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Correlation of MIC with Outcome for *Candida* Species Tested against Voriconazole: Analysis and Proposal for Interpretive Breakpoints

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Developing interpretive breakpoints for any given organism-drug combination requires integration of the MIC distribution, pharmacokinetic and pharmacodynamic parameters, and the relationship between the in vitro activity and outcome from both in vivo and clinical studies. Using data generated by standardized broth microdilution and disk diffusion test methods, the Antifungal Susceptibility Subcommittee of the Clinical and Laboratory Standards Institute has now proposed interpretive breakpoints for voriconazole and *Candida* species. The MIC distribution for voriconazole was determined using a collection of 8,702 clinical isolates. The overall MIC_{90} was 0.25 μg/ml and 99% of the isolates were inhibited at ≤1 μg/ml of voriconazole. Similar results were obtained for 1,681 *Candida* isolates (16 species) from the phase III clinical trials. Analysis of the available data for 249 patients from six phase III voriconazole clinical trials demonstrated a statistically significant correlation ($P = 0.021$) between MIC and investigator end-of-treatment assessment of outcome. Consistent with parallel pharmacodynamic analyses, these data support the following MIC breakpoints for voriconazole and *Candida* species: susceptible (S), ≤1 μg/ml; susceptible dose dependent (SDD), 2 μg/ml; and resistant (R), ≥4 μg/ml. The corresponding disk test breakpoints are as follows: S, ≥17 mm; SDD, 14 to 16 mm; and R, ≤13 mm.

Voriconazole is a triazole antifungal agent that is structurally related to fluconazole, and, like other azoles, it acts by inhibiting the cytochrome P450-dependent 14-α-lanosterol demethylase enzyme, resulting in disruption of fungal ergosterol synthesis (7, 12, 16, 40). The drug is available for oral administration as film-coated tablets (50 or 200 mg) or as a powder for oral suspension, and as an intravenous formulation (200 mg) using sulfobutyl ether β-cyclodextrin sodium as the solubilizing agent.

Voriconazole is administered as an initial loading dose (6 mg/kg of body weight intravenously every 12 h twice) followed by maintenance dosing (3 mg/kg to 4 mg/kg intravenously or 200 mg by mouth every 12 h). If patient response is inadequate, the oral maintenance dose may be increased from 200 to 300 mg every 12 h.

In May 2002, the Food and Drug Administration approved voriconazole for treatment of invasive aspergillosis and for serious infections caused by *Fusarium* spp. and by *Scedosporium apiospermum* in patients who are intolerant of or refractory to other antifungal agents (15, 26). In November 2003, an additional indication for the treatment of esophageal candidiasis was added (1) and in January 2005, an indication for primary treatment of invasive candidiasis (including candidemia) in nonneutropenic patients was approved (17). In Europe, voriconazole has been approved by the European Medicines Agency (EMEA) for treatment of invasive aspergillosis, serious infections caused by *Fusarium* spp. and *S. apiospermum*, and fluconazole-resistant serious invasive *Candida* infections (including those due to *C. krusei*) (14). The EMEA has also recently adopted an extension to the indications for voriconazole to include the treatment of candidemia in nonneutropenic patients (EMEA press release, 25 October 2004).

There is now a very broad experience testing voriconazole against *Candida* species using the broth microdilution (BMD) and disk diffusion methods standardized and recommended by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards) (5, 9, 10, 23, 25, 27–29, 31, 34, 35, 41). In addition to standardized testing methods, the CLSI Antifungal Subcommittee has approved quality control limits for both MIC and disk test methods with voriconazole (20, 22).

During their January 2005 meeting, the CLSI Antifungal Subcommittee used the accumulated microbiological and clinical data to propose interpretive breakpoints for MIC and disk testing of voriconazole against *Candida* spp. The analytical model followed that used previously for fluconazole (38) and as outlined for all types of antimicrobial testing in CLSI document M23-A2 (19), the committee considered the MIC distribution profile, pharmacokinetic and pharmacodynamic (PK/

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PD) parameters, and the relationship between in vitro activity (MIC) and clinical outcome, as determined by the investigators in a total of six phase III efficacy studies. These analyses are summarized below.

MATERIALS AND METHODS

Organisms. All isolates of *Candida* spp. used to generate the MIC distribution profiles, the disk zone diameter histograms, and MIC-versus-zone diameter scattergrams were obtained from the ARTEMIS Global Antifungal Surveillance Program sponsored by Pfizer, Inc. (13, 29, 31, 32, 34, 35). A total of 8,702 isolates of *Candida* spp. (14 different species from 91 study centers), collected from blood and normally sterile body sites, were sent to the ARTEMIS central reference laboratory (University of Iowa) for identification and susceptibility testing by CLSI BMD MIC (M27-A2) and disk diffusion (7,301 isolates tested according to CLSI M44-A) methods. An additional 75,809 clinical isolates of *Candida* spp. (16 different species), representing all clinically significant isolates, regardless of body source, were tested by CLSI M44-A disk diffusion methods at individual ARTEMIS participating sites (115 study centers in 35 countries) during 2001 through 2003 (34). Cross-resistance studies with fluconazole and voriconazole were performed at the University of Iowa using a collection of 13,338 clinical isolates of *Candida* spp. from more than 200 medical centers worldwide.

