text{g/ml}$ ) categories.

<sup>e</sup> NA, data not available.

which it has been used clinically, isolates of *C. glabrata* collected between 2001 and 2004 appear to be progressively more resistant (Table 3) (D. J. Diekema, S. Messer, L. Boyken, S. Tendolkar, R. Hollis, and M. A. Pfaller, Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-2238, 2005). An increase in both MIC<sub>90</sub> (16  $\mu\text{g/ml}$  to 64  $\mu\text{g/ml}$ ) and the percent R (7% to 12%) was observed over the 4-year period despite an overall trend toward a decrease in the frequency of *C. glabrata* detected as a cause of BSI at the various surveillance sites (Table 3). *C. glabrata* remains the focus for concern regarding fluconazole resistance (71). Although fluconazole may serve as a safe, efficacious, and cost-effective treatment option for infections due to *C. glabrata* (59), the susceptibility of this species to fluconazole, or other azoles, is not predictable and requires confirmation by “real-time” antifungal susceptibility testing (9, 28, 31, 71, 112).

TABLE 3. Trends in the frequency of occurrence and resistance to fluconazole among BSI isolates of *C. glabrata*: ARTEMIS Global Antifungal Surveillance Program, 2001–2004<sup>a</sup>

Yr	Total no. of <i>Candida</i> BSI isolates	No. (%) of <i>C. glabrata</i> BSI isolates	MIC ( $\mu\text{g/ml}$ ) <sup>b</sup>		% Resistant <sup>c</sup>
			50%	90%	
2001	1,932	302 (15.6)	8	16	7
2002	2,387	346 (14.5)	8	32	9
2003	2,495	325 (13.0)	16	32	8
2004	1,892	211 (11.2)	16	64	12

<sup>a</sup> A total of 8,706 isolates from 91 institutions worldwide (Diekema et al., 45th ICAAC).

<sup>b</sup> Broth microdilution MICs were determined in accordance with CLSI M27-A2 (50). 50% and 90%, MIC encompassing 50% and 90% of isolates tested, respectively.

<sup>c</sup> Percent resistant to fluconazole at a MIC of  $\geq 64$   $\mu\text{g/ml}$ .

#### FLUCONAZOLE MICs FOR *CANDIDA* SPP. ISOLATED IN RANDOMIZED TRIALS OF FLUCONAZOLE IN THE TREATMENT OF CANDIDEMIA

There have been two randomized multicenter clinical trials examining the efficacy of fluconazole in the treatment of candidemia (81, 87). The first trial compared fluconazole to amphotericin B in the treatment of candidemia in non-neutropenic patients (81), and the second compared high-dose (800 mg/day) fluconazole plus placebo with high-dose fluconazole plus amphotericin B also in the treatment of candidemia in non-neutropenic patients (87). The isolates from these two clinical trials, plus additional isolates collected during an observational study conducted concurrently with the high-dose fluconazole trial (58), were tested for susceptibility to fluconazole by CLSI broth dilution methods (54, 83) (Table 4). The five major species from these studies are identical to those shown in Table 1 and in other multicenter surveys (Table 2). Among these more common species (2,190 isolates), the relative susceptibility to fluconazole was similar to that shown in Table 1, with *C. albicans* representing the most susceptible species (MIC<sub>90</sub>, 1 to 2  $\mu\text{g/ml}$ ; <1 to 5% R) and *C. krusei* (MIC<sub>90</sub>, >64  $\mu\text{g/ml}$ ; 34% R) and *C. glabrata* (MIC<sub>90</sub>, 32  $\mu\text{g/ml}$ ; 8 to 10% R) the least susceptible species (Table 4). Interestingly, both of these studies reported high fluconazole MICs for *C. tropicalis* compared to those in Tables 1 and 2. *C. tropicalis* has been shown to exhibit a high frequency of trailing (incomplete inhibition of growth), which may result in artificially high MICs (7, 54). Indeed, in both studies the MICs for *C. tropicalis* were considerably lower, and in concert with those in Table 1, when the isolates were retested by CLSI BMD and the MICs read at 24 h of incubation (to minimize trailing) (54, 83). Thus, the large-scale surveys produce both MICs and species distribution profiles that are entirely representative of those seen in more formal clinical trials of fluconazole efficacy.



TABLE 4. Species distribution and susceptibility to fluconazole among *Candida* isolates obtained in two randomized trials of fluconazole in the treatment of candidemia

Species	Clinical study, time period <sup>a</sup>							
	Rex (83), 1989–1993 <sup>b</sup>				Ostrosky-Zeichner (54), 1995–1999 <sup>c</sup>			
	n	MIC <sub>50</sub>	MIC <sub>90</sub>	%R	n	MIC <sub>50</sub>	MIC <sub>90</sub>	%R
<i>C. albicans</i>	129	0.25	1	<1	733	0.25	2	5
<i>C. glabrata</i>	31	16	32	10	458	8	32	8
<i>C. parapsilosis</i>	23	1	4	0	391	1	2	2
<i>C. tropicalis</i>	40	1 <sup>d</sup>	>64 <sup>d</sup>	25 <sup>d</sup>	307	0.5	16	8
<i>C. krusei</i>	6	32	ND	NA	50	32	>64	34
<i>C. lusitanae</i>	3	0.5	ND	NA	20	0.5	2	0

<sup>a</sup> MIC<sub>50</sub> and MIC<sub>90</sub>, MIC encompassing 50% and 90% of isolates tested, respectively; %R, percent resistant at MIC of  $\geq 64$   $\mu\text{g/ml}$ ; ND, not done (<10 isolates); NA, not available.

