Journal of Clinical Microbiology

Results from the ARTEMIS DISK Global Antifungal Surveillance Study: a 6.5-Year Analysis of Susceptibilities of *Candida* and Other Yeast Species to Fluconazole and Voriconazole by Standardized Disk Diffusion Testing

M. A. Pfaller, D. J. Diekema, M. G. Rinaldi, R. Barnes, B. Hu, A. V. Veselov, N. Tiraboschi, E. Nagy and D. L. Gibbs
J. Clin. Microbiol. **2005, 43(12):5848. DOI: 10.1128/JCM.43.12.5848-5859.2005.**

Updated information and services can be found at:
http://jcm.asm.org/content/43/12/5848

These include:

REFERENCES

This article cites 31 articles, 16 of which can be accessed free at: http://jcm.asm.org/content/43/12/5848#ref-list-1

CONTENT ALERTS

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»

Information about commercial reprint orders: **http://journals.asm.org/site/misc/reprints.xhtml**
To subscribe to to another ASM Journal go to: **http://journals.asm.org/site/subscriptions/**

Journals.ASM.org

Downloaded from http://jcm.asm.org/ on February 25, 2014 by Cardiff Univ

JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2005, p. 5848–5859 Vol. 43, No. 12
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.12.5848–5859.2005
Copyright © 2005, American Society for Microbiology. All Rights Reserved.

Results from the ARTEMIS DISK Global Antifungal Surveillance Study: a 6.5-Year Analysis of Susceptibilities of *Candida* and Other Yeast Species to Fluconazole and Voriconazole by Standardized Disk Diffusion Testing

M. A. Pfaller,[1]* D. J. Diekema,[1] M. G. Rinaldi,[2] R. Barnes,[3] B. Hu,[4] A. V. Veselov,[5] N. Tiraboschi,[6] E. Nagy,[7] D. L. Gibbs,[8] and the Global Antifungal Surveillance Group

University of Iowa College of Medicine, Iowa City, Iowa[1]; University of Texas Health Science Center, San Antonio, Texas[2]; University of Wales College of Medicine, Cardiff, United Kingdom[3]; Zhong Shan Hospital, Shanghai, China[4]; Institute of Antimicrobial Chemotherapy, Smolensk, Russia[5]; Hospital de Clinicas "Jose de San Martin," Buenos Aires, Argentina[6]; Institute of Clinical Microbiology, Faculty of Medicine, University of Szeged, Szeged, Hungary[7]; and Giles Scientific, Inc., Santa Barbara, California[8]

Received 12 July 2005/Returned for modification 17 August 2005/Accepted 12 September 2005

Fluconazole in vitro susceptibility test results for 140,767 yeasts were collected from 127 participating investigators in 39 countries from June 1997 through December 2003. Data were collected on 79,343 yeast isolates tested with voriconazole from 2001 through 2003. All investigators tested clinical yeast isolates by the CLSI (formerly NCCLS) M44-A disk diffusion method. Test plates were automatically read and results were recorded with the BIOMIC Vision Image Analysis System. Species, drug, zone diameter, susceptibility category, and quality control results were collected quarterly via e-mail for analysis. Duplicate (the same patient, same species, and same susceptible-resistant biotype profile during any 7-day period) and uncontrolled test results were not analyzed. The 10 most common species of yeasts all showed less resistance to voriconazole than to fluconazole. *Candida krusei* showed the largest difference, with over 70% resistance to fluconazole and less than 8% to voriconazole. All species of yeasts tested were more susceptible to voriconazole than to fluconazole, assuming proposed interpretive breakpoints of ≥17 mm (susceptible) and ≤13 mm (resistant) for voriconazole. MICs reported in this study were determined from the zone diameter in millimeters from the continuous agar gradient around each disk, which was calibrated with MICs determined from the standard CLSI M27-A2 broth dilution method by balanced-weight regression analysis. The results from this investigation demonstrate the broad spectrum of the azoles for most of the opportunistic yeast pathogens but also highlight several areas where resistance may be progressing and/or where previously rare species may be "emerging."

Antifungal resistance surveillance with a focus on *Candida* is now widespread (5, 10, 17, 20, 29, 32). Most of these surveillance efforts are by necessity limited in terms of the numbers of participating sentinel sites and isolates tested. Furthermore, none of the programs is extensive enough to provide temporal and geographic data concerning the occurrence and resistance profiles of the less common *Candida* species and other, noncandidal opportunistic yeasts (21).

The ARTEMIS Global Antifungal Surveillance Program is among the most comprehensive and long-running fungal surveillance programs (6, 12, 17, 19, 22, 24, 25, 27). The ARTEMIS Program is made up of two components: (i) a broad international network of participating sites (127 sites in 39 countries), each of which performs Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical and Laboratory Standards [NCCLS])-recommended disk diffusion testing (M44-A) (14) of fluconazole and voriconazole against consecutive yeast isolates from a variety of clinical sources (ARTEMIS DISK Surveillance Study) (6), and (ii) a central reference laboratory (University of Iowa, Iowa City), where CLSI-recommended broth microdilution (BMD) MIC and disk diffusion testing (M27-A2 and M44-A, respectively) (13, 14) is performed on blood and normally sterile-site isolates of *Candida* and other opportunistic yeasts and molds that are referred according to protocol from the participating ARTEMIS study sites (19, 22, 24, 25, 27). As such, the ARTEMIS Program has been designed to address many of the potential limitations of resistance surveillance studies (7): (i) it is both longitudinal (1997 to present) and global (127 participating sites in 39 countries) in scope, (ii) it employs standardized antifungal susceptibility test methods (CLSI disk [M44-A] and BMD MIC [M27-A2]) (13, 14), (iii) both internal quality control (QC) performed in each participating laboratory and external quality assurance measures are used to validate test results (25, 27), (iv) results are recorded electronically using the BIOMIC image analysis plate reader system (Giles Scientific, Santa Barbara, Calif.) (6, 19, 25, 27) and are stored in a central database, and (v) both *Candida* and non-*Candida* yeast isolates obtained from consecutive clinical samples from all body sites are tested locally, thus avoiding misleading results based on biased selective testing. This so-called "routine" testing is augmented by testing of isolates from blood and normally sterile sites in the central reference laboratory (25, 27). Thus, the ARTEMIS Program generates massive amounts of data

* 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.

Downloaded from http://jcm.asm.org/ on February 25, 2014 by Cardiff Univ

that have been externally validated and that can be used to identify temporal and geographic trends in the species distribution of *Candida* and other opportunistic yeasts, as well as the resistance profiles of these organisms to fluconazole and voriconazole as determined by standardized CLSI disk diffusion testing.

In the present study, we utilized the results from the ARTEMIS DISK Surveillance Program to evaluate global trends in the susceptibility of yeasts to fluconazole over a 6.5-year period (140,767 isolates from 127 study sites in 39 countries; June 1997 through December 2003). We also report results of voriconazole susceptibility testing performed on 79,343 isolates collected from 2001 to 2003. The scope of this study provides an unprecedented look at the occurrence and azole susceptibilities of several rare species of *Candida*, as well as several of the other opportunistic yeasts. The study is limited in that the numbers of isolates from certain regions are small and the time frame over which voriconazole data are available is relatively short.

MATERIALS AND METHODS

Organisms and test sites. A total of 134,715 isolates of *Candida* spp. and 6,052 isolates of noncandidal yeasts obtained from 127 different medical centers in Asia (23 sites), Latin America (16 sites), Europe (74 sites), the Middle East (2 sites), and North America (12 sites) were collected and tested against fluconazole between June 1997 and December 2003. In addition, a total of 79,343 isolates (75,810 isolates of *Candida* spp. and 3,533 other yeasts) from 115 study sites in 35 countries were tested against voriconazole between 2001 and 2003. All yeasts considered pathogens from all body sites (e.g., blood, normally sterile body fluids, deep tissue, genital tract, gastrointestinal tract, respiratory tract, skin, and soft tissue) and isolates from patients in all in-hospital locations during the study period were tested. Yeasts considered by the local site investigator to be colonizers, that is, not associated with an obvious pathology, were excluded, as were duplicate isolates from a given patient (the same species and the same susceptible-resistant biotype profile within any 7-day period). Identification of isolates was performed in accordance with each site's routine methods.

Susceptibility test method. Disk diffusion testing of fluconazole and voriconazole was performed as described by Hazen et al. (6) and in CLSI document M44-A (14). Agar plates (150-mm diameter) containing Mueller-Hinton agar (obtained locally at all sites) supplemented with 2% glucose and 0.5 μg of methylene blue per ml (MH-MB) at a depth of 4.0 mm were used. The agar surface was inoculated by using a swab dipped in a cell suspension adjusted to the turbidity of a 0.5 McFarland standard. Fluconazole (25-μg) and voriconazole (1-μg) disks (Becton Dickinson, Sparks, Md.) were placed onto the surfaces of the plates, and the plates were incubated in air at 35 to 37°C and read at 18 to 24 h. Slowly growing isolates, primarily members of the genus *Cryptococcus*, were read after 48 h of incubation. Zone diameter endpoints were read at 80% growth inhibition by using the BIOMIC image analysis plate reader system (version 5.9; Giles Scientific, Santa Barbara, Calif.) (6, 19).

The interpretive criteria for the fluconazole and voriconazole disk diffusion tests were those of the CLSI (1a, 14): susceptible (S), zone diameters of ≥19 mm (fluconazole) and ≥17 mm (voriconazole); susceptible dose dependent (SDD), zone diameters of 15 to 18 mm (fluconazole) and 14 to 16 mm (voriconazole); and resistant (R), zone diameters of ≤14 mm (fluconazole) and ≤13 mm (voriconazole). The corresponding MIC breakpoints (13) are as follows: S, MIC of ≤8 μg/ml (fluconazole) and ≤1 μg/ml (voriconazole); SDD, MIC of 16 to 32 μg/ml (fluconazole) and 2 μg/ml (voriconazole); R, MIC of ≥64 μg/ml (fluconazole) and ≥4 μg/ml (voriconazole).

QC. QC was performed in accordance with CLSI document M44-A (14) by using *Candida albicans* ATCC 90029 and *C. parapsilosis* ATCC 22019. A total of 5,865 and 5,484 QC results were obtained for fluconazole and voriconazole, respectively, of which more than 99% were within the acceptable limits.