In addition to the isolates noted above, all *Candida* spp. isolated from subjects with definite infections in voriconazole phase III primary or salvage/compassionate therapy studies (numbers 608, 603, 309/604, and 301/606) were identified and tested by CLSI BMD MIC methods in either of two mycology reference laboratories located in the United States (Medical College of Virginia) and the United Kingdom (HPA Centre for Infections). A total of 1,681 isolates of *Candida* spp. (16 different species) were obtained from the phase III clinical trials, of which 249 represented baseline isolates from subjects eligible for this analysis.

Antifungal susceptibility testing. All isolates were tested using CLSI recommended methods (20, 22). MIC testing of voriconazole (and fluconazole to determine cross-resistance) was performed exactly as outlined in CLSI document M27-A2 (20) using RPMI 1640 broth, an inoculum of 0.5 to 2.5 $\times$ 10^3 CFU per ml, incubation at 35°C for 48 h, and MIC endpoint criteria of prominent (~50% or MIC-2) inhibition of growth relative to the growth control.

Disk diffusion testing was performed exactly as outlined in CLSI document M44-A (22) using Mueller-Hinton agar supplemented with 2% glucose and 0.5 μg of methylene blue per ml (MH-MB). The inoculum was standardized to the turbidity of a 0.5 McFarland standard and streaked evenly over the MH-MB agar plates (150-mm diameter and 4-mm deep). Voriconazole disks (1 μg; supplied by Becton Dickinson, Sparks, MD) were placed onto the surface of the inoculated agar plates, which were then incubated at 35°C for 18 to 24 h. The zones of inhibition surrounding the voriconazole disks were read with the BIOMIC Vision Image Analysis system (Giles Scientific Inc., www.biomic.com) using endpoint criteria of 80% inhibition (29, 32, 34, 35).

Quality control. Quality control for both MIC and disk testing of voriconazole was performed using CLSI recommended strains *Candida parapsilosis* ATCC 22019 (disk and MIC testing), *C. krusei* ATCC 6258 (disk and MIC testing), and *Candida albicans* 90028 (disk testing only).

Phase III clinical trials. The clinical trial data (patient outcomes and baseline isolates) used in this analysis included data from studies 608 (Global Candidemia Study), 603 (Empirical Therapy Study [data from subjects with baseline infections confirmed by the study's blinded review committee were used]), 309/604 (Global Rare and Refractory Studies), 301 (Compassionate Use Protocol), and 606 (Emergency Use Protocol-U.S. and Canada). These were all multi-institutional studies, the details of which are described elsewhere (17, 26, 42) (Pfizer data on file). In each study, voriconazole was administered intravenously with a loading dose of 6 mg/kg every 12 h for the first 24 h, followed by either 3 mg/kg (studies 603 and 608) or 4 mg/kg every 12 h for ≥3 days, after which patients were given 200 mg by mouth twice daily. If oral therapy was administered initially, a loading dose of 400 mg every 12 h on day 1 was followed by a maintenance dose of 200 mg twice daily thereafter. Response to voriconazole therapy was determined by the investigator at the end of therapy as either cured, improved, or failed. A cured or improved response was classified as success and all other responses as failure.

In vivo correlation. Clinical outcomes as determined by the investigators at the end of therapy were compared to the voriconazole MIC for each baseline *Candida* isolate. Where more than one baseline pathogen was identified per patient, the isolate for which the MIC was highest was used. The analysis incorporated the voriconazole MICs of all *Candida* isolates from the study and, in voriconazole-treated patients, logistic modeling with SAS version 8.2 assessed the relationship between voriconazole baseline MIC and therapeutic response. Sensitivity analyses also examined the influence of various parameters, including age, sex, race, site of infection, and mean voriconazole plasma concentration.

Development of MIC interpretive breakpoints. The MIC interpretive breakpoints for voriconazole and *Candida* spp. were developed by taking into account the available microbiologic data, animal modeling data, pharmacokinetic and pharmacodynamic data, and human clinical data as described previously for fluconazole (38). Important observations included formal PK data in volunteers documenting serum levels above 1 μg/ml throughout the dosing interval (11, 24, 36, 37; voriconazole package insert), MIC distribution data showing that the MIC for 99% of isolates was ≤1 μg/ml (27), consideration of cross-resistance between voriconazole and fluconazole (27, 31), and a PD target for therapeutic success of a free drug 24-h area-under-the-curve (AUC)/MIC ratio of 20 (3, 4). The clinical data analysis, in addition to the results of the logistic modeling described above, also considered the previously described "90/60 rule" in establishing the resistant breakpoint (39). In addition to susceptible (S) and resistant (R) categories, a category of susceptible dose dependent (SDD) was included similar to that of fluconazole (38), to reflect both the nonlinear PK and dosing flexibility of voriconazole (11, 24; voriconazole package insert).