<sup>b</sup> Isolates tested by broth macrodilution (NCCLS, M27-A) (48).

<sup>c</sup> Isolates tested by CLSI M27-A2 broth microdilution (50).

<sup>d</sup> Trailing artifact. When retested using CLSI broth microdilution and reading MIC at 24 h of incubation, MIC<sub>50/90</sub> values were 0.25 and 1  $\mu\text{g/ml}$ , respectively (83).

### CROSS-RESISTANCE BETWEEN FLUCONAZOLE AND OTHER AZOLES

Previous *in vitro* studies have suggested that cross-resistance may occur with fluconazole and other azole compounds (19, 54, 64, 66, 69, 70, 73, 74). The mechanistic basis for such cross-resistance has been clearly demonstrated in a number of elegant studies and most often involves the upregulation of genes encoding the ATP-binding cassette efflux transporters, the so-called CDR pumps (12, 90, 92, 93, 96, 110).

Isolates of *Candida* spp. for which fluconazole MICs are  $\geq 64$   $\mu\text{g/ml}$  also tend to be less susceptible (MIC  $\geq 2$   $\mu\text{g/ml}$ ) to itraconazole (73), posaconazole (69), ravuconazole (65, 70), and voriconazole (69, 77). In general, among *Candida* isolates there is a strong positive correlation ( $R = 0.9$ ) between fluconazole MICs and those of itraconazole, posaconazole, ravuconazole, and voriconazole, suggesting some degree of cross-resistance (54, 69, 70, 77). This is especially true for *C. glabrata* (69, 77).

### CLINICAL CORRELATION AND SUPPORT OF CLSI MIC BREAKPOINTS FOR FLUCONAZOLE

The previously established CLSI MIC interpretive breakpoints for *Candida* spp. tested against fluconazole were based on an analysis of treatment outcomes in both mucosal (411 patient-episode-isolate events) and invasive (108 patient-episode-isolate events) disease (84, 85). In addition to the traditional categories of “susceptible” and “resistant,” the interpretive breakpoints included a novel category, “susceptible-dose dependent” (SDD) (84). The SDD category encompassed MICs of 16 and 32  $\mu\text{g/ml}$ . Isolates for which fluconazole MICs were above this range ( $\geq 64$   $\mu\text{g/ml}$ ) were termed resistant (R), whereas those inhibited at lower concentrations ( $\leq 8$   $\mu\text{g/ml}$ ) were labeled susceptible (S). The purpose of the SDD category was to emphasize the importance of attaining maximal fluconazole levels in blood and tissue for isolates with higher MICs (85). The maximal dose was defined as being at least 400 mg/day in a 70-kg adult with normal renal function (59). The overall clinical response rate for these 519 patient-episode-

isolate events was 87%, including 92% (370/403) for those episodes in which the fluconazole MIC for the isolate was  $\leq 8$   $\mu\text{g/ml}$  (S), 82% (45/55) for those episodes in which the MIC was 16 to 32  $\mu\text{g/ml}$  (SDD), and 56% (34/61) for those episodes in which the MIC was  $\geq 64$   $\mu\text{g/ml}$  (R) (84). For patients with mucosal disease (411 patient-episode-isolate events), the response rates were 92%, 82%, and 41% at MICs of  $\leq 8$   $\mu\text{g/ml}$ , 16 to 32  $\mu\text{g/ml}$ , and  $\geq 64$   $\mu\text{g/ml}$ , respectively. The corresponding success rates for patients with invasive disease (108 patient-episode-isolate events) were 71%, 91%, and 58%, respectively (84). The weaknesses of these data were noted to be that (i) the majority of the results were drawn from cases of mucosal candidiasis, (ii) the number of episodes involving isolates for which the fluconazole MICs were elevated ( $>8$   $\mu\text{g/ml}$ ) was small, and (iii) the concept of dose-dependent susceptibility was most clearly proven only for mucosal disease (85).

Subsequent to this analysis, there have been several additional studies in which the efficacy of fluconazole therapy has been examined relative to the fluconazole MIC or susceptibility category (S, SDD, and R) of the infecting isolate, as determined by standardized (CLSI or EUCAST) susceptibility testing methods (Table 5). In each instance the isolate with the highest MIC from each episode of infection from each patient was defined as a separate patient-episode-isolate event (84). A total of 1,295 patient-episode-isolate events in which patients were infected with *Candida* spp., received fluconazole therapy, and were characterized as treatment successes or failures at the end of therapy were included in 12 published clinical databases (Table 5). These studies included seven (692 patient-episode-isolate events) in which the infection was mucosal in nature and where the daily dose of fluconazole was typically 100 mg/day (6, 14, 15, 37, 80, 84, 88) and five (603 patient-episode-isolate events) in which invasive candidiasis (bloodstream, tissue, normally sterile-site infection) was treated with higher doses (usually 400 mg/day) of fluconazole (5, 18, 38, 84, 100). Although the clinical response was not stratified by species of *Candida*, the species distribution in these studies was typical for the two broad infection types: mucosal (80% *C. albicans*, 20% non-*C. albicans*) and invasive (40% *C. albicans*, 60% non-*C. albicans*).

