JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2003, p. 1087–1090 Vol. 41, No. 3
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.3.1087–1090.2003

Evaluation of Etest Method for Determining Fluconazole and Voriconazole MICs for 279 Clinical Isolates of *Candida* Species Infrequently Isolated from Blood

M. J. Maxwell,[1] S. A. Messer,[1] R. J. Hollis,[1] L. Boyken,[1] S. Tendolkar,[1] D. J. Diekema,[1,2] M. A. Pfaller,[1,3]* and the International Fungal Surveillance Participant Group†

Departments of Pathology,[1] Medicine,[2] and Epidemiology,[3] University of Iowa College of Medicine and College of Public Health, Iowa City, Iowa 52242

Received 11 October 2002/Returned for modification 17 November 2002/Accepted 8 December 2002

The performance of Etest in fluconazole and voriconazole testing of 279 isolates of uncommon *Candida* spp. was assessed in comparison with the National Committee for Clinical Laboratory Standards (NCCLS)-approved standard broth microdilution (BMD) method. The NCCLS method employed RPMI 1640 broth medium, and MICs were read after incubation for 48 h at 35°C. Etest MICs were determined with RPMI agar containing 2% glucose and were read after incubation for 48 h at 35°C. The isolates include *Candida krusei*, *C. lusitaniae*, *C. guilliermondii*, *C. kefyr*, *C. rugosa*, *C. lipolytica*, *C. pelliculosa*, *C. dubliniensis*, *C. famata*, *C. zeylanoides*, *C. inconspicua*, and *C. norvegensis*. Overall agreement between Etest and BMD MICs was 96% for fluconazole and 95% for voriconazole. Where a discrepancy was observed between Etest and the reference method, the Etest tended to give lower values with both fluconazole and voriconazole. The Etest method using RPMI agar appears to be a useful method for determining fluconazole and voriconazole susceptibilities of uncommon species of *Candida*.

The Etest stable agar gradient MIC method (AB BIODISK, Solna, Sweden) has been shown to be useful in testing *Candida* spp. against a variety of antifungal agents, including fluconazole and voriconazole (1, 3, 9, 11, 12, 14, 16, 17, 22, 24). The species of *Candida* tested in these studies generally represent those most commonly isolated from clinical sources and are dominated by *Candida albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*, which account for 95 to 97% of all clinical isolates of *Candida* spp. (8, 13, 15, 18). Thus, although Etest has been validated for the four most common species of *Candida*, the evidence supporting its use in testing the less common species is lacking.

Among the approximately 17 species of *Candida* reported to cause bloodstream infections (BSI) (8), 12 or 13 of these species account for less than 5% of all *Candida* BSI (19). These rare species include among others, *C. krusei*, *C. lusitaniae*, *C. guilliermondii*, *C. kefyr*, *C. rugosa*, and *C. dubliniensis*, several of which may pose problems with antifungal resistance and nosocomial spread (4, 5, 7, 23, 26). Although less common than *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*, these species may pose difficult management problems for individual patients, which may benefit from the application of antifungal susceptibility testing (21). Given the use of Etest for antifungal susceptibility testing of the common *Candida* spp. causing BSI, it is reasonable to validate its use for testing systemically active agents, such as fluconazole and voriconazole, against these less common species as well.

* Corresponding author. Mailing address: Medical Microbiology Division, C606 GH, Department of Pathology, University of Iowa College of Medicine Iowa City, IA 52242. Phone: (319) 384-9566. Fax: (319) 356-4916. E-mail: michael-pfaller@uiowa.edu.

† Members of the International Fungal Surveillance Participant Group are listed in Acknowledgments.

The purpose of the present study is to expand the Etest database for fluconazole and voriconazole by testing an international collection of 279 clinical BSI isolates of 12 uncommon species of *Candida* obtained from 68 different locations in 26 nations. The fluconazole and voriconazole MICs determined by Etest are compared to MICs determined by the National Committee for Clinical Laboratory Standards (NCCLS) reference broth microdilution (BMD) method, NCCLS M27-A (10).