Development of disk interpretive breakpoints. The diameters of the zones of inhibition surrounding the voriconazole disks at 18 to 24 h of incubation for 7,301 isolates of *Candida* spp. were plotted against their respective BMD MICs read at 48 h (6). The interpretive breakpoints for the voriconazole disk diffusion test were assigned using the error rate-bounded method (18) whereby the number of discrepancies between the zone diameter and MIC categories was minimized. The overall categorical agreement between the disk diffusion and BMD MIC results was determined for voriconazole using the MIC interpretive categories as the reference (29). Major errors were identified as a classification of resistant by the disk diffusion test and susceptible by BMD, very major errors were identified as a classification of susceptible by the disk diffusion method and resistant by BMD, and minor errors occurred when the result of one of the tests was susceptible or resistant and that of the other test was susceptible dose dependent.

RESULTS AND DISCUSSION

Voriconazole MIC distribution profile for *Candida* species. The cumulative percentage of isolates for each species of *Candida* inhibited at each concentration throughout the BMD dilution series is shown in Table 1. These results were all determined in a single reference laboratory using CLSI-recommended BMD methods. This large data set represents recent (2001 to 2004), clinically important (blood and normally sterile site) isolates from 91 different medical centers throughout the world. The overall MIC_{90} for voriconazole was 0.25 μg/ml and 99% of the 8,702 isolates tested were inhibited by ≤1 μg/ml of voriconazole.

The MIC_{90} for *Candida glabrata* (1 μg/ml) was considerably higher than that observed for all other species (range, 0.015 to 0.25 μg/ml) with the exception of *C. krusei* (0.5 μg/ml). Nevertheless, 92% of *C. glabrata* and 100% of *C. krusei* isolates were inhibited by ≤1 μg/ml, a concentration that, in phase I volunteer subjects, is exceeded throughout the dosing interval at standard doses of voriconazole (11, 24, 36, 37; voriconazole package insert). As noted previously (27, 31, 33), 99% of the isolates of *C. glabrata* for which voriconazole MICs were ≥4 μg/ml were resistant to fluconazole (data not shown). These data, including the species distribution rank order (Table 1), are highly representative of those published in numerous in vitro studies (5, 8, 9, 10, 23, 24, 27, 29, 31, 34, 41).

Voriconazole MICs for *Candida* spp. isolated in phase III clinical trials. A total of 1,681 isolates of 16 *Candida* spp. were obtained from clinical specimens from more than 400 subjects during the six phase III clinical trials. The five major species (1,630 isolates) from these studies are identical to those shown

TABLE 1. Susceptibility of *Candida* species to voriconazole by MIC: ARTEMIS Global Antifungal Surveillance Program, 2001 to 2004[a]

Species (no. of isolates tested)	Cumulative % of strains at MIC (μg/ml):										
	0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8
C. albicans (4,701)	80	94	98	99	99	99	99	99	99	100	
C. glabrata (1,183)	1	1	3	14	40	70	86	92	95	99	100
C. parapsilosis (1,253)	19	61	81	90	95	98	99	99	99	99	100
C. tropicalis (963)	9	27	64	93	99	99	99	99	99	99	100
C. krusei (243)				1	13	61	95	100			
C. lusitaniae (110)	70	92	94	97	98	98	99	100			
C. guilliermondii (128)		6	15	66	93	98	99	99	100		
C. kefyr (38)	47	87	97	100							
C. pelliculosa (28)			4	4	64	93	100				
Candida spp. (55)[b]	18	40	51	80	89	98	98	100			
All *Candida* isolates (8,702)	48	64	75	82	88	94	98	99	99	99	100

[a] Broth microdilution MICs determined in accordance with CLSI M27-A2.
[b] Includes *C. dubliniensis* (4 isolates), *C. famata* (16 isolates), *C. lipolytica* (8 isolates), *C. rugosa* (13 isolates), and *C. zeylanoides* (7 isolates).

in Table 1 and in other large surveys (Table 2) (23). Among these five species (1,630 isolates), the relative susceptibility to voriconazole was similar to that shown in Table 1, with *C. albicans*, *C. parapsilosis*, and *C. tropicalis* representing the most susceptible species (MIC_{90}, 0.06 to 0.25 μg/ml) and *C. glabrata* the least susceptible (MIC_{90}, 4 μg/ml).