Analysis of results. All yeast disk test results were read by electronic image analysis and interpreted and recorded with a BIOMIC Plate Reader System (Giles Scientific Inc.). Test results were sent by e-mail to Giles Scientific for analysis. The zone diameter, susceptibility category (S, SDD, or R), and QC test results were all recorded electronically. In addition, MICs were calculated for each drug-organism pair by the BIOMIC System software. The MIC-versus-zone-diameter regression data used by the BIOMIC software were generated previously by ARTEMIS investigators (M.A.P. and M.G.R.) using CLSI BMD MIC and disk test methods (19, 25, 27). Patient and doctor names, duplicate test results (the same patient, the same species, and the same biotype results), and uncontrolled results were automatically eliminated by the BIOMIC system prior to analysis.

RESULTS

Isolation rates by species. A total of 140,767 yeast isolates were collected and tested at 127 study sites between June 1997 and December 2003 (Table 1). *Candida* species accounted for 95 to 97% of all isolates in each study year (overall, 95.7%). More than 16 different species of *Candida* were isolated, of which *Candida albicans* was the most common (overall, 66.2% of all *Candida* spp.). A decreasing trend in the rate of *C. albicans* isolation (overall decrease, 10 to 11%) was noted over the 6.5-year period. In contrast, increased rates of isolation of *C. tropicalis* (an increase of 2.9% from 1997 to 2003) and *C. parapsilosis* (an increase of 3.1% from 1997 to 2003) were noted. Neither *C. glabrata* nor *C. krusei* showed a consistent increase or decrease in isolation rate. Although isolates of more unusual *Candida* species, such as *C. guilliermondii*, *C. kefyr*, *C. rugosa*, and *C. famata*, constituted only a small percentage of the *Candida* isolates, the isolation rates of these four species increased from 2- to 10-fold over the course of the study. Likewise, although *C. inconspicua*, *C. norvegensis*, *C. lipolytica*, *C. pelliculosa*, and *C. zeylanoides* are rare species of *Candida*, the sheer size of the ARTEMIS database provides a significant number of each of these species for study.

Among the noncandidal yeasts, *Cryptococcus neoformans* (21% of 6,052 isolates), *Saccharomyces* spp. (6.8%), *Trichosporon* spp. (6.5%), and *Rhodotorula* spp. (2.3%) were the most commonly identified species (Table 1). Unidentified ("other") yeasts represented 0.46 to 3.05% of all isolates. As noted previously (6), this percentage decreased somewhat over the course of the study as more isolates were identified to the species level.

Fluconazole and voriconazole susceptibilities of *Candida* spp. Table 2 summarizes the in vitro susceptibilities of 78,463 and 75,787 isolates of *Candida* spp. to fluconazole and voriconazole, respectively, as determined by CLSI disk diffusion testing. These isolates were obtained from 115 institutions in 35 countries during the period 2001 through 2003. The distribution of zone diameters and their respective interpretive categories are shown in Fig. 1 for both agents. The percentages of isolates in each category (S, SDD, and R) were 89.6%, 4.0%, and 6.4% and 94.6%, 2.3%, and 3.1% for fluconazole and voriconazole, respectively. Fluconazole was most active against *C. albicans* (97.8% S), *C. parapsilosis* (93.2% S), *C. lusitaniae* (93.3% S), *C. kefyr* (95.3% S), *C. dubliniensis* (96.8% S), and *C. pelliculosa* (94.7% S). Decreased susceptibility to fluconazole was seen with *C. glabrata* (66.7% S; 16.6% R), *C. krusei* (9.4% S; 77.2% R), *C. guilliermondii* (73.3% S; 9.8% R), *C. rugosa* (39.3% S; 51.8% R), *C. famata* (79.8% S; 11.9% R), *C. inconspicua* (25.7% S; 49.2% R), *C. norvegensis* (50.0% S; 38.0% R), *C. lipolytica* (54.7% S; 39.6% R), and *C. zeylanoides* (54.1% S; 37.8% R). These findings confirm previously reported data for the more common species (e.g., *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. krusei*) and markedly expand our understanding of the susceptibility, or lack thereof, of less common

Downloaded from http://jcm.asm.org/ on February 25, 2014 by Cardiff Univ

TABLE 1. Species distribution of *Candida* and other yeast isolates by year: ARTEMIS DISK Surveillance Program, 1997 to 2003[a]

Organism	1997–1998		1999		2000		2001		2002		2003	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Candida	22,533	95.2	20,998	95.7	11,698	97.0	21,804	96.3	24,680	95.3	33,002	95.5
C. albicans	16,514	69.77	14,667	66.87	7,961	66.02	14,268	62.99	15,147	58.51	20,576	59.56
C. glabrata	2,475	10.46	2,047	9.33	1,112	9.22	2,431	10.73	2,635	10.18	3,974	11.50
C. tropicalis	1,036	4.38	1,117	5.09	843	6.99	1,634	7.21	1,838	7.10	2,487	7.20
C. parapsilosis	955	4.03	1,028	4.68	650	5.39	1,501	6.63	1,632	6.30	2,406	6.96
C. krusei	372	1.57	459	2.09	376	3.12	544	2.40	639	2.47	884	2.56
C. guilliermondii	111	0.47	168	0.77	88	0.73	163	0.72	239	0.92	260	0.75
C. lusitaniae	115	0.49	99	0.45	62	0.51	122	0.54	131	0.51	211	0.61
C. kefyr	34	0.14	84	0.38	64	0.53	86	0.38	87	0.34	171	0.49
C. rugosa	7	0.03	7	0.03	21	0.17	151	0.67	150	0.58	116	0.34
C. famata	19	0.08	51	0.23	53	0.44	54	0.24	110	0.42	89	0.26
C. inconspicua					9	0.07	30	0.13	44	0.17	113	0.33
C. norvegensis	1	0.0	1	0.0	9	0.07	32	0.14	18	0.07	42	0.12
C. dubliniensis					1	0.01	19	0.08	26	0.10	18	0.05
C. lipolytica					7	0.06	14	0.06	14	0.05	25	0.07
C. zeylanoides					4	0.03	19	0.08	5	0.02	13	0.04
C. pelliculosa					1	0.01	14	0.06	12	0.05	12	0.03
Candida spp.	894	3.78	1,260	5.74	437	3.62	722	3.19	1,953	7.54	1,605	4.65
Other yeasts	1,131	4.8	950	4.3	361	3.0	849	3.7	1,210	4.7	1,547	4.5
Cryptococcus neoformans	275	1.16	334	1.52	79	0.66	312	1.38	575	2.22	463	1.34
Trichosporon spp.	68	0.29	77	0.35	107	0.89	134	0.59	118	0.46	139	0.40
Saccharomyces spp.	36	0.15	130	0.59	81	0.67	101	0.44	141	0.54	200	0.58
Rhodotorula spp.	33	0.14	31	0.14	17	0.14	17	0.08	42	0.16	80	0.23
Blastoschizomyces capitatus					1	0.01	17	0.08	22	0.08	16	0.05
Cryptococcus spp.					3	0.02	17	0.08	12	0.05	43	0.12
Pichia spp.					7	0.06	5	0.02	7	0.03	15	0.04
Hansenula anomala					10	0.08	2	0.01	9	0.03	2	0.01
Other yeast	723	3.05	378	1.72	56	0.46	244	1.08	284	1.10	589	1.70
Total	23,668		21,948		12,059		22,653		25,890		34,549	

[a] Includes all specimen types and all locations in hospitals from a total of 121 different institutions.

species, such as *C. rugosa*, *C. inconspicua*, and *C. norvegensis*, to fluconazole (5, 15, 16, 18, 21, 23).

Voriconazole was significantly more active than fluconazole against virtually every species, with the exception of *C. tropicalis* (89.1% S to fluconazole versus 87.1% S to voriconazole) (Table 2). Among the species with decreased susceptibility to fluconazole, more than 80% were susceptible to voriconazole, including *C. glabrata* (81.7% S), *C. krusei* (83.2% S), *C. guilliermondii* (91.2% S), *C. famata (*89.5% S), *C. inconspicua* (89.2% S), and *C. norvegensis* (92.3% S). Among the fluconazole-resistant (zone diameter, ≤14 mm) isolates of *C. glabrata*, ~30% remained susceptible (zone diameter, ≥17 mm) to voriconazole; however, all voriconazole-resistant strains were also resistant to fluconazole (reference 22 and data not shown). Although voriconazole was more active than fluconazole against *C. rugosa* (61.4% S versus 39.3% S, respectively), *C. lipolytica* (67.3% S versus 54.7% S, respectively), and *C. zeylanoides* (74.3% S versus 54.1% S, respectively), these species were markedly less susceptible and more resistant (11.4% to 26.4%) to voriconazole than all other species of *Candida*. Again, these data confirm and extend previous observations, especially with the less common species of *Candida* (18, 20, 23, 24). Importantly, it is readily apparent from these data that although some degree of cross-resistance may be seen between fluconazole and voriconazole, it varies by species and should not be assumed in the absence of species identification and susceptibility testing results.

TABLE 2. In vitro susceptibilities of *Candida* spp. to fluconazole and voriconazole as determined by CLSI disk diffusion testing: ARTEMIS DISK Surveillance Program, 2001 to 2003[a]

Species	Susceptibility					
	Fluconazole[b]			Voriconazole[b]		
	n	%S	%R	*n*	%S	%R
C. albicans	49,991	97.8	1.3	47,584	98.6	1.0
C. glabrata	9,040	66.7	16.6	8,719	81.7	10.1
C. tropicalis	5,959	89.1	5.0	5,643	87.1	6.7
C. parapsilosis	5,539	93.2	3.6	5,233	96.8	1.8
C. krusei	2,067	9.4	77.2	1,996	83.2	7.5
C. guilliermondii	662	73.3	9.8	633	91.2	4.9
C. lusitaniae	464	93.3	4.1	445	96.4	2.0
C. rugosa	417	39.3	51.8	394	61.4	26.4
C. kefyr	344	95.3	3.5	331	99.1	0.6
C. famata	253	79.8	11.9	238	89.5	5.5
C. inconspicua	187	25.7	49.2	186	89.2	5.4
C. norvegensis	92	50.0	38.0	91	92.3	1.1
C. dubliniensis	63	96.8	3.2	63	100.0	0.0
C. lipolytica	53	54.7	39.6	52	67.3	19.2
C. pelliculosa	38	94.7	0.0	38	100.00	0.0
C. zeylanoides	37	54.1	37.8	35	74.3	11.4
C. sake	12	83.3	8.3	12	100.0	0.0
Candida spp.[c]	4,245	86.6	8.2	4,094	92.7	4.7

[a] Isolates obtained from 115 institutions in 35 countries.
[b] Fluconazole and voriconazole disk diffusion testing was performed in accordance with CLSI M44-A. Interpretive breakpoints: S, fluconazole ≥19 mm, voriconazole ≥17 mm: R, fluconazole ≤14 mm, voriconazole ≤13 mm.
[c] *Candida* species not otherwise identified.