MATERIALS AND METHODS

Organisms. A total of 279 clinical isolates of *Candida* spp. obtained from 68 medical centers in North America (39 centers), Latin America (6 centers), Europe (15 centers), and the Asia-Pacific region (8 centers) were tested. The collection included the following numbers of isolates: 118, *C. krusei*; 56, *C. lusitaniae*; 53, *C. guilliermondii*; 11, *C. kefyr*; 10, *C. rugosa*; 8, *C. lipolytica*; 8, *C. pelliculosa*; 7, *C. dubliniensis*; 3, *C. famata*; 3, *C. zeylanoides*; 1, *C. inconspicua*; and 1, *C. norvegensis*. All were incident isolates obtained from blood cultures of 279 different patients with candidemia. Isolates were identified by using Vitek and API yeast identification systems (bioMerieux, Inc., Hazelwood, Mo.) and were supplemented by conventional methods as needed (25). Isolates were stored as water suspensions until used. Prior to testing, each isolate was passaged at least twice on potato dextrose agar (Remel, Lenexa, Kans.) to ensure purity and viability.

Susceptibility testing. Reference antifungal susceptibility testing of *Candida* spp. was performed by BMD, as described by the NCCLS (10). Reference powders of fluconazole and voriconazole were obtained from Pfizer Pharmaceuticals (Groton, Conn.).

Etest strips for fluconazole and voriconazole were provided by AB BIODISK. MICs using Etest were determined as described previously (11, 14) by using 150-mm-diameter plates containing RPMI agar with 2% glucose (RPG; Remel), an inoculum suspension adjusted to the turbidity of a 0.5 McFarland standard (10^6 cells/ml), and incubation at 35°C for 48 h. Both fluconazole and voriconazole strips were placed on the same plate. The MICs of both fluconazole and voriconazole were read at the lowest concentration at which the border of the elliptical inhibition zone intercepted the scale on the strip. Any growth, such as microcolonies, throughout a discernible inhibition ellipse was ignored.

MIC interpretive criteria for fluconazole were those published by Rex et al.

TABLE 1. In vitro susceptibility of uncommon *Candida* spp. to fluconazole determined by BMD and Etest

Species (no. of isolates tested)	Test method	Cumulative no. of isolates (%) susceptible at MIC (μg/ml):										% Agreement[a]
		0.12	0.25	0.5	1	2	4	8	16	32	64	
C. krusei (118)	BMD	0	0	0	0	0	0	2 (2)	13 (11)	59 (50)	112 (95)	
	Etest	0	0	0	0	0	0	1 (1)	3 (3)	37 (31)	81 (69)	97 (114/118)
C. lusitaniae (56)	BMD	2 (4)	18 (32)	41 (73)	48 (86)	52 (93)	52 (93)	53 (95)	54 (96)	54 (96)	56 (100)	
	Etest	5 (9)	18 (32)	36 (64)	47 (84)	52 (93)	52 (93)	53 (95)	58 (95)	55 (98)	55 (98)	100 (56/56)
C. guilliermondii (53)	BMD	0	0	1 (2)	6 (11)	16 (30)	33 (62)	44 (83)	49 (92)	49 (92)	49 (92)	
	Etest	0	4 (8)	8 (15)	12 (23)	27 (51)	41 (77)	50 (94)	51 (96)	52 (98)	52 (98)	91 (48/56)
C. kefyr (11)	BMD	0	6 (55)	10 (91)	11 (100)							
	Etest	1 (9)	7 (64)	10 (91)	11 (100)							100 (11/11)
C. rugosa (10)	BMD	0	0	0	1 (10)	3 (30)	6 (60)	7 (70)	9 (90)	10 (100)		
	Etest	0	0	0	1 (10)	4 (40)	6 (60)	7 (70)	7 (70)	8 (80)	10 (100)	100 (10/10)
C. lipolytica (8)	BMD	0	0	0	0	0	5 (63)	6 (75)	6 (75)	6 (75)	7 (88)	
	Etest	0	0	0	0	1 (13)	5 (63)	6 (75)	6 (75)	7 (88)	7 (88)	100 (8/8)
C. pelliculosa (8)	BMD	0	0	0	0	0	6 (75)	8 (100)				
	Etest	0	0	1 (13)	1 (13)	3 (38)	4 (50)	8 (100)				100 (8/8)
C. dubliniensis (7)	BMD	1 (14)	7 (100)									
	Etest	4 (57)	6 (86)	7 (100)								100 (7/7)
C. famata (3)	BMD	0	1 (33)	2 (67)	2 (67)	2 (67)	2 (67)	2 (67)	3 (100)			
	Etest	1 (33)	1 (33)	2 (67)	2 (67)	3 (100)						67 (2/3)
C. zeylanoides (3)	BMD	1 (33)	1 (33)	2 (67)	3 (100)							
	Etest	1 (33)	1 (33)	2 (67)	3 (100)							100 (3/3)
C. inconspicua (1)	BMD	0	0	0	0	0	0	1 (100)				
	Etest	0	0	0	0	0	1 (100)					100 (1/1)
C. norvegensis (1)	BMD	0	0	0	0	0	0	0	1 (100)			
	Etest	0	0	0	0	0	0	0	0	0	0	0 (0/1)
All species (279)	BMD	4 (1)	33 (12)	63 (23)	78 (28)	94 (34)	125 (45)	144 (52)	165 (59)	212 (76)	268 (96)	
	Etest	12 (4)	37 (13)	66 (24)	84 (30)	111 (40)	133 (48)	150 (54)	153 (55)	192 (69)	238 (85)	96 (268/279)