Although the modal MIC for each species was either identical to or within one twofold dilution of that shown in Table 1, higher MICs were observed for a few isolates of each species tested in the phase III trials. This can be attributed to the fact that four of the six trials were either salvage therapy or compassionate-use studies, in which the patients enrolled had already failed one or more prior courses of antifungal therapy. The MIC_{90} values for fluconazole were also higher for these isolates compared to those in Table 1 (data not shown). Given these comparative data, the isolates obtained from phase III clinical trials represent a rigorous challenge to the therapeutic efficacy of voriconazole.

Cross-resistance between fluconazole and voriconazole. Previous in vitro studies have suggested that the MICs for voriconazole may be elevated against isolates of *Candida* with reduced susceptibility to fluconazole, suggesting that cross-resistance may occur with voriconazole and other azole compounds (9, 23, 27, 28, 30, 31). Figure 1 demonstrates the relationship between fluconazole and voriconazole MICs for a collection of 13,338 clinical isolates of *Candida* (18 different species) collected from more than 200 different medical centers between 1992 and 2003. Although the MICs for voriconazole were at least 16- to 32-fold lower than the corresponding fluconazole MICs for each isolate tested, there was strong positive correlation ($R = 0.9$) between voriconazole and fluconazole MICs. Among all isolates tested against both agents, 100% of fluconazole-susceptible (12,087 isolates), 98% of fluconazole-susceptible dose-dependent (836 of 855 isolates), and 48.5% (192 of 396 isolates) of resistant isolates were susceptible to voriconazole at a concentration of ≤1 μg.

The extent of cross-resistance varies considerably with the species of *Candida*. It is most prominent with *C. glabrata* (33) and appears to be negligible with most other species of *Candida* (23, 24, 37, 41). Among 1,966 isolates of *C. glabrata*, the voriconazole MICs were ≤1 μg/ml for 100% of fluconazole-susceptible isolates (1,217 isolates) and 98% (557 of 568 isolates) of fluconazole-SDD isolates. Among the 181 fluconazole-resistant isolates of *C. glabrata*, the MICs for voriconazole were ≤1 μg/ml for 30 isolates (17%), 2 μg/ml for 79 isolates (44%), and ≥4 μg for 72 isolates (39%).

In particular contradistinction to the susceptibility pattern noted with fluconazole, 99% (310 of 312 isolates) of the *C. krusei* isolates tested were inhibited by ≤1 μg voriconazole per ml. Thus, despite a strong positive correlation between the fluconazole and voriconazole MICs, the majority (1,028 of 1,251, 82%) of isolates that were nonsusceptible (MICs ≥ 16 μg/ml) to fluconazole were inhibited by concentrations of voriconazole (≤1 μg/ml) that can be achieved clinically using recommended doses of voriconazole.

As voriconazole is only 58% protein bound (4, 11, 24) and in vitro studies show only a minimal effect of added protein, a comparison of unadjusted human plasma concentrations with MIC data was considered reasonable at this stage of the analysis; a pharmacodynamic analysis that uses free drug concentrations is discussed below. In previously published clinical studies, 17 of 22 patients (77%) infected with fluconazole-resistant *C. albicans* and seven of nine patients (78%) infected with fluconazole-resistant non-*C. albicans Candida* species responded to voriconazole, suggesting that from a clinical perspective, the impact of cross-resistance was minimal (14, 26).

In vivo correlation of in vitro data. A total of 249 patients enrolled in the phase III clinical trials were infected with *Candida* spp., received voriconazole therapy, and were characterized as treatment successes or failures at the end of therapy by

TABLE 2. Voriconazole MIC results for 1,630 *Candida* isolates from phase III clinical studies[a]

Species	No. of isolates tested	MIC (μg/ml)[b]		
		Range	50%	90%
C. albicans	886	0.004–16	0.008	0.25
C. glabrata	285	0.008–16	0.5	4
C. tropicalis	240	0.004–16	0.06	0.25
C. parapsilosis	178	0.004–0.125	0.03	0.06
C. krusei	41	0.125–1	0.25	1

[a] Broth microdilution MICs determined in accordance with CLSI M27-A2.
[b] 50% and 90%, MIC encompassing 50% and 90% of isolates tested, respectively.