Downloaded from http://jcm.asm.org/ on February 25, 2014 by Cardiff Univ

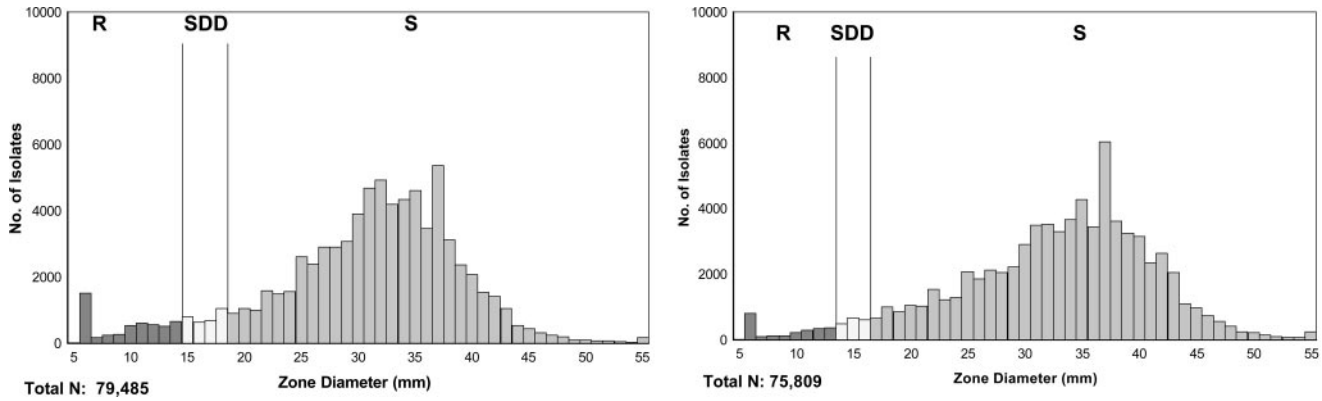

FIG. 1. Fluconazole (left) and voriconazole (right) zone diameter (in mm) distribution for all *Candida* spp.: 79,485 isolates tested against fluconazole and 75,809 isolates tested against voriconazole. The isolates were obtained from 115 institutions in 34 countries from 2001 through 2003. Interpretive breakpoints: S, ≥19 mm (fluconazole) and ≥17 mm (voriconazole); SDD, 15 to 18 mm (fluconazole) and 14 to 16 mm (voriconazole); R, ≤14 mm (fluconazole) and ≤13 mm (voriconazole).

Trends in resistance to fluconazole among *Candida* spp. over a 6.5-year period. The longitudinal nature of the ARTEMIS DISK Surveillance Program allows one to examine trends in fluconazole resistance among clinical isolates of *Candida* spp. with the important advantage of sufficient numbers of isolates of each species, all tested by a single standardized method (Table 3). Among the 10 species listed in Table 3, no consistent increase or decrease in fluconazole resistance was seen over time with *C. albicans* (range, 0.8% to 1.5%) or *C. glabrata* (range, 14.3% to 22.8%). Although resistance among *C. tropicalis* isolates appeared to decline from 1997-1998 (4.2%) thru 2001 (3.0%), increases were seen in 2002 (6.6%) and 2003 (5.0%). A slight increase in resistance was noted over time among *C. parapsilosis* and *C. kefyr*, whereas a major increase in resistance was detected among isolates of *C. rugosa*, where 61.2 to 66.0% resistance was observed in the last 2 years of data collection. In contrast, following a peak of 26.1% R in 2000, resistance among isolates of *C. guilliermondii* decreased steadily between 2001 (11.7% R) and 2003 (8.1% R). Although *C. famata* appeared to be quite resistant to fluconazole in 1997 and 1998 (47.4% of 19 isolates), this was likely due to the small number of isolates tested. As the numbers of *C. famata* isolates increased to >50 per year over the next 5 years, the level of resistance stabilized at 10 to 12%. Despite the increase in the overall percentage of isolates of *C. krusei* that tested as resistant to fluconazole, this is not an important finding, as the species must be considered to be clinically resistant to fluconazole. The CLSI recommends that *C. krusei* not be tested against fluconazole (13, 14). All such isolates should be reported as fluconazole resistant.

Trends in resistance to voriconazole among *Candida* spp., 2001 to 2003. Voriconazole has been used clinically since 2001 and since that time has been tested against *Candida* in the ARTEMIS Global Surveillance Program (Table 4). Overall, there has been a slight increase in the percentage of *Candida* isolates that appear to be resistant (zone diameter, ≤13 mm) to voriconazole, from 2.6% in 2001 to 3.5% in 2003. This may be accounted for by increases in resistance observed with *C. glabrata* (9.8% to 11.0%), *C. tropicalis* (4.7% to 7.0%), *C. rugosa* (3.1 to 38.0%), *C. lipolytica* (7.7% to 12.0%), and un-

TABLE 3. Trends in in vitro resistance to fluconazole among *Candida* spp. as determined by CLSI disk diffusion testing over a 6.5-year period: ARTEMIS DISK Surveillance Program, 1997 to 2003[a]

Species	Resistance[b]											
	1997–1998		1999		2000		2001		2002		2003	
	n	%R	*n*	%R	*n*	%R	*n*	%R	*n*	%R	*n*	%R
C. albicans	16,514	0.8	14,677	0.8	7,961	1.5	14,268	1.0	15,147	1.5	20,576	1.4
C. glabrata	2,475	18.5	2,047	22.8	1,112	14.3	2,431	18.3	2,635	14.7	3,974	16.9
C. tropicalis	1,036	4.2	1,117	3.5	843	3.1	1,634	3.0	1,838	6.6	2,487	5.0
C. parapsilosis	955	2.0	1,028	2.8	650	2.9	1,501	4.2	1,632	3.9	2,406	3.0
C. krusei	372	56.5	459	71.5	376	68.1	544	70.4	639	78.9	884	80.2
C. guilliermondii	111	6.3	168	9.5	88	26.1	163	11.7	239	10.5	260	8.1
C. lusitaniae	115	2.6	99	4.0	62	1.6	122	6.6	131	4.6	211	2.4
C. kefyr	34	0.0	84	4.8	64	3.1	86	2.3	87	5.7	171	2.9
C. rugosa	7	28.6	7	14.3	21	42.9	151	30.5	150	66.0	116	61.2
C. famata	19	47.4	51	9.8	53	13.2	54	14.8	110	10.9	89	11.2
Candida spp.[c]	894	15.5	1,260	7.1	437	10.1	722	9.6	1,953	52	1,605	11.4

[a] Isolates from all specimen types and all hospital locations in 127 institutions.
[b] Zone ≤14 mm. Fluconazole disk diffusion testing performed in accordance with CLSI M44-A.
[c] *Candida* species not otherwise identified.

Downloaded from http://jcm.asm.org/ on February 25, 2014 by Cardiff Univ

TABLE 4. Trends in in vitro resistance to voriconazole among *Candida* spp. as determined by CLSI disk diffusion testing over a 3-year period: ARTEMIS DISK Surveillance Program, 2001 to 2003[a]

Species	Resistance[b]					
	2001		2002		2003	
	n	%R	*n*	%R	*n*	%R
C. albicans	11,980	0.8	15,086	1.0	20,518	1.0
C. glabrata	2,123	9.8	2,625	8.0	3,971	11.0
C. tropicalis	1,350	4.7	1,820	8.0	2,473	7.0
C. parapsilosis	1,205	1.9	1,627	2.0	2,401	1.0
C. krusei	474	8.0	635	6.0	887	8.0
C. guilliermondii	142	4.2	235	6.0	256	5.0
C. lusitaniae	106	2.8	129	2.0	210	2.0
C. rugosa	129	3.1	149	38.0	116	38.0
C. kefyr	75	1.3	85	1.0	171	0.0
C. famata	39	10.3	110	2.0	89	8.0
C. inconspicua	30	6.7	43	5.0	113	5.0
C. norvegensis	31	0.0	18	0.0	42	2.0
C. dubliniensis	19	0.0	26	0.0	18	0.0
C. lipolytica	13	7.7	14	43.0	25	12.0
C. pelliculosa	14	0.0	12	0.0	12	0.0
Candida spp.[c]	590	4.2	1,958	3.0	1,616	7.0
All *Candida*	18,320	2.6	24,572	3.0	32,918	3.5

[a] Isolates obtained from 115 institutions in 35 countries.
[b] Zone ≤13 mm. Voriconazole disk diffusion testing was performed in accordance with CLSI M44-A.
[c] *Candida* species not otherwise identified.

identified *Candida* species (4.2% to 7.0%). In contrast, no change or a decrease in resistance was seen with *C. albicans*, *C. parapsilosis*, *C. krusei*, *C. lusitaniae*, *C. kefyr*, *C. famata*, *C. inconspicua*, *C. dubliniensis*, and *C. pelliculosa*. Thus, the picture for voriconazole, in terms of spectrum and potency versus *Candida* spp., looks quite favorable. Emerging resistance, especially among *C. glabrata*, *C. tropicalis*, and *C. rugosa*, bears close monitoring.

Geographic variation in the susceptibilities of *C. albicans* and *C. glabrata* to fluconazole and voriconazole. Table 5 presents the in vitro susceptibility results for fluconazole and voriconazole tested against the two most common species of *Candida*, *C. albicans* and *C. glabrata*, stratified by geographic region and country of origin for the time period 2001 to 2003. With the exception of those from India, isolates of *C. albicans* were highly susceptible to both fluconazole and voriconazole. The only other countries where the percentages of *C. albicans* susceptible to either agent dropped below 94% were Colombia (fluconazole, 91.2% S, 6.1% R) and Ecuador (fluconazole, 91.6% S, 4.9% R). Overall, there was no meaningful difference in the fluconazole or voriconazole susceptibility profile for *C. albicans* when stratified by specimen type (96.7% to 99.3% S to fluconazole; 97.9% to 99.3% S to voriconazole) or by hospital location (95.3 to 99.1% S to fluconazole; 97.2% to 99.4% S to voriconazole) (data not shown).