Among the isolates causing mucosal infection, 77% (533/692) were susceptible (MIC  $\leq 8$   $\mu\text{g/ml}$ ), 8% (58/692) were SDD (MIC, 16 to 32  $\mu\text{g/ml}$ ), and 15% (101/692) were R to fluconazole (Table 5). Similarly, 76% (460/603) of the isolates from invasive infections were classified as S, 12% (72/603) were SDD, and 12% (71/603) were R. By comparison to the MIC distribution profiles shown in Tables 1 and 4, these isolates tended to be less susceptible to fluconazole. These differences may be explained by the fact that the MIC data shown in Tables 1 and 4 represent that of the incident isolate (first isolate) from each episode of infection, whereas those shown in Table 5 generally represent the highest MIC determined for each patient-episode. Thus, the patient-episode-isolate events summarized in Table 5 represent a rigorous challenge to the therapeutic efficacy of fluconazole and provide significant numbers of isolates for which fluconazole MICs are elevated. Additionally, the studies shown in Table 5 serve to expand the number of patient-episode-isolate events representing more serious invasive disease and thus strengthen the potential ap-

TABLE 5. Correlations of fluconazole susceptibility testing with clinical response for mucosal and invasive *Candida* infections treated with fluconazole<sup>a</sup>

Reference	Type of infection	Dose (mg/day)	MIC ( $\mu\text{g/ml}$ ) used to determine susceptibility class			No. of events <sup>b</sup>	% Success ( $n/N$ ) <sup>f</sup> by susceptibility class		
			S	SDD	R		S	SDD	R
84	Mucosal	100	$\leq 8$	16–32	$\geq 64$	302	98 (248/253)	78 (21/27)	73 (16/22)
6	Mucosal	100–200	$\leq 8$	16–32	$\geq 64$	48	80 (28/35)		46 (6/13)
15	Mucosal	100	$\leq 8$	16–32	$\geq 64$	66	96 (49/51)	0 (0/7)	0 (0/8)
88	Mucosal	100–400	$\leq 32$		$\geq 64$	21	88 (14/16)		0 (0/5)
37	Mucosal	100	$\leq 0.39$	1.56	$\geq 3.12$	27	93 (14/15)	0 (0/2)	30 (3/10)
14	Mucosal	NA <sup>c</sup>	$\leq 8$	16–32	$\geq 64$	73	75 (42/56)	50 (2/4)	23 (3/13)
80	Mucosal	100–800	$\leq 8$	16–32	$\geq 64$	155	87 (93/107)	72 (13/18)	43 (13/30)
Total mucosal						692	92 (488/533)	62 (36/58)	41 (41/101)
84	Invasive <sup>d</sup>	>100	$\leq 8$	16–32	$\geq 64$	217 <sup>e</sup>	81 (122/150)	86 (24/28)	46 (18/39)
38	Invasive	400	$\leq 8$	16–32	$\geq 64$	32	79 (19/24)	67 (4/6)	0 (0/2)
5	Invasive	400	$\leq 8$	16–32	$\geq 64$	80	92 (54/59)	75 (6/8)	54 (7/13)
18	Invasive	50–400	$\leq 8$	16–32	$\geq 64$	32	67 (14/21)	20 (1/5)	0 (0/6)
100	Invasive	200	$\leq 8$	16–32	$\geq 64$	242	70 (144/206)	64 (16/25)	55 (6/11)
Total invasive						603	77 (353/460)	71 (51/72)	44 (31/71)
Total invasive plus mucosal						1,295	85 (841/993)	67 (87/130)	42 (72/172)

<sup>a</sup> All studies were performed by CLSI or EUCAST broth dilution methods. Percent clinical success was determined by investigators at the end of treatment.

<sup>b</sup> Number of individual patient-episode-isolate events.

<sup>c</sup> NA, data not available.

<sup>d</sup> Invasive infections including bloodstream, deep tissue, and other normally sterile sites.

<sup>e</sup> Ninety-nine patient-episode-isolate events represented invasive infection, with response rates of 71% (S), 91% (SDD), and 58% (R) (84).

<sup>f</sup> Abbreviations:  $n$ , number of successful events;  $N$ , number of total patient-episode-isolate events.

plicability of the MIC interpretive criteria to infections other than mucosal.

The overall success rate for the 1,295 patient-episode-isolate events shown in Table 5 was 77% (1,000/1,295), including 85% (841/993) for those episodes in which the fluconazole MIC was  $\leq 8 \mu\text{g/ml}$ , 67% (87/130) for those episodes in which the MIC was 16 to 32  $\mu\text{g/ml}$ , and 42% (72/172) for those episodes with resistant (MIC  $\geq 64 \mu\text{g/ml}$ ) isolates. These aggregate data clearly support the interpretive breakpoints proposed earlier by the CLSI Subcommittee for Antifungal Testing and are consistent with the “90-60” rule described previously for both antibacterial and antifungal susceptibility testing (86).