[a] % agreement signifies the percentage of Etest MICs within 2 dilutions of the reference BMD MICs determined following 48 h of incubation.

(20) and the NCCLS (10). Breakpoints were as follows: susceptible (S), ≤ 8 μg/ml; susceptible-dose dependent, 16 to 32 μg/ml; and resistant (R), ≥ 64 μg/ml. Interpretive breakpoints have not yet been established for voriconazole.

QC. Quality control (QC) was performed for BMD and Etest in accordance with NCCLS document M27-A by using *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 (2, 10). QC determinations made on each day of testing were within the control limits for fluconazole and voriconazole described by Barry et al. (2, 3).

Analysis of results. Etest MICs for fluconazole and voriconazole read at 48 h were compared to reference BMD MICs read at 48 h. The reference microdilution MICs and Etest MICs were determined in two different time periods and were read independently by two different individuals; i.e., the testing was blinded. Etest MICs were rounded to the next even $\log_2$ concentration in order to simplify analysis (3, 11, 14). Discrepancies of no more than 2 dilutions were used to calculate the percent agreement.

The interpretive breakpoints described by NCCLS (10) were utilized to determine the categorical agreement between the Etest and BMD results for fluconazole. Major errors were identified as a classification of R by Etest and S by BMD, very major errors were when the Etest results was S and BMD was R, and minor errors were when one of the test results was S or R and the other was susceptible-dose dependent.

RESULTS AND DISCUSSION

Tables 1 and 2 summarize the in vitro susceptibilities of 279 BSI isolates of uncommon *Candida* spp. to fluconazole and voriconazole, respectively, as determined by the reference BMD and Etest methods. The data are presented in a continuous fashion as cumulative percentages of organisms susceptible at each dilution throughout the dilution series. The fluconazole MICs obtained by both BMD and Etest demonstrate excellent activity (≥ 95% S) against *C. lusitaniae*, *C. kefyr*, *C. pelliculosa*, *C. dubliniensis*, *C. zeylanoides*, and *C. inconspicua* and relatively poor activity against *C. krusei*, *C. guilliermondii*, *C. rugosa*, *C. lipolytica*, and *C. norvegensis* (Table 1).