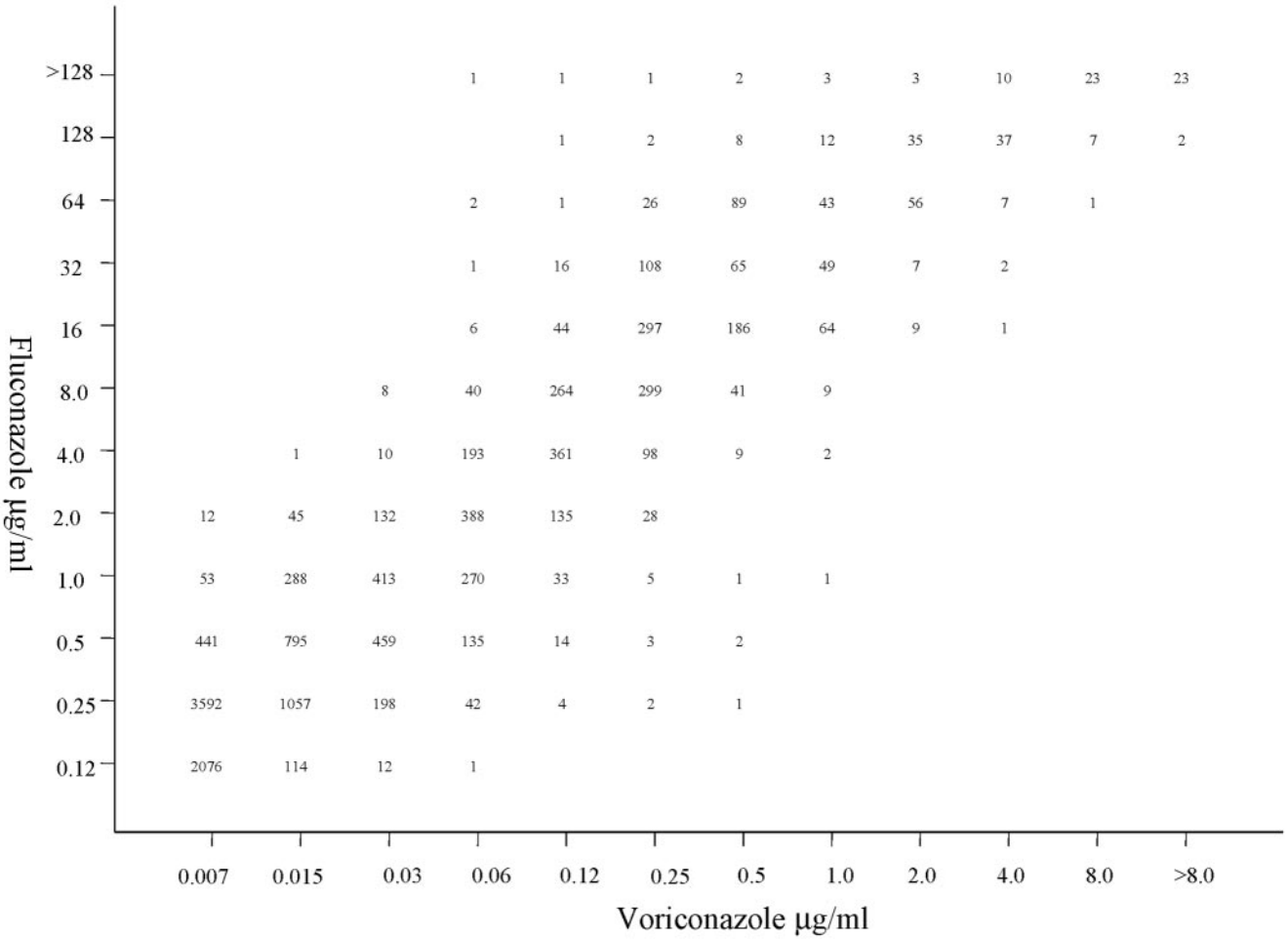

FIG. 1. Scatterplot showing the relationship between fluconazole and voriconazole MICs obtained with 13,338 isolates of *Candida* species.

the site investigators. The geometric mean MICs for the baseline isolates of each species of *Candida* and the percentage of cases in which voriconazole treatment was judged to be successful are shown in Table 3. The geometric mean MIC for each of these species is consistent with that shown in Table 1: *C. albicans* was the most susceptible species and *C. glabrata* was the least susceptible to voriconazole. The clinical efficacies of voriconazole were comparable for all of the species (72 to 92% success) with the exception of *C. glabrata* (55%).

The logistic analysis of these data shows a significant relationship between the MIC of the baseline isolates and the investigator end-of-therapy assessment of efficacy (Fig. 2). The linear logistic model in Fig. 2 indicates that the predicted slope is significantly different from zero ($P = 0.02$) and the 60% response rate is at a voriconazole MIC of 4 μg/ml. The significance of the linear log MIC term in the logistic model of investigator response is largely due to MIC differences among species, particularly *C. glabrata*. When species differences in MIC are included ($P = 0.0310$), the addition of the log MIC term (standing for within-species MIC differences) is no longer significant. Thus, knowledge of the species is also useful because of the differences in MICs among species. However, as is the case in most infectious diseases (2), the more that is known about each subject in regard to other predictive factors (e.g., age and underlying diseases), the less important the MIC or species of the infecting organism becomes; the two become statistically nonsignificant.