Although a successful outcome was more strongly predicted by a result of S (MIC  $\leq 8 \mu\text{g/ml}$ ) in the case of mucosal candidiasis compared to invasive disease, the complexities surrounding those patients in which candidemia or other invasive disease develops are well known and clearly contribute to a poorer outcome irrespective of the potency of the antifungal agent administered (31, 86, 100). Perhaps more important than the ability of an antifungal test to predict clinical success when the MIC is low (i.e., susceptible) is the ability to predict failure when the result is high (i.e., resistant). In this regard, fluconazole susceptibility testing functions quite well (Table 5). Only 42% of the patient-episode-isolate events were successfully treated with fluconazole when the MIC for the infecting isolate was  $\geq 64 \mu\text{g/ml}$ . This was true irrespective of whether the infection was mucosal or invasive. Thus, the evidence most strongly supports the usefulness of the R category. Consideration of the overall MIC distribution (Table 1), the cross-resistance and resistance mechanism data, the PK of the drug, and the relationship between MIC and clinical outcome (Table 5) are very compelling and supportive of this concept. It is clear

that *Candida* isolates for which fluconazole MICs are  $\geq 64 \mu\text{g/ml}$  (i) are predominantly *C. glabrata* and *C. krusei* (Table 1), (ii) represent a concentration of fluconazole that cannot be maintained over the dosing interval with currently recommended doses, (iii) exhibit several different resistance mechanisms (Fig. 1), and (iv) are significantly less likely to respond clinically to fluconazole therapy (Table 5).

The fact that most of the fluconazole-resistant isolates are *C. glabrata* and *C. krusei* raises some issues that may confound the relationship between MIC and outcome. Specifically, patients at risk for infections with *C. glabrata* and *C. krusei* may have other factors, such as advanced age, relapsed leukemia, fluconazole prophylaxis, and corticosteroid therapy, that can also be associated with poor outcome irrespective of the antifungal agent used to treat the infections (58, 100, 104, 112). Evidence to support the conclusion that decreased susceptibility to fluconazole does in fact contribute to a poor outcome may be found in several salvage therapy studies in which patients failing fluconazole therapy (and infected with isolates of *Candida* spp. [including *C. glabrata* and *C. krusei*] with decreased susceptibility to fluconazole) were treated successfully with voriconazole (55), micafungin (56), caspofungin (35), or amphotericin B (47, 112).

The data also support the SDD category as one in which the clinical response may approach that of S isolates as long as higher doses of fluconazole are used. This was seen most prominently in invasive disease, where the higher doses administered in such infections might be expected to be more effective in treating infections with organisms in the SDD category (2, 3, 85, 105). It is also apparent that the rate of successful therapy was higher with SDD isolates than that seen with isolates that were R. It should be noted that the concept of dose-dependent

TABLE 6. Relationship between dose/MIC ratio and clinical response in fluconazole treatment of mucosal and invasive candidiasis

Dose/MIC	% Clinical success (n/N) <sup>a</sup>				Total
	Rex et al. (85) <sup>b</sup>	Clancy et al. (18) <sup>c</sup>	Lee et al. (38) <sup>c</sup>	Takakura et al. (100) <sup>c</sup>	
≥400	99 (115/116)	89 (8/9)			98 (123/125)
100–300	98 (129/132)	60 (6/10)			95 (135/142)
50–75	92 (34/37)	0 (0/1)	79 (19/24)		86 (53/62)
25–37.5	91 (30/33)	0 (0/1)	67 (4/6)	70 (144/206)	72 (178/246)
6.26–12.5	74 (35/47)	20 (1/5)		64 (16/25)	68 (52/77)
<6.25	65 (30/46)	0 (0/6)	0 (0/2)	55 (6/11)	55 (36/65)

<sup>a</sup> Abbreviations: n, number of successful treatment events; N, number of total patient-episode-isolate events.

<sup>b</sup> Mucosal infection study.

<sup>c</sup> Invasive candidiasis (candidemia) study.

susceptibility pertains only to species such as *C. glabrata*, where resistance is inducible or acquired, and not to intrinsically resistant species such as *C. krusei*.

These data provide strong validation of the CLSI MIC interpretive breakpoints for fluconazole and *Candida* spp. and document their applicability in both mucosal and invasive disease. In performing this analysis we have addressed the aforementioned weaknesses of fluconazole MIC testing by expanding the number of invasive cases as well as the number of episodes of infection involving isolates for which fluconazole MICs were elevated. Furthermore, we provide additional data to support the concept of dose-dependent susceptibility for infections more serious than mucosal disease. Such a concept is even more strongly supported by PD considerations (2, 3, 18).