The MICs obtained by both BMD and Etest methods demonstrated that voriconazole was very active against all of the uncommon species of *Candida* (92 to 100% S if MIC ≤ 1 μg/ml) with the exception of *C. lipolytica* (88% S at MIC if 1 μg/ml) (Table 2). Overall, 97 and 99% of isolates were inhibited at ≤1μg/ml by BMD and Etest, respectively (Table 2).

As reported previously for the more common *Candida* spp. (3, 11, 14), the agreement between BMD and Etest for both fluconazole and voriconazole with the uncommon *Candida* spp. was excellent (Tables 1 and 2). The agreement was 96% for fluconazole and 95% for voriconazole.

The agreement between fluconazole Etest and BMD MICs was > 97% for all species, with the exception of *C. guilliermondii* (91%), *C. famata* (two of three, 67%), and *C. norvegensis* (zero of one, 0%) (Table 1). When discrepancies were observed between the results obtained by Etest and BMD for fluconazole, the Etest generally provided lower MICs, although Etest MICs tended to be higher than BMD MICs if *C. krusei* was being tested. The overall categorical agreement between Etest and BMD results for fluconazole was 81%, with 1.1% very major errors, 0% major errors, and 17.9% minor errors (data not shown). This agreement is lower than that reported by Barry et al. (3) when the more common species of *Candida* are tested, largely due to a greater number of minor errors attributed to *C. krusei*.

The agreement between voriconazole Etest and BMD MICs was > 99% for all species, with the exception of *C. guilliermondii* (79%), *C. pelliculosa* (seven of eight, 88%), and *C. famata* (two of three, 67%) (Table 2). As with fluconazole, when discrepancies occurred between the results obtained by

TABLE 2. In vitro susceptibility of uncommon *Candida* spp. to voriconazole determined by BMD and Etest

Species (no. of isolates tested)	Test method	Cumulative no. of isolates (%) susceptible at MIC (μg/ml):											% Agreement[a]
		0.07	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	
C. krusei (118)	BMD	0	0	0	1 (1)	13 (11)	62 (53)	110 (93)	116 (98)	118 (100)			
	Etest	0	0	0	4 (3)	16 (14)	84 (71)	113 (96)	117 (99)	117 (99)	118 (100)		99 (117/118)
C. lusitaniae (56)	BMD	38 (68)	51 (91)	53 (95)	53 (95)	53 (95)	54 (96)	56 (100)					
	Etest	37 (66)	51 (91)	52 (93)	53 (95)	53 (95)	54 (96)	56 (100)					100 (56/56)
C. guilliermondii (53)	BMD	3 (6)	5 (9)	10 (19)	27 (51)	35 (66)	43 (81)	49 (92)	49 (92)	51 (96)	51 (96)	51 (96)	
	Etest	7 (13)	12 (23)	28 (53)	46 (87)	49 (92)	51 (96)	52 (98)	53 (100)				79 (42/53)
C. kefyr (11)	BMD	4 (36)	8 (73)	11 (100)									
	Etest	0	6 (55)	11 (100)									100 (11/11)
C. rugosa (10)	BMD	1 (10)	2 (20)	3 (30)	6 (60)	7 (70)	10 (100)						
	Etest	1 (10)	4 (40)	7 (70)	7 (70)	9 (90)	10 (100)						100 (10/10)
C. lipolytica (8)	BMD	0	0	1 (13)	3 (38)	6 (75)	6 (75)	6 (75)	7 (88)	7 (88)	8 (100)		
	Etest	0	1 (13)	4 (50)	6 (75)	6 (75)	6 (75)	7 (88)	7 (88)	7 (88)	7 (88)	7 (88)	100 (8/8)
C. pelliculosa (8)	BMD	0	0	0	0	4 (50)	8 (100)						
	Etest	0	1 (13)	4 (50)	6 (75)	6 (75)	6 (75)	7 (88)	7 (88)	7 (88)	7 (88)	7 (88)	100 (8/8)
C. pelliculosa (8)	BMD	0	0	0	0	4 (50)	8 (100)						
	Etest	0	1 (13)	2 (25)	3 (38)	5 (63)	8 (100)						88 (7/8)
C. dubliniensis (7)	BMD	6 (86)	7 (100)										
	Etest	7 (100)											100 (7/7)
C. famata (3)	BMD	1 (33)	1 (33)	2 (67)	2 (67)	2 (67)	3 (100)						
	Etest	2 (67)	2 (67)	3 (100)									67 (2/3)
C. zeylanoides (3)	BMD	2 (67)	2 (67)	2 (67)	2 (67)	3 (100)							
	Etest	2 (67)	2 (67)	3 (100)									100 (3/3)
C. inconspicua (1)	BMD	0	0	0	0	1 (100)							
	Etest	0	0	1 (100)									100 (1/1)
C. norvegensis (1)	BMD	0	0	0	0	1 (100)							
	Etest	0	0	0	0	0	1 (100)						100 (1/1)
All species (279)	BMD	55 (20)	76 (27)	89 (32)	112 (40)	143 (51)	209 (75)	265 (95)	272 (97)	276 (99)	277 (99)	277 (99)	
	Etest	62 (22)	91 (33)	118 (42)	144 (52)	163 (58)	239 (86)	272 (97)	277 (99)	277 (99)	278 (99)	278 (99)	95 (265/279)