Fluconazole and voriconazole susceptibilities of *C. glabrata* isolates varied considerably among the various countries and geographic regions. Susceptibilities to fluconazole were lowest (<50%) in Venezuela (29.2% S), Malaysia (34.0% S), Belgium (39.7% S), the Czech Republic (44.8% S), and South Africa (49.6%) and highest (>80%) in India and the Middle East (100% S), Brazil (94.9% S), Greece (93.9% S), Canada (90.6% S), Portugal (87.1% S), Mexico (86.7% S), Poland (86.4% S), South Korea (83.7% S), Turkey (82.4% S), and Italy (81.3% S). Overall rates of resistance to fluconazole among *C. glabrata* isolates were 10.6% in the Asia-Pacific region, 13.2% in Latin America, 16.5% in Europe, and 18.0% in North America (data not shown). These rates of fluconazole resistance are considerably higher for each geographic region than those reported previously for blood and normally sterile-site infection isolates of *C. glabrata* (range, 2 to 9% R) tested by BMD between 1992 and 2000 (20).

In contrast to that seen with *C. albicans*, the susceptibility of *C. glabrata* isolates to fluconazole varied according to specimen type and hospital location. Isolates from blood and normally sterile sites were the most susceptible (71% S; 14.8% R) and genital tract isolates were the least susceptible (53.6% S; 21.2% R) to fluconazole (data not shown). The highest rates of resistance were seen in isolates of *C. glabrata* from the surgical intensive-care unit (21.3%), the obstetrics and gynecology service (21.5%), the hematology/oncology service (22.6%), and the neonatal intensive-care unit (35.0%) (data not shown).

Voriconazole was equally or more active than fluconazole against *C. glabrata* isolates from all countries and geographic regions (Table 5). Susceptibilities to voriconazole were lowest (<70%) in Venezuela (32.7% S), Belgium (53.2% S), Malaysia (59.5% S), the Czech Republic (65.3% S), and Ecuador (66.7% S) and highest (>90%) in India, Turkey and the Middle East (100% S), Brazil (96.8% S), Canada (95.7% S), Greece (95.5%), Thailand (92.5%), and Portugal (90.0%). Overall rates of resistance to voriconazole among *C. glabrata* isolates were 4.1% in the Asia-Pacific region, 5.4% in Latin America, 5.6% in Europe, and 9.0% in North America (data not shown). Our previous results using BMD MIC testing found resistance rates of 2.2 to 5.4% among blood and normally sterile-site isolates of *C. glabrata* tested in 2001 and 2002 (22). Similar to that seen with *C. albicans*, there was little variation in the susceptibility of *C. glabrata* to voriconazole when stratified by specimen type. Isolates from blood and normally sterile sites were the most susceptible (81%) and genital tract isolates were the least susceptible (70%) to voriconazole (data not shown). The rates of resistance to voriconazole ranged from 2.5% (neonatal intensive-care unit) to 8.2% (hematology/oncology service) across the different hospital locations.

Activities of fluconazole and voriconazole against other opportunistic yeasts and yeast-like fungi. Although they comprise only 3 to 5% of all of the isolates tested in this study, the number of noncandidal yeasts tested against fluconazole and voriconazole exceeds that published in the current literature (1, 3, 21, 26). Lack of standardized methods for testing most of these fungi may be considered problematic; however, the vast majority grew well on the MH-MB agar plates, and the zone diameters were easily determined. For the purposes of this study, we utilized the interpretive breakpoints for *Candida*, and we recognize that they may be adjusted for noncandidal yeasts in the future. Nevertheless, the data generated for these organisms are not dissimilar to those obtained using CLSI BMD MIC methods (1, 3, 21, 26). Using *Cryptococcus neoformans* as an example, the susceptibilities of the isolates shown in Table 6 indicated moderate susceptibility to fluconazole and a very high level of activity for voriconazole. Very similar findings for these two agents using BMD MIC methods were

Downloaded from http://jcm.asm.org/ on February 25, 2014 by Cardiff Univ

TABLE 5. Geographic variation in the in vitro susceptibilities of *C. albicans* and *C. glabrata* to fluconazole and voriconazole as determined by CLSI disk diffusion testing: ARTEMIS DISK Global Surveillance Program, 2001 to 2003[a,b]

Region/country	Antifungal agent	Susceptibility					
		C. albicans			*C. glabrata*		
		n	%S	%R	*n*	%S	%R
Asia-Pacific							
Australia	Fluconazole	207	96.6	2.4	74	58.1	16.2
	Voriconazole	207	99.0	1.0	74	77.0	9.5
China	Fluconazole	1,071	97.1	1.7	307	74.9	13.4
	Voriconazole	1,055	98.7	0.7	307	83.4	8.5
India	Fluconazole	60	70.0	23.3	6	100.0	0.0
	Voriconazole	60	78.3	20.0	6	100.0	0.0
Malaysia	Fluconazole	4,327	99.1	0.3	623	34.0	24.1
	Voriconazole	3,602	99.5	0.0	518	59.5	0.8
South Africa	Fluconazole	3,324	99.4	0.3	355	49.6	21.1
	Voriconazole	3,286	99.9	0.1	333	73.3	3.0
South Korea	Fluconazole	1,928	99.5	0.2	49	83.7	12.2
	Voriconazole	1,848	99.7	0.3	47	83.0	10.6
Taiwan	Fluconazole	1,395	95.8	2.7	352	75.6	12.2
	Voriconazole	1,389	98.5	1.0	349	84.0	4.9
Thailand	Fluconazole	290	97.6	1.4	93	71.0	5.4
	Voriconazole	289	98.6	1.0	93	92.5	2.2
Europe							
Belgium	Fluconazole	1,761	99.5	0.3	224	39.7	42.0
	Voriconazole	1,683	99.7	0.3	216	53.2	18.5
Czech Republic	Fluconazole	2,633	99.6	0.2	429	44.8	27.5
	Voriconazole	2,402	99.9	0.0	412	65.3	8.7
France	Fluconazole	1,149	97.7	1.1	265	77.4	15.1
	Voriconazole	936	99.4	0.4	202	85.1	5.9
Germany	Fluconazole	1,214	94.2	2.8	634	58.5	15.9
	Voriconazole	1,214	98.3	0.8	634	72.6	4.3
Greece	Fluconazole	223	96.4	3.1	66	93.9	4.5
	Voriconazole	223	97.8	2.2	66	95.5	3.0
Hungary	Fluconazole	5,036	98.3	0.7	860	58.4	16.9
	Voriconazole	4,889	99.6	0.3	821	74.2	3.9
Italy	Fluconazole	2,638	97.5	1.8	582	81.3	9.5
	Voriconazole	2,638	99.1	0.5	582	88.5	4.1
Netherlands	Fluconazole	1,815	98.8	1.0	190	76.3	8.9
	Voriconazole	1,815	99.3	0.6	190	87.9	3.7
Norway	Fluconazole	187	98.9	1.1	14	71.4	14.3
	Voriconazole	187	100.0	0.0	14	71.4	14.3
Poland	Fluconazole	476	99.4	0.2	59	86.4	8.5
	Voriconazole	351	99.7	0.3	58	89.7	3.4
Portugal	Fluconazole	754	97.2	2.7	70	87.1	4.3
	Voriconazole	754	98.1	1.7	70	90.0	1.4
Russia	Fluconazole	916	98.8	0.9	64	60.9	20.3
	Voriconazole	906	99.1	0.9	64	79.7	6.3
Slovakia	Fluconazole	1,362	99.0	0.4	155	78.1	4.5
	Voriconazole	1,362	98.6	1.3	155	89.7	1.9
Spain	Fluconazole	2,617	98.5	0.9	321	62.3	24.0
	Voriconazole	2,614	99.5	0.2	321	75.4	7.2
Switzerland	Fluconazole	566	97.9	1.1	144	78.5	9.7
	Voriconazole	565	99.5	0.5	144	83.3	4.2
Turkey	Fluconazole	519	98.3	1.3	17	82.4	11.8
	Voriconazole	475	98.3	1.5	15	100.0	0.0
United Kingdom	Fluconazole	4,340	98.8	0.5	868	68.4	14.2
	Voriconazole	4,055	99.4	0.4	837	77.9	6.0
Middle East							
Israel	Fluconazole	12	100.0	0.0	1	100.0	0.0
	Voriconazole	12	100.0	0.0	1	100.0	0.0
Saudi Arabia	Fluconazole	20	100.0	0.0	2	100.0	0.0
	Voriconazole	20	100.0	0.0	2	100.0	0.0
Latin America							
Argentina	Fluconazole	1,595	97.6	1.4	335	74.0	14.9
	Voriconazole	1,458	99.5	0.0	318	84.9	4.4

Continued on following page

Downloaded from http://jcm.asm.org/ on February 25, 2014 by Cardiff Univ

TABLE 5—*Continued*

Region/country	Antifungal agent	Susceptibility					
		C. albicans			*C. glabrata*		
		n	%S	%R	*n*	%S	%R
Brazil	Fluconazole	1,401	99.6	0.1	410	94.9	3.9
	Voriconazole	1,288	99.8	0.0	405	96.8	1.0
Colombia	Fluconazole	1,315	91.2	6.1	99	75.8	17.2
	Voriconazole	1,263	94.9	4.0	90	80.0	7.8
Ecuador	Fluconazole	1,169	91.6	4.9	33	60.6	27.3
	Voriconazole	1,093	96.4	0.7	33	66.7	12.1
Mexico	Fluconazole	186	98.4	1.1	15	86.7	13.3
	Voriconazole	179	98.3	1.7	15	73.3	6.7
Venezuela	Fluconazole	550	94.4	2.7	48	29.2	60.4
	Voriconazole	525	96.6	2.5	49	32.7	38.8
North America							
Canada	Fluconazole	297	100.0	0.0	117	90.6	5.1
	Voriconazole	297	100.0	0.0	117	95.7	1.7
United States	Fluconazole	2,638	94.8	4.4	1,159	75.9	19.3
	Voriconazole	2,644	97.0	2.2	1,161	82.1	9.7

[a] Isolates obtained from 115 institutions in 35 countries.
[b] Fluconazole and voriconazole disk diffusion testing was performed in accordance with CLSI M44-A. Interpretive breakpoints: S, ≥19 mm (fluconazole), ≥17 mm (voriconazole); R, ≤14 mm (fluconazole), ≤13 mm (voriconazole).

recently reported from our laboratory (26). As noted previously (21), most of these noncandidal yeasts were substantially less susceptible to both fluconazole and voriconazole than *Candida* species. Although voriconazole was more active than fluconazole for each of these different genera, it is notable that less than 80% of *Trichosporon beigelii*/*Trichosporon cutaneum*, *Trichosporon asahi*, and *Rhodotorula* spp. were susceptible to either of these agents. The diverse array of opportunistic yeasts and yeast-like fungi and their variable susceptibilities to these azole antifungals emphasize the need for prompt identification of noncandidal yeasts from clinical material. The flexibility of the CLSI disk diffusion method may well be an advantage in assessing the antifungal susceptibilities of these "emerging" pathogens.