Development of MIC interpretive breakpoints. Given the MIC distributions shown in Tables 1 and 2 and the clinical correlation between MIC and efficacy, what are the possible interpretive breakpoints for BMD MIC testing of *Candida* and voriconazole? Consideration of the overall MIC distribution

TABLE 3. *Candida* species, geometric mean MICs, and investigator-assessed response to voriconazole therapy[a]

Species	No. of isolates tested	Geometric mean MIC (μg/ml)	% Success
C. albicans	96	0.0164	72
C. parapsilosis	34	0.0266	85
Candida spp.	12	0.0712	92
C. tropicalis	51	0.1283	73
C. krusei	9	0.3650	78
C. glabrata	47	0.7937	55

[a] Broth microdilution MICs were determined in accordance with CLSI M27-A2. Baseline isolates from studies 603, 608, 309/604, and 301/606 were used.

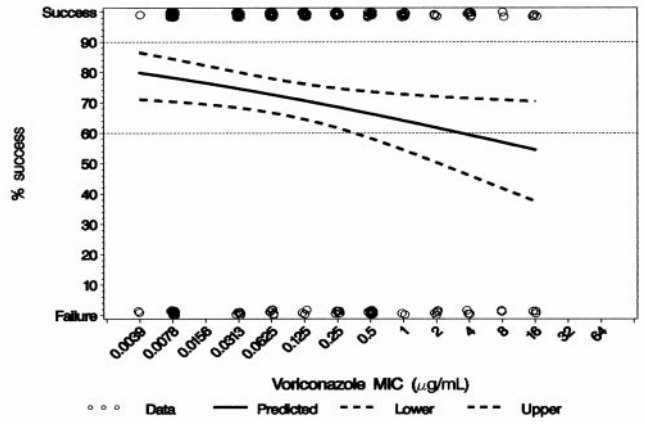

FIG. 2. Binomial data and logistic fit for investigator outcome versus baseline MICs for *Candida* species in primary and salvage therapy studies 603, 608, 309/604, and 301/606 ($n = 249$; $P = 0.021$).

(Table 1), the cross-resistance data (Fig. 1), the PK of the drug and the relationship between MIC and clinical outcome (Fig. 2 and Table 3) allows the reasonable assignment of an MIC breakpoint of ≥4 μg/ml as resistant. It is clear that *Candida* isolates for which voriconazole MICs are ≥4 μg/ml (i) are predominantly *C. glabrata* (93%), 99% of which are also resistant to fluconazole, (ii) represent a concentration of voriconazole that cannot be maintained over the dosing interval with the currently recommended doses, and (iii) are significantly less likely to respond clinically to voriconazole therapy (Fig. 2). The 60% clinical response rate at a voriconazole MIC of ≥4 μg/ml constitutes a reasonable resistance breakpoint when applying the 90/60 rule (39).

Regarding the category of susceptible, breakpoints at 0.25, 0.5, and 1 μg/ml were considered. In this context, a consideration arises related to the MIC distributions of various populations of organisms (43): whenever possible, breakpoints that bisect a large population of organisms should be avoided. It is clear from the data presented in Table 1 that breakpoints below 1.0 μg/ml would bisect the population of *C. glabrata* isolates, creating a situation in which a one-dilution change in MIC (within the error of the test) would frequently result in a categorical change in reporting. Given the importance and frequency of isolation of *C. glabrata*, these breakpoints would be problematic at best.

In support of a susceptibility breakpoint of ≤1 μg/ml, plasma concentrations of 1 μg/ml or greater are routinely observed throughout the dosing interval when voriconazole is administered at recommended doses to phase I volunteers (11, 24, 36, 37; voriconazole package insert). Likewise, an MIC of ≤1 μg/ml encompasses 99% of all clinical isolates of *Candida* and is at least two dilutions removed from the modal MIC of *C. glabrata* (Table 1). The clinical success associated with this MIC breakpoint is also significantly different from that of the resistant category (Table 4).

Finally, a susceptible breakpoint of ≤1 μg/ml can be supported by pharmacodynamic data (3, 4). In a murine model of disseminated candidiasis, the AUC/MIC ratio was the PK-PD parameter most strongly predictive of treatment outcomes (4). As with other azoles (3), a free drug 24-h AUC/MIC ratio of 20 was predictive of efficacy (4). Given the plasma protein binding (58%) of voriconazole, it is necessary to use free drug AUCs rather than total drug when considering PD targets (4).

If one considers the kinetics of voriconazole in humans, either an oral dose of 200 mg to 300 mg or an intravenous dose of 4 mg/kg every 12 h would produce free drug AUCs of approximately 20 μg · h/ml (4, 36; voriconazole package insert). Given a PD target of a free drug AUC/MIC ratio of 20, one could predict that these voriconazole dosing regimens could successfully be used for treatment of infections due to *Candida* spp. for which MICs are as high as 1 μg/ml (4). Thus, a susceptible breakpoint of ≤1 μg/ml for voriconazole and *Candida* is supported by the MIC population distribution, by PK-PD parameters, by analysis of cross-resistance, and by correlation with an in vivo outcome.