**PD SUPPORT FOR FLUCONAZOLE BREAKPOINTS AND DOSE-DEPENDENT SUSCEPTIBILITY OF *CANDIDA* SPP.**

Correlation of human PK and clinical trial outcome with several anti-infective agents has suggested that the PD parameter magnitude that produces efficacy in animal models also predicts efficacy in humans (2, 3). In vivo PD studies of fluconazole and invasive candidiasis have identified the AUC/MIC ratio as the key PD parameter and a magnitude of ~25 as predictive of efficacy (2–4, 42). It is known that the AUC for healthy adults given fluconazole is almost exactly equal to the daily dose, in milligrams (85). Thus, a 70-kg adult given 400 mg of fluconazole will have an AUC of 400 mg · h/liter (42, 85). Given this information, the CLSI Subcommittee for Antifungal Testing used the dose/MIC ratio as a surrogate for AUC/MIC in analyzing the relationship between drug dose, organism MIC, and clinical outcome for fluconazole treatment of mucosal candidiasis (85). The data showed a clear relationship between the dose/MIC ratio and clinical outcome (Table 6) (85). When the fluconazole dose/MIC ratio exceeded a value of 25, clinical treatment success was observed in 91% to 99% of patients (Table 6). When this PD value fell below 25, treatment failures were reported in 26% to 35% of cases (85). Thus, the dose/MIC ratio of ~25 was predictive of outcome in humans, as was the AUC/MIC ratio in animal model PD studies (2, 3). Notably, the dose/MIC magnitude of ~25 was supportive of the susceptibility breakpoint guidelines suggested in the CLSI M27 document (85).

Subsequent analysis of several smaller patient series provides additional support for this concept as it applies to oro-

pharyngeal candidiasis (2). Application of this PD analysis to patients with candidemia has been more problematic, largely due to lack of data from patients with invasive candidiasis who were treated with fluconazole and in which MICs were high enough to demonstrate a relationship between drug dose, MIC, and outcome (2, 3, 85). Three such studies have now been published, the results of which are compared with those of mucosal infection in Table 6. Although the rate of clinical success in these three studies was considerably lower than that in patients with mucosal infection, a strong relationship was observed between MIC, fluconazole dose, and outcome. Taken together, these three studies show that clinical success was observed in 70% of patients (181/257) when the dose/MIC ratio was 25 or greater and was 47% (23/49) when the PD value fell below 25 (Table 6). Combining the mucosal and invasive candidiasis databases shows a clear decrement in clinical response with a decreasing dose/MIC ratio (Table 6).

Looking at fluconazole MICs in the critical range of 8 to 32 µg/ml in light of the target dose/MIC ratio of 25, one can see that a dose of 800 mg/day would be required to treat an isolate for which the fluconazole MIC was 32 µg/ml and 200 mg/day would be required for an isolate for which the fluconazole MIC was 8 µg/ml (Table 7). The dose-dependent nature of the clinical response is very apparent in this critical MIC range, which spans the S and SDD susceptibility categories. In further support of the dose-dependent nature of response in candidemia are the experiences of Graninger et al. (24) and Voss and de Pauw (105), both of which demonstrated increased rates of successful therapy for candidemia when the dose of

TABLE 7. Analysis of the fluconazole dose/MIC ratio highlights important ranges for MICs and daily dosages

Dose (mg/day)	MIC (µg/ml)	Dose/MIC	Anticipated % success <sup>a</sup>
800	8	100	95
	16	50	86
	32	25	72
400	8	50	86
	16	25	72
	32	12.5	68
200	8	25	72
	16	12.5	68
	32	6.25	55

<sup>a</sup> Data from Table 6.

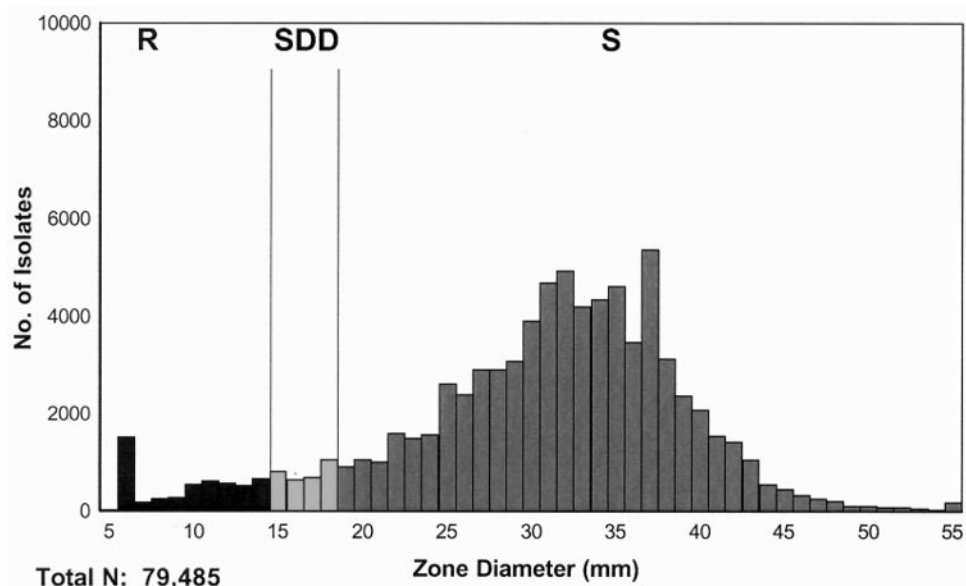


FIG. 2. Fluconazole zone diameter distributions for all *Candida* spp.: 79,485 isolates tested against fluconazole. Isolates were obtained from 115 institutions in 34 countries from 2001 through 2003. Interpretive breakpoints: susceptible (S),  $\geq 19$  mm; susceptible-dose dependent (SDD), 15 to 18 mm; resistant (R),  $\leq 14$  mm. (Reprinted from reference 75.)

fluconazole was increased from 400 to 800 mg/day in successive cohorts of patients.