TABLE 6. In vitro susceptibilities of non-*Candida* yeasts to fluconazole and voriconazole as determined by CLSI disk diffusion testing: ARTEMIS DISK Surveillance Program, 2001 to 2003[a]

Species	Susceptibility					
	Fluconazole[b]			Voriconazole[b]		
	n	%S	%R	*n*	%S	%R
Cryptococcus neoformans	1,281	79.1	9.8	1,266	97.2	11.7
Cryptococcus spp.[c]	50	68.0	16.0	50	86.0	10.0
Saccharomyces cerevisiae	413	86.9	6.8	401	94.8	3.2
Trichosporon spp.[d]	291	80.8	11.3	270	92.9	2.6
T. beigelii/*T. cutaneum*	80	73.8	15.0	78	79.5	15.4
T. mucoides	14	100.0	0.0	14	100.0	0.0
T. asahii	6	50.0	33.3	6	66.7	33.3
Rhodotorula spp.[e]	139	30.9	65.5	137	43.1	51.1
Blastoschizomyces capitatus	55	76.4	16.4	55	89.1	5.5
Pichia spp.[f]	27	81.5	11.1	26	100.0	0.0
Hansenula anomala	13	69.2	7.7	13	92.3	7.7

[a] Isolates obtained from 115 institutions in 35 countries.
[b] Fluconazole and voriconazole disk diffusion testing was performed in accordance with CLSI M44-A. Interpretive breakpoints: S, ≥19 mm (fluconazole), ≥17 mm (voriconazole); R, ≤14 mm (fluconazole), ≤13 mm (voriconazole).
[c] *Cryptococcus* species other than *C. neoformans*.
[d] *Trichosporon* species not otherwise identified.
[e] *Rhodotorula* species not otherwise identified.
[f] *Pichia* species not otherwise identified.

Conversion of zone diameters to MICs. In addition to using image analysis technology to measure and record the zones of inhibition surrounding an antifungal disk, the BIOMIC system uses previously developed scatter plots and regression analysis to calculate MICs based on the relationship between the zone diameter and the MIC (Fig. 2). The data in Fig. 2 show the correlation between the MIC and the zone diameter for voriconazole with *Candida* spp. As seen previously with fluconazole (6, 19, 25), an excellent correlation was observed. Based on these data, the voriconazole MICs for *Candida* spp. were calculated and the data were compared to BMD MICs published previously (24) for the same species (Table 7). Although the numbers of isolates tested are considerably different in the two groups, it is readily apparent that the MIC_{50} and MIC_{90} values are very close for each species, as is the percent resistant. Thus, the large amount of qualitative disk diffusion data presented here can be converted to quantitative MIC data for purposes of comparing the activities of fluconazole and voriconazole for individual species (Fig. 3) or potentially for following trends across time. Additional work in this area is warranted.

DISCUSSION

The ARTEMIS Global Antifungal Surveillance Program is the largest and most comprehensive program of its kind and the only one to incorporate many of the features that arguably constitute an "ideal" resistance surveillance program (7–9, 11, 30). It is longitudinal and global, employs standardized methods used for "routine" testing in participating laboratories and for "reference" testing in a central reference laboratory, uses electronic data capture and storage in a central database, and conducts external validation of the data generated by participating laboratories. The current report from the ARTEMIS

Downloaded from http://jcm.asm.org/ on February 25, 2014 by Cardiff Univ

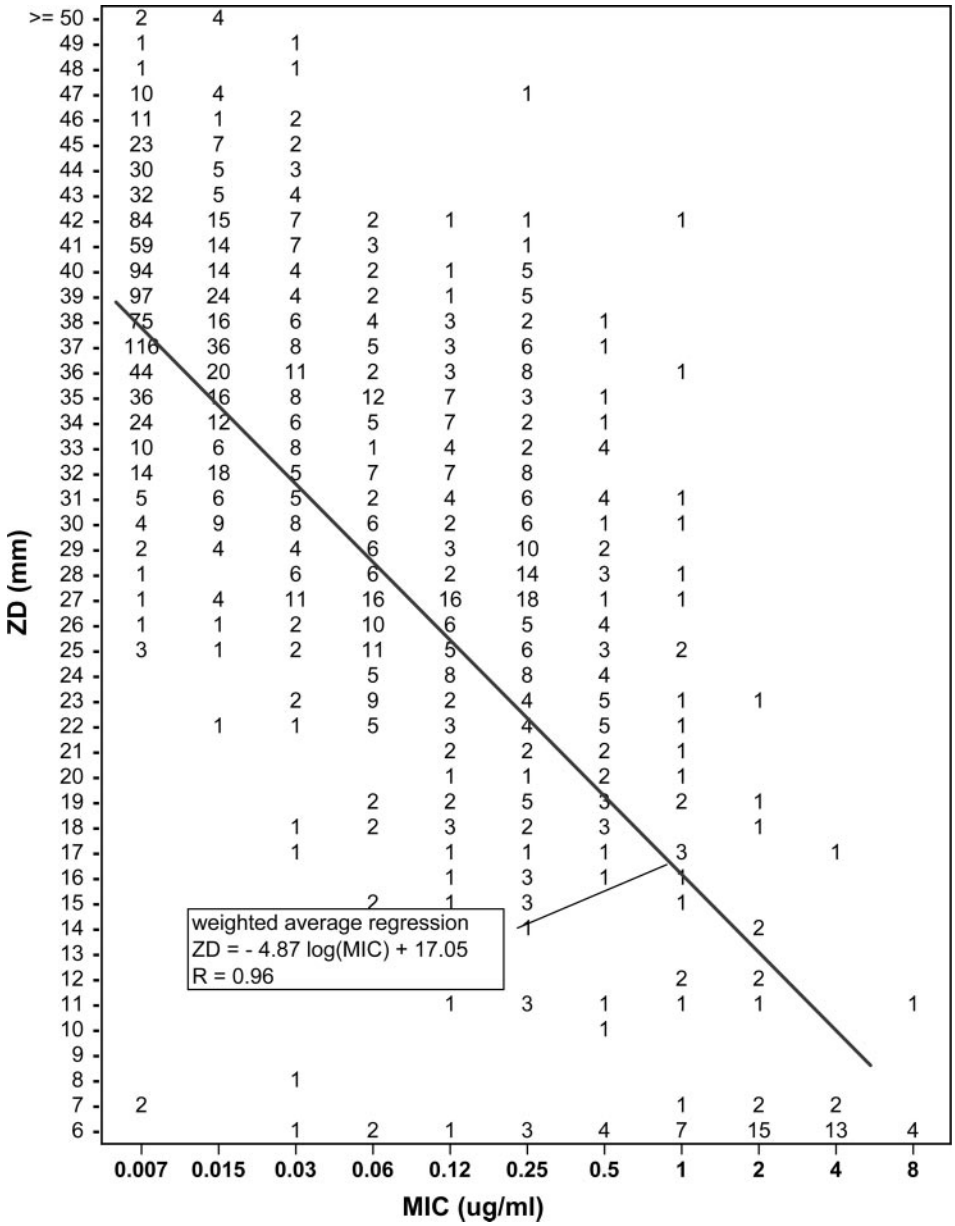

FIG. 2. Correlation of broth microdilution MICs and disk diffusion zone diameters with *Candida* (1,670 isolates) and voriconazole. ZD, zone diameter.

DISK Surveillance Study includes more than 140,000 opportunistic yeast isolates and is by far the largest and most geographically diverse study of antifungal susceptibility and resistance to date (5, 15, 16, 20, 28). Important findings regarding species distribution include a steady decrease in the isolation of *C. albicans* and an increase in the isolation of *C. tropicalis* and *C. parapsilosis*. Although they are still rare, it appears that *C. rugosa*, *C. famata*, *C. inconspicua*, and *C. norvegensis* may be "emerging" in recent years. Among the noncandidal yeasts, *Cryptococcus neoformans*, *Saccharomyces*, *Trichosporon*, and *Rhodotorula* species are prominent and may prove to be important due to their decreased susceptibilities to several antifungal agents (21).