[a] % agreement signifies the percentage of Etest MICs within 2 dilutions of the reference BMD MICs determined following 48 h of incubation.

Etest and BMD for voriconazole, the Etest provided lower MICs.

The results of this study confirm and extend those of previous reports regarding the ability of Etest to generate fluconazole and voriconazole MIC data for the less common species of *Candida* (1, 6, 9, 11, 14). Previously, agreement was demonstrated between Etest (by using RPG) and the reference BMD method of 94% for fluconazole and 98% for voriconazole in studies where the vast majority of isolates were the more common species of *Candida* (11, 14). Testing smaller numbers of *C. krusei*, *C. lusitaniae*, and *C. guilliermondii* isolates revealed agreements between Etest and BMD of 97 to 100% for fluconazole (11) and 100% for voriconazole (14). Favel et al. (6) reported an agreement between Etest and BMD of 92% when testing fluconazole against 35 isolates of *C. lusitaniae.*

In summary, we have provided documentation of the ability of Etest to generate fluconazole and voriconazole MIC data for uncommon species of *Candida* that are comparable to those obtained by the NCCLS BMD method. RPMI agar with 2% glucose may be used to determine reference quality MICs of fluconazole and voriconazole against these rare agents of candidemia as well as the more common species detected in the clinical laboratory. Although the species tested in this study are uncommon causes of BSI, the fact that several may exhibit innate or acquired resistance to both amphotericin B and fluconazole (4, 5, 7, 23, 26) emphasizes the importance of reference quality antifungal testing capabilities in aiding management decisions (21). Given these concerns, the potent in vitro activity of voriconazole against these species is notable (Table 2).

ACKNOWLEDGMENTS

Linda Elliott provided excellent support in the preparation of the manuscript.

The International Fungal Surveillance Program was supported in part by research grants from Bristol-Myers Squibb (SENTRY Program), Pfizer (ARTEMIS Program), and Schering Plough (Global Surveillance Program).

We express our appreciation to all International Fungal Surveillance Program participants: L. Steele-Moore, Christiana Care Health Services, Wilmington, Del.; G. Denys, Clarion Health Methodist Hospital, Indianapolis, Ind.; C. Staley, Henry Ford Hospital, Detroit, Mich.; J. R. Dipersio, Summa Health System, Akron, Ohio; M. Saubolle, Good Samaritan Regional Medical Center, Phoenix, Ariz.; M. L. Wilson, Denver General Hospital, Denver, Colo.; G. D. Overturf, University of New Mexico Hospital, Albuquerque; L. R. Peterson, Northwestern Memorial Hospital, Chicago, Ill.; P. C. Schreckenberger, University of Illinois at Chicago, Chicago; G. V. Doern, University of Iowa Hospitals and Clinics, Iowa City; et al.