#### DEVELOPMENT OF DISK INTERPRETIVE BREAKPOINTS

The CLSI has standardized an agar disk diffusion test method for fluconazole and *Candida* spp. (51). The method employs a 25- $\mu$ g disk and Mueller-Hinton agar supplemented with 2% glucose and 0.5  $\mu$ g of methylene blue per ml (10, 11). In contrast to the 48-h incubation requirement for the BMD test, disk diffusion test results may be determined after only 24 h of incubation (50, 51). Several studies have shown good correlation between fluconazole BMD MICs and disk diffusion test zone diameters (10, 67, 72). Fluconazole disk tests have been used to great advantage in conducting antifungal resistance surveillance in the ARTEMIS DISK Global Antifungal Surveillance Study (30, 75). Previously, we documented that fluconazole disk testing can be performed with a high degree of accuracy as part of routine laboratory testing (72).

Fluconazole disk testing has been performed in more than 115 laboratories in 35 countries between 2001 and the present as part of the ARTEMIS program (72, 75). The frequency distribution of fluconazole zone diameters for 79,485 isolates of *Candida* spp. is shown in Fig. 2. The frequency distribution of zone diameters is similar to that of the MIC distribution (Table 1), with the vast majority of isolates showing large zones of inhibition indicative of susceptibility to fluconazole.

The relationship between fluconazole MICs and zone diameters is shown in Fig. 3 for 2,949 clinical isolates of *Candida*. These isolates represent the same species distribution as that shown in Table 1. Using the MIC breakpoints for fluconazole of  $\leq 8$   $\mu$ g/ml, 16 to 32  $\mu$ g/ml, and  $\geq 64$   $\mu$ g/ml to represent the S, SDD, and R categories, respectively, one can then derive zone diameter breakpoints by the error-rate bounded method (45), whereby the number of discrepancies between the zone

diameter and MIC categories is minimized (Fig. 3 and Table 8). The overall categorical agreement between the disk diffusion and BMD MIC results was determined for fluconazole with the MIC interpretive categories used as the reference. Major errors (ME) were identified as a classification of resistant by the disk diffusion test and susceptible by BMD, very major errors (VME) were identified as a classification of susceptible by the disk diffusion method and resistant by BMD, and minor errors (M) occurred when the result of one of the tests was susceptible or resistant and that of the other test was SDD.

With zone diameter breakpoints of  $\geq 19$  mm (S), 15 to 18 mm (SDD), and  $\leq 14$  mm (R), the overall categorical agreement between the disk diffusion test results and the MIC test results was excellent (92.8%), with very few VME or ME (Table 8). On the basis of these findings, it appears that the disk diffusion test is a useful method for testing the activity of fluconazole against *Candida* spp.

#### CLINICAL IMPORTANCE OF ANTIFUNGAL SUSCEPTIBILITY TESTING AS IT PERTAINS TO FLUCONAZOLE AND *CANDIDA*

Candidiasis is clearly a very important infectious disease (29, 34, 58, 79). Candidemia alone occurs at a rate of 8 to 10 infections per 100,000 population per year (29, 34, 79) and is associated with an excess mortality of 30 to 50% (27, 107). It is estimated that the excess cost attributable to candidemia in the United States approaches \$1 billion per year (46, 60, 111). Although much of this cost can be attributed to length of stay in hospital, the cost of antifungal therapy, especially the newer lipid formulations of amphotericin B, the echinocandins, and the extended-spectrum triazoles, is not inconsequential (41, 46, 60, 111). Selection of the optimal antifungal therapeutic strategy is becoming increasingly complex and is often complicated further by concerns of emerging antifungal resistance (9, 41, 59).