Despite the use of a standard protocol, it is recognized that any surveillance program based on susceptibility tests performed by the participating laboratories needs to include some measure of quality assurance, beyond simple QC testing, in order to provide an independent assessment of laboratory performance and validation of the results generated by the various laboratories (7, 9, 31). One approach to cross-validation that has been suggested is to use centralized testing with high-quality microbiology to confirm the trends in routine data obtained from participating sentinel sites (7–9, 11, 30). Comparison of results obtained for isolates tested in participating laboratories with results obtained for the same organisms tested in a central reference laboratory would accomplish this

Downloaded from http://jcm.asm.org/ on February 25, 2014 by Cardiff Univ

TABLE 7. Voriconazole MICs for *Candida* spp. calculated from disk zone diameter measurements and MIC versus zone diameter regression plots: comparison with ARTEMIS BMD MIC results[a]

Species	Calculated MIC (μg/ml) and %R[b]				ARTEMIS BMD MIC (μg/ml) and %R[b,c]			
	n	50%[d]	90%[d]	%R	*n*	50%[d]	90%[d]	%R[b]
C. albicans	47,584	0.02	0.09	0.7	2,359	0.007	0.015	1.0
C. glabrata	8,719	0.36	2.08	5.8	607	0.25	1.0	4.0
C. parapsilosis	5,233	0.02	0.16	0.8	439	0.015	0.12	1.0
C. tropicalis	5,643	0.11	0.86	2.4	319	0.06	0.12	1.0
C. krusei	1,996	0.24	0.95	1.7	114	0.25	0.5	0.0
C. lusitaniae	445	0.01	0.12	1.6	42	0.007	0.06	0.0
C. pelliculosa	38	0.09	0.26	0.0	16	0.25	0.5	0.0

[a] BMD testing was performed as described in CLSI M27-A2, and disk diffusion testing was performed as described in CLSI M44-A.
[b] %R, percent resistant to voriconazole (MIC ≥ 4 μg/ml).
[c] Data abstracted from Pfaller et al. (24).
[d] 50% and 90%, MIC encompassing 50% and 90% of isolates tested, respectively.

goal (8, 9). This approach has been used to validate and support the epidemiologic relevance of findings from antibacterial surveillance programs (9, 30). Most recently, we have used the same approach to validate fluconazole and voriconazole disk test results generated by laboratories participating in the ARTEMIS Program (25, 27). More than 2,900 isolates of *Candida* obtained from blood and normally sterile-site infections were tested against fluconazole and voriconazole by ARTEMIS participating laboratories (CLSI disk test) and by the central reference laboratory (CLSI disk and BMD MIC tests) (25, 27). Categorical agreement between the reference MIC results and the disk diffusion test results performed in the participant laboratories was 87.4% and 94.1% for fluconazole and voriconazole, respectively (Table 8). A similar level of agreement was seen when the disk test results obtained in the reference laboratory were compared with those from the participant laboratories (references 25 and 27 and data not shown). It was noted that participating laboratories tended to err on the side of calling isolates more resistant than the reference laboratory did; however, the numbers of major and very major discrepancies were quite small (Table 8). This external quality assurance data, coupled with excellent QC performance, ensures the generation of accurate and useful surveillance data in the ARTEMIS DISK Surveillance Program.

The data reported here for the more common species of *Candida* (i.e., *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*) confirm most of the previously published data regarding their susceptibilities to fluconazole and voriconazole (5, 16, 20, 24). The activity of fluconazole remains high against *C. albicans*, *C. parapsilosis*, and *C. tropicalis*, although resistance may be increasing among *C. tropicalis* isolates. Flucon-

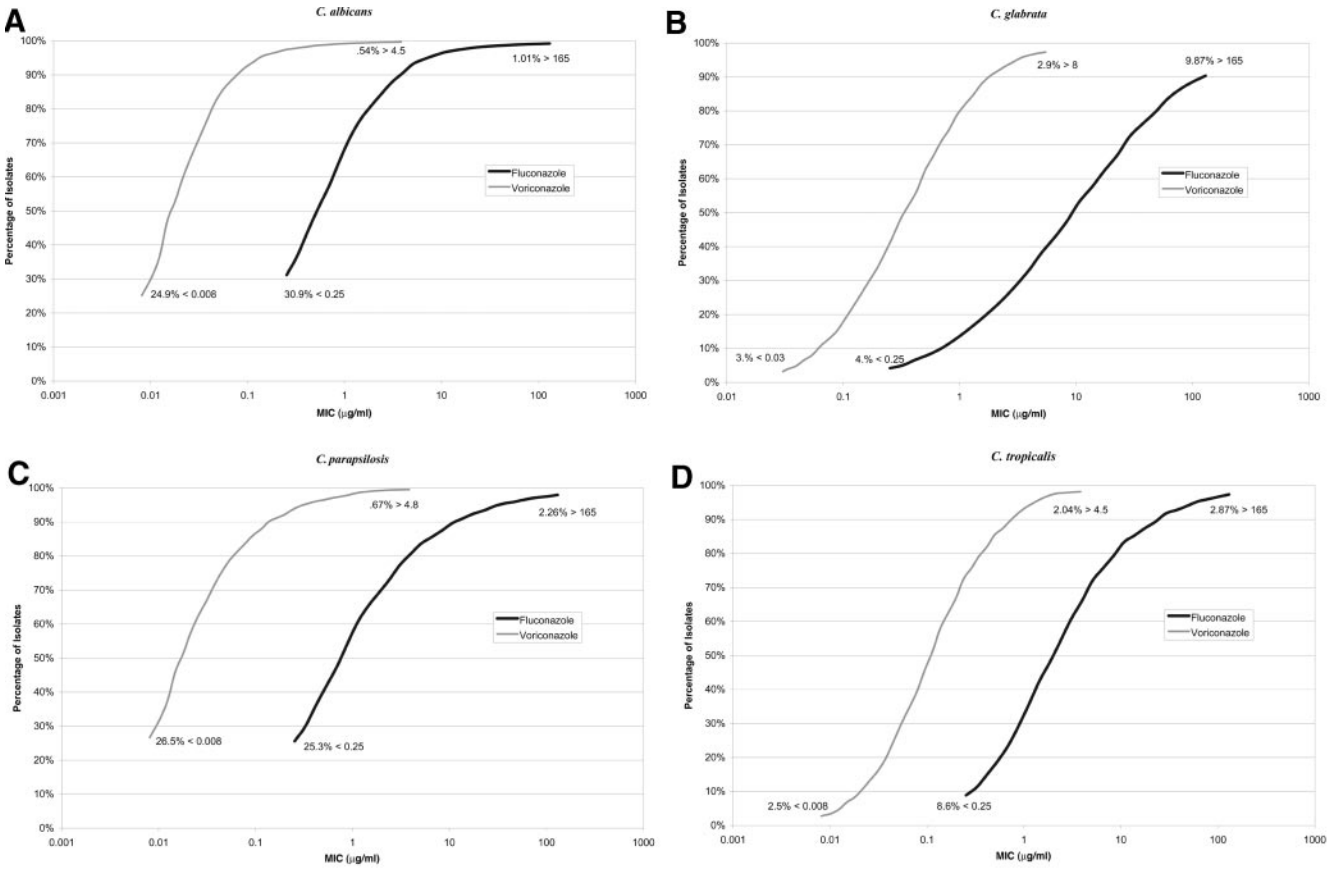

FIG. 3. Cumulative susceptibilities of *Candida* species to fluconazole and voriconazole using calculated MICs: (A) *C. albicans* (47,584 isolates [voriconazole]; 49,991 isolates [fluconazole]); (B) *C. glabrata* (8,719 isolates [voriconazole]; 9,040 isolates [fluconazole]); (C) *C. parapsilosis* (5,233 isolates [voriconazole]; 5,539 isolates [fluconazole]); (D) *C. tropicalis* (5,643 isolates [voriconazole]; 5,959 isolates [fluconazole]).

Downloaded from http://jcm.asm.org/ on February 25, 2014 by Cardiff Univ

TABLE 8. Interpretive agreement between results of fluconazole and voriconazole disk diffusion tests and standard 48-h BMD[a,b]

Antifungal agent (no. tested)	Test method[e]	% of results[c]			% Agreement[f]	% Errors[d]		
		S	SDD	R		VME	ME	M
Fluconazole (2,949)	Ref.-MIC	91.6	6.7	1.7				
	Part.-disk	87.9	4.5	7.6	87.4	0.2	3.3	9.1
Voriconazole (2,934)	Ref.-MIC	99.4	0.2	0.4				
	Part.-disk	94.0	2.1	3.9	94.1	0.1	3.4	2.4

[a] Fluconazole and voriconazole disk diffusion testing was performed according to CLSI M44-A, and BMD MIC testing was performed according to CLSI M27-A2.

[b] Data compiled from references 25 and 27.

[c] MIC and disk zone interpretive categories were as follows: fluconazole, S, MIC ≤ 8 μg/ml (≥19 mm), SDD, MIC 16 to 32 μg/ml (15 to 18 mm), R, MIC ≥ 64 μg/ml (≤14 mm); voriconazole, S, MIC ≤ 1 μg/ml (≥17 mm), SDD, MIC = 2 μg/ml (14 to 16 mm), R, MIC ≥ 4 μg/ml (≤13 mm).

[d] % Errors: VME, very major error; ME, major error; M, minor error.

[e] Ref.-MIC, MIC testing performed by ARTEMIS reference laboratory; Part.-disk, disk diffusion testing performed by ARTEMIS participants.

[f] % Agreement, percent categorical agreement between disk diffusion and MIC test results.

azole resistance was considerable among isolates of *C. glabrata*, although the extent of resistance varied widely throughout the world. Fortunately, voriconazole remains quite active against this species. It is notable, however, that resistance to voriconazole has increased among *C. glabrata* isolates over the 3-year period of this study and was quite high in certain countries, such as Belgium (18.5%) and Venezuela (38.8%), where fluconazole resistance was also widespread. Again, our previous studies have shown that compared to reference laboratory testing of *C. glabrata* by MIC and disk methods, the fluconazole and voriconazole disk test results reported by ARTEMIS participating sites tended to overestimate resistance (25, 27). Thus, the rates of resistance to fluconazole and voriconazole reported in this study for *C. glabrata* may be somewhat higher than previously reported in the literature. Nevertheless, the geographical and temporal comparisons and differences remain important.

The ARTEMIS database is most valuable as it pertains to the less common species of *Candida* (Table 2). The excellent activity of voriconazole against *C. krusei* was confirmed by the results from almost 2,000 clinical isolates. Similarly, the high levels of activity of both azoles against *C. lusitaniae*, *C. kefyr*, *C. dubliniensis*, and *C. pelliculosa* were clearly demonstrated, confirming previous results based on comparatively few isolates (21, 23). Equally important was the demonstration of generally poor activities of fluconazole against *C. guilliermondii*, *C. rugosa*, *C. famata*, *C. inconspicua*, *C. norvegensis*, *C. lipolytica*, and *C. zeylanoides*. In most instances, these findings confirm what can only be called preliminary observations (21); however, for some of these species, these constitute new data and serve to underscore the imperative to identify *Candida* to the species level. Although voriconazole is active against the vast majority of these rare species, it is notable that decreased susceptibility to this agent, as well as to fluconazole, is seen with *C. rugosa*, *C. lipolytica*, and *C. zeylanoides*. These findings are especially important for *C. rugosa*, as the frequency of isolation of this species appears to be increasing over time (Table 1), it has been shown to cause clusters of nosocomial infection that are poorly responsive to amphotericin B (2, 4), and it was previously considered highly susceptible to voriconazole based on results for less than 20 clinical isolates (21).