REFERENCES

1. **Arendrup, M., B. Lundgren, I. M. Jensen, B. S. Hansen, and N. Frimodt-Møller.** 2001. Comparison of Etest and a tablet diffusion test with the NCCLS broth microdilution method for fluconazole and amphotericin B susceptibility testing *Candida* isolates. J. Antimicrob. Chemother. **47:**521–526.
2. **Barry, A. L., M. A. Pfaller, S. D. Brown, A. Espinel-Ingroff, M. A. Ghannoum, C. Knapp, R. P. Rennie, J. H. Rex, and M. G. Rinaldi.** 2000. Quality control limits for broth microdilution susceptibility tests of ten antifungal agents. J. Clin. Microbiol. **38:**3457–3459.
3. **Barry, A. L., M. A. Pfaller, R. P. Rennie, P. C. Fuchs, and S. D. Brown.** 2002. Precision and accuracy of fluconazole susceptibility tests by broth microdilution, Etest, and disk diffusion methods. Antimicrob. Agents Chemother. **46:**1781–1784.

4. **Dick, J. D., R. R. Rosengard, W. G. Merz, R. K. Stuart, G. M. Hutchins, and R. Saral.** 1985. Fatal disseminated candidiasis due to amphotericin B-resistant *Candida guilliermondii*. Ann. Intern. Med. **102:**67–68.
5. **Dubé, M. P., P. N. R. Heseltine, M. G. Rinaldi, S. Evans, and B. Zawacki.** 1994. Fungemia and colonization with nystatin-resistant *Candida rugosa* in a burn unit. Clin. Infect. Dis. **18:**77–82.
6. **Favel, A., A. Michel-Nguyen, C. Chastin, F. Trousson, A. Penaud, and P. Regli.** 1997. In-vitro susceptibility pattern of *Candida lusitaniae* and evaluation of the Etest method. J. Antimicrob. Chemother. **39:**591–596.
7. **Hadfield, T. L., M. B. Smith, R. E. Winn, M. G. Rinaldi, and C. Guerra.** 1987. Mycoses caused by *Candida lusitaniae*. Rev. Infect. Dis. **9:**1006–1012.
8. **Hazen, K. C.** 1995. New and emerging yeast pathogens. Clin. Microbiol. Rev. **8:**462–478.
9. **Morace, G., G. Amato, F. Bistoni, G. Fadda, P. Marone, M. T. Montagna, S. Oliveri, L. Polonelli, R. Rigoli, I. Mancuso, S. LaFace, L. Masucci, L. Romano, C. Napoli, D. Tato, M. G. Buscema, C. M. C. Belli, M. M. Piccirillo, S. Conti, S. Covan, F. Fanti, C. Cavanna, F. D'Alo, and L. Pitzuna.** 2002. Multicenter comparative evaluation of six commercial systems and the National Committee for Clinical Laboratory Standards M27-A broth microdilution method for fluconazole susceptibility testing of *Candida* species. J. Clin. Microbiol. 40:2953–2958.
10. **National Committee for Clinical Laboratory Standards.** 1997. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard M27-A. National Committee for Clinical Laboratory Standards, Wayne, Pa.
11. **Pfaller, M. A., S. A. Messer, Å. Karlsson, and A. Bolmström.** 1998. Evaluation of the Etest method for determining fluconazole susceptibilities of 402 clinical yeast isolates by using three different agar media. J. Clin. Microbiol. **36:**2586–2589.
12. **Pfaller, M. A., S. A. Messer, and A. Bolmström.** 1998. Evaluation of Etest for determining in vitro susceptibility of yeast isolates to amphotericin B. Diagn. Microbiol. Infect. Dis. **32:**223–227.
13. **Pfaller, M. A., S. A. Messer, R. J. Hollis, R. N. Jones, G. V. Doern, M. E. Brandt, and R. A. Hajjeh.** 1999. Trends in species distribution and susceptibility to fluconazole among bloodstream isolates of *Candida* species in the United States. Diagn. Microbiol. Infect. Dis. **33:**217–222.