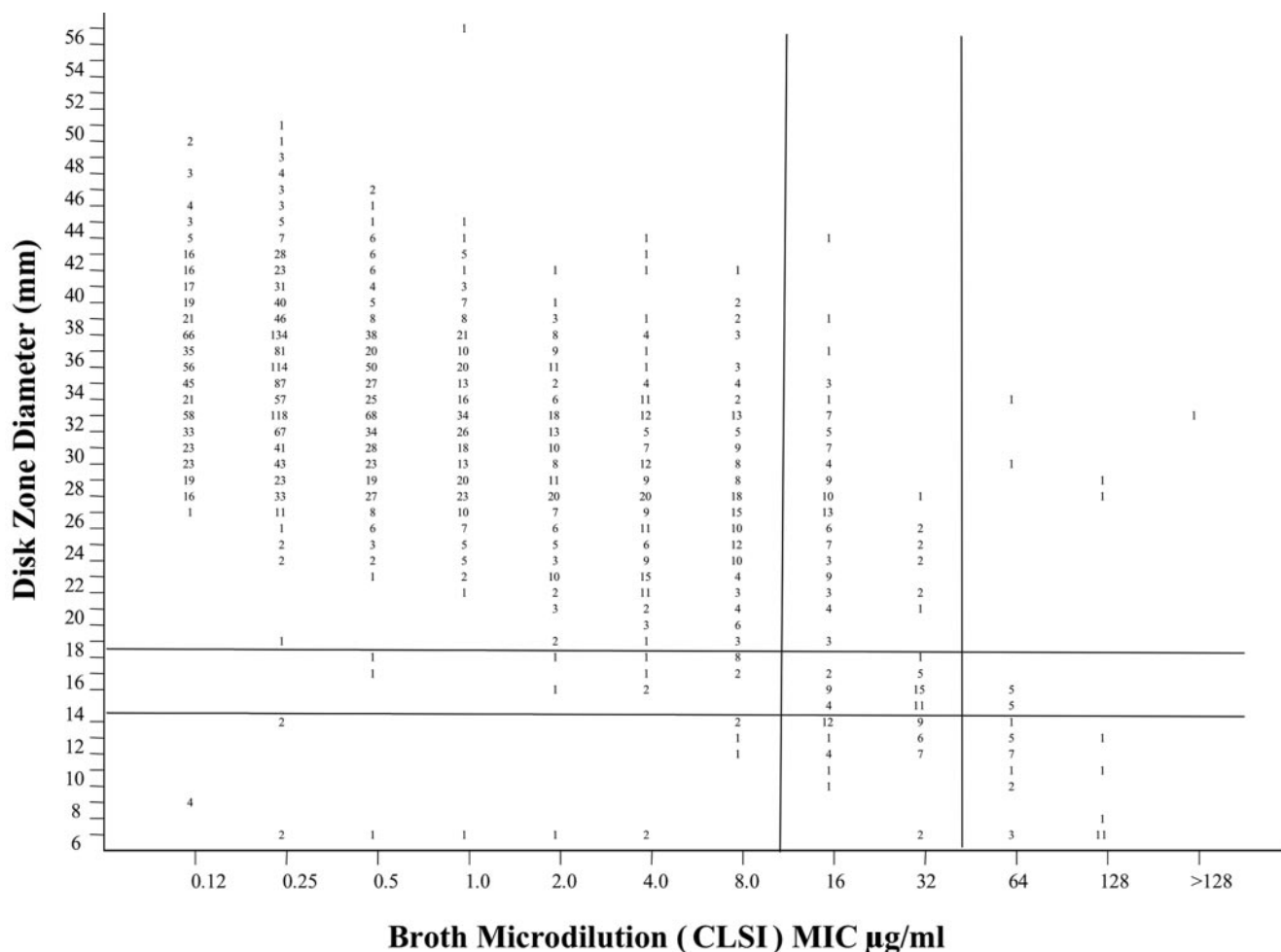


FIG. 3. Scatterplot showing the relationship between fluconazole MICs and zone diameters for 2,949 isolates of *Candida* species. The proposed interpretive breakpoints for each test method are indicated by the horizontal and vertical lines. (Adapted from reference 72.)

In light of these concerns, one might expect that antifungal susceptibility testing would be useful as an aid in selecting the most appropriate antifungal agent, especially for treating more serious forms of candidiasis. However, routine use of antifungal susceptibility testing has been relatively uncommon, and its clinical utility is often limited by delays in the availability of results (9, 28, 31, 57, 59). Given the advances in standardiza-

tion of antifungal susceptibility testing and the proposed interpretive breakpoints for fluconazole described herein, several authors and consensus groups have recommended routine fluconazole susceptibility testing of *Candida* spp. from sterile sites after identification of the clinical isolate (9, 31, 59, 85, 86).

Recent studies examining the clinical utility of “real-time” antifungal susceptibility testing in the treatment of candidemia have shown that when such testing is available on site, physicians find the results helpful and not infrequently alter therapy on the basis of the results (9, 28). At the University of Alabama, Baddley et al. (9) found that the most common change was a switch from amphotericin B to fluconazole. Furthermore, physicians felt reassured in continuing patients on fluconazole if the MIC demonstrated susceptibility of the *Candida* isolate. On the other hand, detection of resistance to fluconazole is clearly desirable, and early recognition of resistance in clinical isolates may contribute to successful clinical and microbiologic outcomes (86, 101, 112). Hadley et al. (28) reported the use of real-time antifungal susceptibility testing in modifying treatment decisions in several patients with invasive fungal infections. They documented resistance to fluconazole in two patients with *C. albicans* infection and noted that al-

TABLE 8. Categorical agreement between fluconazole BMD MIC and disk test results for 2,949 clinical isolates of *Candida*<sup>a</sup>

Method <sup>b</sup>	% by interpretive category <sup>c</sup>			% Agreement <sup>d</sup>	% Errors <sup>e</sup>		
	S	SDD	R		VME	ME	M
BMD	91.6	6.7	1.7				
Disk	94.1	2.2	3.7	92.8	0.1	0.4	6.6

<sup>a</sup> Compiled from reference 72.