As is the case for the less common *Candida* species, new information for noncandidal yeasts is provided by this data set. Although the antifungal susceptibility profile of *Cryptococcus neoformans* is well known (1, 26), much less is known of the susceptibilities of *Saccharomyces*, *Trichosporon*, *Rhodotorula*, and *Blastoschizomyces* species to fluconazole and voriconazole (3, 21, 33). The results presented in Table 6 indicate that most of these opportunistic yeasts have decreased susceptibility to fluconazole, and although voriconazole is clearly more active than fluconazole, decreased susceptibility to that agent is also seen with certain species of *Trichosporon* and with *Rhodotorula* spp. The fact that these yeast-like fungi are also nonsusceptible to the echinocandins (they lack β-1,3-D-glucan) and respond variably to amphotericin B highlights the potential for their emergence as difficult-to-treat mycotic pathogens in the future (21, 33).

Finally, the ability of the BIOMIC software to convert disk diffusion zone diameters to MICs is an important feature of the ARTEMIS surveillance program, providing quantitative data that will be valuable in trend analysis. We have extended the previous work of Hazen et al. (6) and have shown that the voriconazole MICs calculated from the disk diffusion data for *Candida* spp. compare very favorably to those obtained by BMD MIC testing performed centrally (Table 7).

In summary, we present a tremendous volume of data describing temporal and geographic trends in the isolation and azole susceptibilities of opportunistic yeast pathogens. The data point to the strength of azole coverage for most of these organisms but also highlight several areas where resistance may be progressing and/or previously rare species may be "emerging." The strength of the ARTEMIS Global Surveillance Program is in the overall design, incorporating standardized test methods, "routine" and centralized testing of isolates, and a broad international network of study sites providing consistent data over time. The continued efforts of this surveillance program will provide data on pathogen frequency and antifungal susceptibility on a global scale.

ACKNOWLEDGMENTS

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

The ARTEMIS DISK Surveillance Program is supported by grants from Pfizer.

We express our appreciation to all ARTEMIS participants. Participants contributing to this study included Jorge Finquelievich, Buenos Aires University, and Nora Tiraboschi, Hospital Escuela Gral., Buenos Aires, Argentina; David Ellis, Women's and Children's Hospital, North Adelaide, Australia; Dominique Frameree, CHU de Jumet, Jumet, Annemarie van den Abeele, St Lucas Campus Heilige Familie, Ghent, and Jean-Marc Senterre, Hôpital de la Citadelle, Liege, Belgium; Arnaldo Colombo, Escola Paulista de Medicina, Sao Paulo, Brazil; Robert Rennie, University of Alberta Hospital, Edmonton, and Steve Sanche, Royal University Hospital, Saskatoon, Canada; Bijie Hu, Zhong Shan Hospital, Shanghai, Yingchun Xu, Peking Union Medical College Hospital, Beijing, Yingyuan Zhang, Hua Shan Hospital, Shanghai, and Nan Shan Zhong, Guangzhou Institute of Respiratory Disease, Guangzhou, China; Pilar Rivas, Inst. Nacional de Cancerología, Bogota, Angela Restrepo and Catalina Bedout, CIB, Medellin, and Ricardo Vega and Matilde Mendez, Hospital Militar Central, Bogota, Colombia; Nada Mallatova, Hospital Ceske Budejovice, Ceske, and Eva Chmelarova, Krajska Hygienicka Stanice, Ostrava, Czech Republic; Julio Ayabaca, Hospital FF. AA HG1, Quito, and Jeannete Zurita, Hospital Vozandes, Quito, Ecuador; M.

Downloaded from http://jcm.asm.org/ on February 25, 2014 by Cardiff Univ

Mallie, Faculte de Pharmacie, Montpellier, and E. Candolfi, Institut de Parasitologie, Strasbourg, France; W. Fegeler, Universitaet Muenster, Münster, A. Haase, RWTH Aachen, Aachen, G. Rodloff, Inst. F. Med. Mikrobiologie, Leipzig, W. Bar, Carl-Thiem Klinikum, Cottbus, and V. Czaika, Humaine Kliniken, Bad Saarow, Germany; George Petrikos, Laikon General Hospital, Athens, Greece; Erzsébet Puskás, BAZ County Institute, Miskolc, Ilona Doczi, University of Szeged, Szeged, Mestyan Gyula, Medical University of Pecs, Pecs, and Radka Nikolova, Szt Laszlo Hospital, Budapest, Hungary; Uma Banerjee, All India Institute of Medical Sciences, New Delhi, India; Nathan Keller, Sheba Medical Center, Tel Hashomer, Israel; Vivian Tullio, Università degli Studi di Torino, Turin, Gian Carlo Schito, University of Genoa, Genoa, Giacomo Fortina, Ospedale di Novara, Novara, Gian Piero Testore, Univerrsita di Roma Tor Vergata, Rome, Domenico D'Antonio, Pescara Civil Hospital, Pescara, Giorgio Scalise, Instituto di Malattie Infettive, Ancona, Pietro Martino, Dept. di Biotechnologie, Rome, and Graziana Manno, Università di Genova, Genova, Italy; Kee Peng, University Malaya, Kuala Lumpur, Malaysia; Celia Alpuche and Jose Santos, Hospital General de Mexico, Mexico City, Eduardo Rodriguez Noriega, Universidad de Guadalajara, Guadalajara, and Mussaret Zaidi, Hospital General O'Horan, Merida, Mexico; Jacques F. G. M. Meis, Canisius Wilhemina Hospital, Nijmegen, The Netherlands; Egil Lingaas, Rikshospitalet, Oslo, Norway; Danuta Dzierzanowska, Children's Memorial Health Institute, Warsaw, and Waclaw Pawliszyn, Pracownia Bakteriologii, Cracow, Poland; Mariada Luz Martins, Inst. de Higiene e Medicina Tropical, Lisbon, Luis Albuquerque, Centro Hospitalar de Coimbra, Coimbra, Laura Rosado, Instituto Nacional de Saude, Lisbon, Rosa Velho, Hosp. da Universidade de Coimbra, Coimbra, and Jose Amorim, Hospital de Santo Antonio, Porto, Portugal; Vera N. Ilina, Novosibirsk Regional Hospital, Novosibirsk, Olga I. Kretchikova, Institute of Antimicrobial Chemotherapy, Smolensk, Galina A. Klyasova, Hematology Research Center, Moscow, Sophia M. Rozanova, City Clinical Hospital No 40, Ekaterinburg, Irina G. Multykh, Territory Center of Laboratory Diagnostics, Krasnodar, Nikolay N. Klimko, Medical Mycology Research Institute, St. Petersburg, Elena D. Agapova, Irkutsk Regional Children's Hospital, Irkutsk, and Natalya V. Dmitrieva, Oncology Research Center, Moscow, Russia; Abdul Mohsen Al-Rasheed, Riyadh Armed Forces Hospital, Riyadh, Saudi Arabia; Jan Trupl, National Cancer Center, Leon Langsadl, NUTaRCH, Alena Vaculikova, Derer University Hospital, and Hupkova Helena, St. Cyril and Metod Hospital, Bratislava, Slovak Republic; Denise Roditi, Groote Schuur Hospital, Cape Town, Anwar Hoosen, GaRankuwa Hospital, Medunsa, H. H. Crewe-Brown, Baragwanath Hospital, Johannesburg, M. N. Janse van Rensburg, Pelanomi Hospital, UOFS, Bloemfontein, and Adriano Duse, Johannesburg General Hospital, Johannesburg, South Africa; Kyungwon Lee, Yonsei University College of Medicine, and Mi-Na Kim, Asan Medical Center, Seoul, South Korea; A. del Palacio, Hospital 12 De Octobre, and Aurora Sanchez-Sousa, Hospital Ramon y Cajal, Madrid, Spain; Jacques Bille, Institute of Microbiology CHUV, Lausanne, and K. Muhlethaler, Universitat Bern, Bern, Switzerland; Shan-Chwen Chang, National Taiwan University Hospital, Taipei, and Jen-Hsien Wang, China Medical College Hospital, Taichung, Taiwan; Malai Vorachit, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; Deniz Gur, Hacettepe University Children's Hospital, Ankara, and Volkan Korten, Marmara Medical School Hospital, Istanbul, Turkey; John Paul, Royal Sussex County Hospital, Brighton, Brian Jones, Glasgow Royal Infirmary, Glasgow, F. Kate Gould, Freeman Hospital, Newcastle, Chris Kibbler, Royal Free Hospital, London, Nigel Weightman, Friarage Hospital, Northallerton, Ian M. Gould, Aberdeen Royal Hospital, Aberdeen, Ruth Ashbee, General Infirmary, P.H.L. S, Leeds, and Rosemarie Barnes, University of Wales College of Medicine, Cardiff, United Kingdom; Jose Vazquez, Harper Hospital, Wayne State University, Detroit, Michigan; Ed Chan, Mt. Sinai Medical Center, New York, and Davise Larone, Cornell Medical Center NYPH, Ithaca, N.Y.; Ellen Jo Baron, Stanford Hospital and Clinics, Stanford, Calif.; Mahmoud A. Ghannoum, University Hospitals of Cleveland, Cleveland, Ohio; Mike Rinaldi, University of Texas Health Science Center, San Antonio, Texas; Kevin Hazen, University of Virginia Health Systems, Charlottesville, Va.; Elyse Foraker, Christiana Care, Wilmington, Del.; and Heidi Reyes, Gen del Este Domingo Luciani, and Axel Santiago, Universitario de Caracas, Caracas, Venezuela.

REFERENCES

1. **Brandt, M. E., M. A. Pfaller, R. A. Hajjeh, R. H. Hamill, P. G. Pappas, A. L. Reingold, D. Rimland, and D. W. Warnock for the Cryptococcal Disease Active Surveillance Group.** 2001. Trends in antifungal drug susceptibility of *Cryptococcus neoformans* isolates in the United States: 1992 to 1994 and 1996 to 1998. Antimicrob. Agents Chemother. **45:**3065–3069.

1a. **CLSI.** 2005. Minutes of the CLSI Antifungal Subcommittee Meeting, 2005. CLSI, Wayne, Pa.

2. **Colombo, A. L., S. A. Melo, R. F. C. Rosas, R. Salomao, M. Briones, R. J. Hollis, S. A. Messer, and M. A. Pfaller.** 2003. Outbreak of *Candida rugosa* candidemia: an emerging pathogen that may be refractory to amphotericin B therapy. Diagn. Microbiol. Infect. Dis. **46:**253–257.