Consistent with what has been done with fluconazole (38), we have designated the category between S and R (i.e., isolates with an MIC of 2 μg/ml) as susceptible dose dependent to take into account both the nonlinear pharmacokinetics and the dosing flexibility of voriconazole (Table 4). Such a category implies that an infection due to the isolate may be appropriately

TABLE 4. Investigator assessment of efficacy versus baseline MIC for *Candida* species in primary and salvage therapy studies 603, 608, 309/604, and 301/606

MIC breakpoint (μg/ml)	Interpretive category	No. of isolates	% Success
≤0.25	S	189	77
0.5–2	SDD	39	54
≥4	R	21	62
≤0.5	S	211	73
1–2	SDD	17	65
≥4	R	21	62
≤1	S	221	74
2	SDD	7	43
≥4	R	21	62

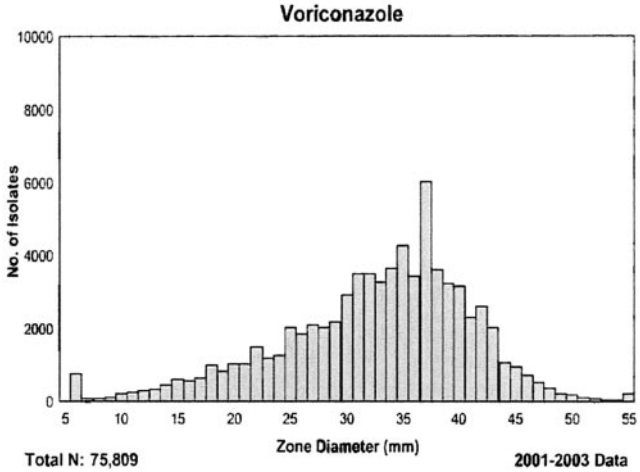

FIG. 3. Voriconazole zone diameter distribution for all *Candida* species (75,809 isolates). Isolates were obtained from 115 institutions in 35 countries from 2001 through 2003.