<sup>b</sup> BMD and disk diffusion testing were performed in accordance with CLSI M27-A2 (50) and M44-A (51), respectively.

<sup>c</sup> Interpretive categories: S, MIC of  $\leq 8$   $\mu\text{g/ml}$  ( $\geq 19$  mm); SDD, MIC of 16 to 32  $\mu\text{g/ml}$  (15 to 18 mm); R, MIC of  $\geq 64$   $\mu\text{g/ml}$  ( $\leq 14$  mm).

<sup>d</sup> Percent categorical agreement between disk diffusion and MIC test results.

<sup>e</sup> VME, very major error; ME, major error; M, minor error.

TABLE 9. Comparative performance in CAP proficiency challenges: antibacterial versus antifungal susceptibility testing<sup>a</sup>

Challenge strain	Antimicrobial agent	No. of results	% Correct
D-10, 2003 ( <i>S. pneumoniae</i> )	Ceftriaxone	1,087	43
	Penicillin	1,932	89
	Trimethoprim-sulfamethoxazole	165	94
F-01, 2003 ( <i>C. albicans</i> )	Fluconazole	96	94
F-07, 2003 ( <i>C. parapsilosis</i> )	Fluconazole	78	91
F-17, 2003 ( <i>C. krusei</i> )	Fluconazole	68	100
CAP, 2003 (all <i>Candida</i> spp.)	Fluconazole	242	95

<sup>a</sup> Data compiled from Pfaller et al. (76).

though fluconazole would normally have been an optimal choice for this species to avoid toxicities and excess costs, a report of resistance supported continued use of amphotericin B. Thus, it would appear that routine antifungal susceptibility testing can serve as an adjunct in the treatment of candidemia in the same way that antibacterial testing aids in the treatment of bacterial infections (9, 28, 86).

It is now apparent that antifungal susceptibility testing of fluconazole against *Candida* is being incorporated into clinical laboratory practice as BMD panels and fluconazole disks become available commercially (62, 67, 72, 75). In the United States, the number of clinical laboratories participating in the College of American Pathologists (CAP) antifungal proficiency testing program has increased from 50 laboratories in 1997 to more than 100 laboratories at present (76). The majority of these laboratories use commercial MIC tests, either YeastOne (Sensititre, Cleveland, OH) or Etest (AB BIODISK, Solna, Sweden), and more than a third of laboratories perform more than 100 MIC tests per year for clinical purposes. The performance of these laboratories in testing fluconazole against *Candida* spp. is excellent and, as seen in Table 9, is easily comparable to the level of performance accuracy achieved by CAP participants in testing a fastidious bacterium such as *Streptococcus pneumoniae* (76).

Fluconazole disk diffusion testing of *Candida* spp. is also increasing worldwide. The ARTEMIS DISK Global Antifungal Surveillance Program monitors the performance of 127 participating laboratories in 39 countries (75). These laboratories all perform fluconazole disk diffusion testing routinely for clinical purposes on isolates of *Candida* spp. and other opportunistic yeasts and report results to a central database (75). External validation of this routine laboratory testing by a central reference laboratory has documented excellent performance (72, 74). The simplicity and flexibility of disk diffusion testing makes it a very appealing method for use in the clinical laboratory (72, 74, 75). Results are clearly available in real time (i.e., 24 h), and innovative efforts to integrate this form of antifungal testing into the work flow of the clinical laboratory are ongoing (101). Thus, fluconazole disk diffusion testing is being performed in clinical laboratories throughout the world

as an aid in selecting the most efficacious, nontoxic, and cost-effective therapy for treatment of candidiasis.

## SUMMARY AND CONCLUSIONS

We have validated CLSI interpretive breakpoints for in vitro susceptibility testing of fluconazole and *Candida* spp.: S MIC (zone diameter),  $\leq 8$   $\mu\text{g/ml}$  ( $\geq 19$  mm); SDD, 16 to 32  $\mu\text{g/ml}$  (15 to 18 mm); R,  $\geq 64$   $\mu\text{g/ml}$  ( $\leq 14$  mm). These interpretive breakpoints are supported by (i) consideration of mechanisms of resistance to fluconazole, (ii) analysis of the MIC population distribution, (iii) consideration of cross-resistance patterns, (iv) analysis of parameters associated with success in pharmacodynamic models, and (v) the results from clinical efficacy studies. The clinical data supporting these breakpoints have been expanded to include more cases with invasive candidiasis and more infections with isolates with higher fluconazole MICs. The strength of the correlation of these breakpoints with clinical outcome is consistent with that from other fungal and bacterial infections: a result of S is associated with a higher success rate than a result of R, but host factors also influence outcome.

This "blueprint" for establishing interpretive breakpoints should be applicable to other antifungal agents, such as voriconazole (77) and the echinocandins; however, the dose/MIC ratio used herein as a surrogate for the AUC/MIC ratio cannot be used for other agents that have less-predictable dose/AUC relationships.

Antifungal susceptibility testing of fluconazole and *Candida* is becoming recognized as a useful aid in optimizing treatment of candidiasis. Standardized MIC and disk diffusion testing can be performed accurately in the routine clinical laboratory, providing real-time results for difficult clinical infections.

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