14. **Pfaller, M. A., S. A. Messer, A. Houston, K. Mills, A. Bolmström, and R. N. Jones.** 2000. Evaluation of the Etest method for determining voriconazole susceptibilities of 312 clinical isolates of *Candida* species by using three different media. J. Clin. Microbiol. **38:**3715–3717.
15. **Pfaller, M. A., D. J. Diekema, R. N. Jones, H. S. Sader, A. C. Fluit, R. J. Hollis, S. A. Messer, and the SENTRY Participant Group.** 2001. International surveillance of bloodstream infections due to *Candida* species: frequency of occurrence and in vitro susceptibilities to fluconazole, ravuconazole, and voriconazole of isolates collected from 1997 through 1999 in the SENTRY Antimicrobial Surveillance Program. J. Clin. Microbiol. **39:**3254–3259.
16. **Pfaller, M. A., S. A. Messer, K. Mills, A. Bolmström, and R. N. Jones.** 2001. Evaluation of Etest method for determining posaconazole MICs for 314 clinical isolates of *Candida* species. J. Clin. Microbiol. **39:**3952–3954.
17. **Pfaller, M. A., S. A. Messer, K. Mills, A. Bolmström, and R. N. Jones.** 2001. Evaluation of Etest method for determining caspofungin (MK-0991) susceptibilities of 726 clinical isolates of *Candida* species. J. Clin. Microbiol. **39:**4387–4389.
18. **Pfaller, M. A., and D. J. Diekema.** 2002. The role of sentinel surveillance of candidemia: trends in species distribution and antifungal susceptibilities. J. Clin. Microbiol. **40:**3551–3557.
19. **Pfaller, M. A., S. A. Messer, R. J. Hollis, R. N. Jones, and D. J. Diekema.** 2002. In vitro activities of ravuconazole and voriconazole compared with those of four approved systemtic antifungal agents against 6,970 clinical isolates of *Candida* spp. Antimicrob. Agents Chemother. **46:**1723–1727.
20. **Rex, J. H., M. A. Pfaller, J. N. Galgiani, M. S. Bartlett, A. Espinel-Ingroff, M. A. Ghannoum, M. Lancaster, M. G. Rinaldi, T. J. Walsh, and A. L. Barry.** 1997. Development of interpretive breakpoints for antifungal susceptibility testing: conceptual framework and analysis of in vitro-in vivo correlation data for fluconazole, itraconazole, and *Candida* infections. Clin. Infect. Dis. **24:**235–247.
21. **Rex, J. H., T. J. Walsh, J. D. Sobel, S. G. Filler, P. G. Pappas, W. E. Dismukes, and J. E. Edwards.** 2000. Practice guidelines for the treatment of candidiasis. Clin. Infect. Dis. **30:**662–678.
22. **Simor, A. E., G. Goswell, L. Louie, M. Lee, and M. Louie.** 1997. Antifungal susceptibility testing of yeast isolates from blood cultures by microbroth dilution and the Etest. Eur. J. Clin. Microbiol. Infect. Dis. **16:**693–697.
23. **Sullivan, D., and D. Coleman.** 1997. *Candida dubliniensis*: an emerging opportunistic pathogen. Curr. Top. Med. Mycol. **8:**15–25.
24. **Warnock, D. W., E. M. Johnson, T. R. Rogers, et al.** 1998. Multi-centre evaluation of the Etest method for antifungal drug susceptibility testing of *Candida* spp. and *Cryptococcus neoformans*. J. Antimicrob. Chemother. **42:**321–331.
25. **Warren, N. G., and K. C. Hazen.** 1999. *Candida*. *Cryptococcus*, and other yeasts of medical importance, p. 1184–1199. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. ASM Press, Washington, D.C.
26. **Wingard, J. R., W. G. Merz, M. G. Rinaldi, T. R. Johnson, J. E. Karp, and R. Saral.** 1991. Increase in *Candida krusei* infection among patients with bone marrow transplantation and neutropenia treated prophylactically with fluconazole. N. Engl. J. Med. **325:**1274–1277.