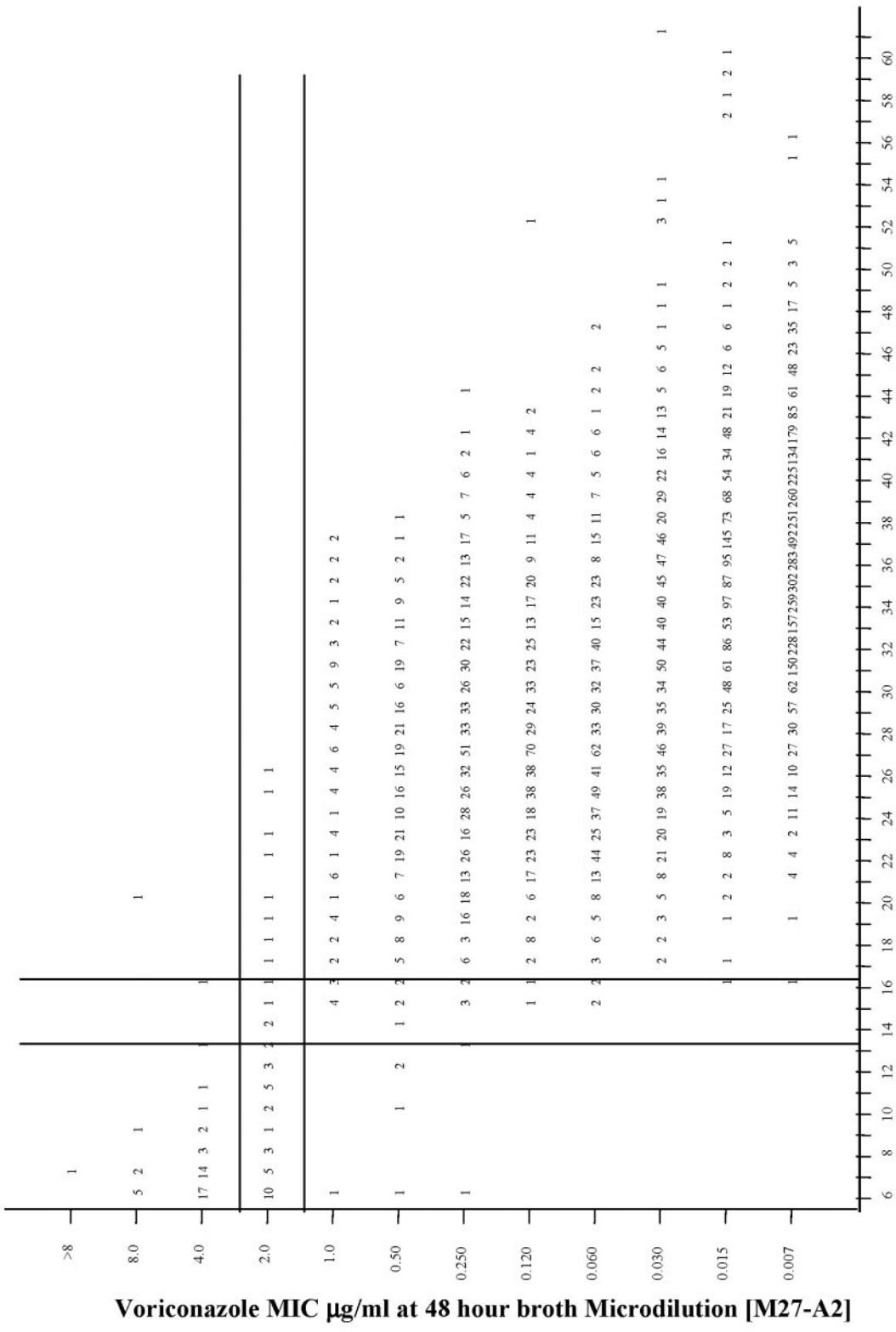

FIG. 4. Scatterplot showing the relationship between voriconazole MICs and zone diameters for 7,301 isolates of *Candida*. The proposed interpretive breakpoints for each test method are indicated by the horizontal and vertical lines.

TABLE 5. Categorical agreement between voriconazole broth microdilution MIC and disk test result for 7,301 clinical *Candida* isolates

Method[a]	% of isolates by interpretive category[b]			% Agreement	% Error[c]		
	S	SDD	R		VME	ME	M
BMD	98.7	0.6	0.7				
Disk	98.4	0.4	1.2	99.0	0.01	0.09	0.9

[a] BMD and disk diffusion testing were performed in accordance with CLSI M27-A2 and M44-A, respectively.
[b] Interpretive categories: S, susceptible, MIC ≤1 μg/ml (≥17 mm); SDD, susceptible dose dependent, MIC = 2 μg/ml (14 to 16 mm); R, resistant, MIC ≥4 μg/ml (≤13 mm).
[c] VME, very major error; ME, major error; M, minor error.

treated in body sites where the drug is physiologically concentrated or when a high dosage of the drug can be used (38, 43). Analogous to that seen with antibacterial testing, it may also serve as a "buffer zone" to prevent small, uncontrolled technical factors from causing major discrepancies in interpretations (21, 43).

Development of disk interpretive breakpoints. The CLSI has standardized agar disk diffusion test methods for both fluconazole and voriconazole and *Candida* spp. (22). Preliminary data have shown good correlation between voriconazole BMD MICs and disk diffusion test zone diameters (27, 32, 35). Both fluconazole and voriconazole disk tests have been used to great advantage in conducting antifungal resistance surveillance in the ARTEMIS DISK Global Antifungal Surveillance Study (13, 34). Previously, we have documented that voriconazole disk testing can be performed with a high degree of accuracy as part of routine laboratory testing (35).

Voriconazole disk diffusion testing has been performed in more than 115 laboratories in 35 countries as part of the ARTEMIS program between 2001 and 2003 (34). The frequency distribution of voriconazole zone diameters for 75,809 isolates of *Candida* spp. is shown in Fig. 3. The frequency distribution of zone diameters is very similar to that of the MIC distribution (Table 1), with the vast majority of isolates showing large zones (>17 mm), indicative of susceptibility to voriconazole.

The relationship between voriconazole MICs and zone diameters is shown in Fig. 4 for 7,301 clinical isolates of *Candida*. These isolates represent the same species distribution as that shown in Table 1. Using the MIC breakpoints of ≤1, 2, and ≥4 μg/ml for voriconazole to represent the S, SDD, and R categories, respectively, one can then assign zone diameter breakpoints such that discrepancies between the MIC and disk diffusion test categories are minimized (Fig. 4 and Table 5). Using zone diameter breakpoints of ≥17 mm (S), 14 to 16 mm (SDD), and ≤13 mm (R), the overall categorical agreement between the disk diffusion test results and the MIC test results was excellent (99.0%), with very few very major or major errors (Table 5). On the basis of these findings, it appears that the disk diffusion test is a useful method for testing the activity of voriconazole against *Candida* spp.

In summary, we have established a susceptible MIC (zone diameter) breakpoint of ≤1 μg/ml (≥17 mm), a susceptible dose-dependent breakpoint of 2 μg/ml (14 to 16 mm), and a resistant breakpoint of ≥4 μg/ml (≤13 mm) for voriconazole and *Candida* species. These interpretive breakpoints are supported by (i) analyses of the MIC population distribution, (ii) consideration of cross-resistance patterns, (iii) analysis of parameters associated with success in pharmacodynamic analyses, and (d) the results from clinical efficacy studies. The strength of the correlation of these breakpoints with the clinical outcome is consistent with that from other antifungal and antibacterial infections: a result of S is associated with a higher success rate than a result of R, but host factors also influence outcome.

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