3. **Diekema, D. J., B. Petroelje, S. A. Messer, R. J. Hollis, and M. A. Pfaller.** 2005. Activities of available and investigational antifungal agents against *Rhodotorula* species. J. Clin. Microbiol. **43:**476–478.

4. **Dube, 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.

5. **Hajjeh, R. A., A. N. Sofair, L. H. Harrison, G. M. Lyon, B. A. Arthington-Skaggs, S. A. Mirza, M. Phelan, J. Morgan, W. Lee-Yang, M. A. Ciblak, L. E. Benjamin, L. Thompson Sanza, S. Huie, S. F. Yeo, M. E. Brandt, and D. W. Warnock.** 2004. Incidence of bloodstream infections due to *Candida* species and in vitro susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program. J. Clin. Microbiol. **42:**1519–1527.

6. **Hazen, K. C., E. J. Baron, A. L. Colombo, C. Girmenia, A. Sanchez-Sousa, A. del Palacio, C. de Bedout, D. L. Gibbs, and The Global Antifungal Surveillance Group.** 2003. Comparison of the susceptibilities of *Candida* spp. to fluconazole and voriconazole in a 4-year global evaluation using disk diffusion. J. Clin. Microbiol. **41:**5623–5632.

7. **Kahlmeter, G., and D. F. J. Brown.** 2002. Resistance surveillance studies—comparability of results and quality assurance of methods. J. Antimicrob. Chemother. **50:**775–777.

8. **Livermore, D. M., E. J. Threlfall, M. H. Reacher, A. P. Johnson, D. James, T. Cheasty, A. Shah, F. Warburton, A. V. Swan, J. Skinner, A. Grahm, and D. C. E. Speller.** 2000. Are routine test data suitable for the surveillance of resistance? Resistance rates amongst *Escherchia coli* from blood and CSF from 1991–1997, as assessed by routine and centralized testing. J. Antimicrob. Chemother. **45:**205–211.

9. **Livermore, D. M., A. P. Macgowan, and M. C. J. Wale.** 2005. Surveillance of antimicrobial resistance: centralized surveys to validate routine data offer a practical approach. Br. Med. J. **317:**614–615.

10. **Luzzati, R., G. Amalfitano, L. Lazzarini, F. Soldori, S. Bellino, M. Solbiatic, M. C. Danzi, S. Vento, G. Todeschini, C. Vivenza, and E. Concia.** 2000. Nosocomial candidemia in non-neutrophenic patients at an Italian tertiary care hospital. Eur. J. Clin. Microbiol. Infect. Dis. **19:**602–607.

11. **Magee, J. T., M. L. Heginbothom, and B. W. Mason.** 2005. Finding a strategy: the case for co-operative research on resistance epidemiology. J. Antimicrob. Chemother. **55:**628–633.

12. **Meis, J., M. Petrou, J. Bille, D. Ellis, D. Gibbs, and the Global Antifungal Surveillance Group.** 2000. A global evaluation of the susceptibility of *Candida* species to fluconazole by disk diffusion. Diagn. Microbiol. Infect. Dis. **36:**215–223.

13. **National Committee for Clinical Laboratory Standards.** 2002. Reference method for broth dilution testing of yeasts. Approved standard, 2nd ed. M27-A2. National Committee for Clinical Laboratory Standards, Wayne, Pa.

14. **National Committee for Clinical Laboratory Standards.** 2004. Method for antifungal disk diffusion susceptibility testing of yeasts: approved guidance M44-A. National Committee for Clinical Laboratory Standards, Wayne, Pa.

15. **Nguyen, M. H., J. E. Peacock, Jr., A. J. Morris, D. C. Tanner, M. L. Nguyen, D. R. Snydman, M. M. Wagener, M. G. Rinaldi, and V. L. Yu.** 1996. The changing face of candidemia: emergence of non-*Candida albicans* species and antifungal resistance. Am. J. Med. **100:**617–623.

16. **Ostrosky-Zeichner, L., J. H. Rex, P. G. Pappas, R. J. Hamill, R. A. Larsen, H. W. Horowitz, W. G. Powderly, N. Hyslop, C. A. Kauffman, J. Cleary, J. E. Mangino, and J. Lee.** 2003. Antifungal susceptibility survey of 2,000 bloodstream *Candida* isolates in the United States. Antimicrob. Agents Chemother. **47:**3149–3154.

17. **Pfaller, M. A., and D. J. Diekema.** 2002. Role of sentinel surveillance of candidemia: trends in species distribution and antifungal susceptibility. J. Clin. Microbiol. **40:**3551–3557.

18. **Pfaller, M. A., D. J. Diekema, S. A. Messer, L. Boyken, R. J. Hollis, R. N. Jones, and the International Fungal Surveillance Participant Group.** 2003. In vitro activities of voriconazole, posaconazole, and four licensed systemic antifungal agents against *Candida* species infrequently isolated from blood. J. Clin. Microbiol. **41:**28–83.

19. **Pfaller, M. A., D. J. Diekema, S. A. Messer, L. Boyken, and R. J. Hollis.** 2003. Activites of fluconazole and voriconazole against 1,586 recent clinical isolates of *Candida* species determined by broth microdilution, disk diffusion, and Etest methods: report from the ARTEMIS Global Antifungal Susceptibility Program, 2001. J. Clin. Microbiol. **41:**1440–1446.

Downloaded from http://jcm.asm.org/ on February 25, 2014 by Cardiff Univ

20. **Pfaller, M. A., and D. J. Diekema.** 2004. Twelve years of fluconazole in clinical practice: global trends in species distribution and fluconazole susceptibility of bloodstream isolates of *Candida*. Clin. Microbiol. Infect. **10**(Suppl. 1)**:**11–23.
21. **Pfaller, M. A., and D. J. Diekema.** 2004. Rare and emerging opportunistic fungal pathogens: concern for resistance beyond *Candida albicans* and *Aspergillus fumigatus*. J. Clin. Microbiol. **42:**4419–4431.
22. **Pfaller, M. A., S. A. Messer, L. Boyken, S. Tendolkar, R. J. Hollis, and D. J. Diekema.** 2004. Geographic variation in the susceptibilities of invasive isolates of *Candida glabrata* to seven systemically active antifungal agents: a global assessment from the ARTEMIS Antifungal Surveillance Program conducted in 2001 and 2002. J. Clin. Microbiol. **42:**3142–3146.
23. **Pfaller, M. A., D. J. Diekema, S. A. Messer, L. Boyken, R. J. Hollis, and R. N. Jones.** 2004. In vitro susceptibilities of rare *Candida* bloodstream isolates to ravuconazole and three comparative antifungal agents. Diagn. Microbiol. Infect. Dis. **48:**101–105.
24. **Pfaller, M. A., S. A. Messer, L. Boyken, R. J. Hollis, C. Rice, S. Tendolkar, and D. J. Diekema.** 2004. In vitro activities of voriconazole, posaconazole, and fluconazole against 4,169 clinical isolates of *Candida* spp. and *Cryptococcus neoformans* collected during 2001 and 2002 in the ARTEMIS global antifungal surveillance program. Diagn. Microbiol. Infect. Dis. **48:**201–205.
25. **Pfaller, M. A., K. C. Hazen, S. A. Messer, L. Boyken, S. Tendolkar, R. J. Hollis, and D. J. Diekema.** 2004. Comparison of results of fluconazole disk diffusion testing for *Candida* species with results from a central reference laboratory in the ARTEMIS Global Antifungal Surveillance Program. J. Clin. Microbiol. **42:**3607–3612.
26. **Pfaller, M. A., S. A. Messer, L. Boyken, C. Rice, S. Tendolkar, R. J. Hollis, G. V. Doern, and D. J. Diekema.** 2005. Global trends in the antifungal susceptibility of *Cryptococcus neoformans* (1990–2004). J. Clin. Microbiol. **43:**2163–2167.
27. **Pfaller, M. A., L. Boyken, S. A. Messer, S. Tendolkar, R. J. Hollis, and D. J. Diekema.** 2005. Comparison of results of voriconazole disk diffusion testing for *Candida* species with results from a central reference laboratory in the ARTEMIS Global Antifungal Surveillance Program. J. Clin. Microbiol. **43:** 5208–5213.
28. **Rees, J. R., R. W. Pinner, R. A. Hajjeh, M. R. Brandt, and A. L. Reingold.** 1998. The epidemiological features of invasive mycotic infection in the San Francisco Bay Area, 1992–1993: results of a population-based laboratory active surveillance. Clin. Infect. Dis. **27:**1138–1147.
29. **Richet, H., P. Roux, C. Des Champs, Y. Esnault, A. Andremont and the French Candidemia Study Group.** 2002. Candidemia in French hospitals: incidence rates and characteristics. Clin. Microbiol. Infect. **8:**405–412.
30. **Stelling, J. M., K. Travers, R. N. Jones, P. J. Turner, T. F. O'Brien, and S. B. Levy.** 2005. Integrating *Escherichia coli* antimicrobiol susceptibility data from multiple surveillance programs. Emerg. Infect. Dis. **11:**873–882.
31. **Tenover, F. C., M. J. Mohammed, J. Stelling, T. O'Brien, and R. Williams.** 2001. Ability of laboratories to detect emerging antimicrobial resistance: proficiency testing and quality control results from the World Health Organizations' external quality assurance system for antimicrobial susceptibility testing. J. Clin. Microbiol. **39:**241–250.
32. **Tortorano, A. M., J. Peman, H. Bernhardt, L. Klingspor, C. C. Kibbler, O. Faure, E. Biraghi, E. Canton, K. Zimmerman, S. Seaton, R. Grillot, and the ECMM Working Group on Candidaemia.** 2004. Epidemiology of candidaemia in Europe: results of 28-month European Confederation of Medical Mycology (ECMM) hospital-based surveillance study. Eur. J. Clin. Microbiol. Infect. Dis. **23:**317–322.
33. **Walsh, T. J., A. Groll, J. Hiemenz, R. Flemming, E. Roilides, and E. Anaissie.** 2004. Infections due to emerging and uncommon medically important fungal pathogens. Clin. Microbiol. Infect. **10**(Suppl. 1)**:**48–66.

Downloaded from http://jcm.asm.org/ on February 25, 2014 by Cardiff Univ