

Geographic Variation in the Frequency of Isolation and Fluconazole (FLU) and Voriconazole (VOR) Susceptibilities of *Candida glabrata*: an Assessment from the ARTEMIS DISK Global Antifungal Surveillance Program

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ABSTRACT

Background: Geographic differences in frequency and azole-resistance among *C. glabrata* (CGLA) may impact empirical antifungal therapy choice. We examined geographic variation in isolation and azole susceptibility of CGLA.

Methods: We examined 23,305 clinical isolates of CGLA during ARTEMIS DISK global surveillance. Susceptibility testing to FLU and VOR was assessed by disk diffusion, and the results grouped by geographic location: North America (NA; 2,470 isolates), Latin America (LA; 2,039), Europe (EU; 12,439), Africa and Middle East (AME; 720) and Asia-Pacific (AP; 5,629).

Results: Overall, CGLA accounted for 11.6% of 201,653 isolates of *Candida*, and varied as a proportion of all *Candida* isolated from 7.4% in LA to 21.1% in NA. Decreased susceptibility (S) to FLU was observed in all geographic regions and ranged from 62.8% in AME to 76.7% in LA. Considerable variation in FLU susceptibility was observed within each region. AP (range 50%–100% S), AME (48%–86.9%), EU (44.8%–88%), LA (43%–92%), and NA (74.5%–91.6%) VOR was more active than FLU (range 82.3–84.2% S) with similar regional variation. Among 22 sentinel sites participating in ARTEMIS from 1997 through 2007 (85,140 total isolates), 8,163 CGLA, the frequency of CGLA isolation increased in 14 sites and the frequency of FLU resistance (R) increased in 11 sites over the decade. The sites with the highest cumulative rates of FLU R were in Poland (22% R), the Czech Republic (27% R), Venezuela (27% R), and Greece (33% R). CGLA was most often isolated from blood, normally sterile body fluids and urine.

Conclusions: There was substantial geographic and institutional variation in both frequency of isolation and azole resistance among CGLA. Prompt species identification and FLU susceptibility testing is necessary to optimize therapy of invasive candidiasis.

INTRODUCTION

Fluconazole is a mainstay for therapy for invasive candidiasis (IC) (8, 9, 36, 41, 43, 46, 52, 61, 67, 72). Unfortunately, the use of this agent has been impacted by the emergence of *Candida* spp. with reduced susceptibility to this agent (2, 4, 5, 7, 10, 21, 33, 38, 40, 43, 45, 66). Foremost among those species of *Candida* with decreased susceptibility to fluconazole is *Candida glabrata* (1, 2, 4, 21, 24, 28, 30, 34, 38, 45, 47, 48, 52, 62, 71, 72). The Infectious Diseases Society of America (IDSA) guidelines for the treatment of IC suggest that although infection due to *C. glabrata* may be treated with fluconazole using a dosing regimen of 0–12 mg/kg/d, such therapy should be guided by antifungal susceptibility whenever possible given the variable frequency of resistance seen in this species (42, 43). Although the echinocandins may provide reliable empirical coverage of *C. glabrata* (55), such agents are much more expensive than fluconazole and de-escalation from initial echinocandin coverage to the more economical fluconazole is encouraged whenever possible (16, 31, 43).

Several studies have attempted to identify clinical parameters that would allow clinicians to identify those patients who are likely to become infected with *C. glabrata* versus *C. albicans* as a means of providing appropriate early therapy (13, 20, 23, 29, 33, 44, 65). Unfortunately, no consensus has come of such studies and thus the most common recommendation is that early empiric therapy be guided by the local epidemiology concerning the frequency of *C. glabrata* as a cause of IC and the institutional antifungal susceptibility profile of this species (2, 16, 31, 33, 37, 43, 44, 61, 72).

Previously we have reported broad geographic trends in the isolation of *C. glabrata* from clinical specimens and the accompanying rates of fluconazole resistance worldwide (47, 48). We now update this information using ARTEMIS Antifungal Surveillance Program data for 2001 through 2007. In addition to broad trends for geographic regions, we will examine trends for 22 institutions that have provided data for each of the seven years of study. The latter analysis is to emphasize the importance of local versus regional epidemiological data.

MATERIALS and METHODS

Organisms and study sites. A total of 201,653 isolates of *Candida* spp., including 23,305 isolates of *C. glabrata*, from 133 different medical centers in the Asia-Pacific region (21 sites), Africa and the Middle East (8 sites), Europe (76 sites), Latin America (15 sites) and North America (13 sites) were and were collected and tested against fluconazole and voriconazole between 2001 and 2007. All *Candida* spp. considered pathogens by the local site investigator from all body sites (e.g. blood, normally sterile body fluids (NSBF), deep tissue biopsy, genital tract, gastrointestinal tract, respiratory tract, skin and soft tissue) were tested. Data for *C. glabrata* were stratified by year of isolation, geographic region, and specimen type. In addition, a group of 22 medical centers that contributed data for each of the seven years (84,140 total isolates), 8,163 *C. glabrata* isolates were analyzed separately in order to determine trends within individual institutions. *Candida* spp. considered by the local site investigator to be colonizers, that is, not associated with clinical infection, were excluded, as were duplicate isolates (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 (Pfaller et al. 2007).

Susceptibility test methods. Disk diffusion testing of fluconazole and voriconazole was performed as described previously (54) and in the Clinical and Laboratory Standards Institute (14) document M44-A (CLSI, 2004). Agar plates (90-, 100-, or 150-mm diameter) containing Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg of methylene blue per ml 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 inoculated plates, and the plates were incubated in air at 35–37 degrees Celsius for 18–24 hours. Zone diameter endpoints were read at 80% growth inhibition by using a BIOMEC i read analysis plate reader system (Giles Scientific, Santa Barbara, CA) (54).

The interpretive criteria for fluconazole and voriconazole disk diffusion tests were those of the CLSI (15, 50, 51): susceptibility (S), zone diameters of ≥ 19 mm (fluconazole) and ≥ 17 mm (voriconazole); susceptible dose dependent (S-DD), zone diameters of 15–18 mm (fluconazole) and 11–16 mm (voriconazole); and resistant (R), zone diameters of ≤ 14 mm (fluconazole) and ≤ 13 mm (voriconazole). The corresponding MIC breakpoints (15, 50, 51) are as follows: S, MICs of ≤ 8 µg (fluconazole) and ≤ 1 µg (voriconazole); S-DD, MICs of 16–32 µg (fluconazole) and 2 µg (voriconazole); and R, MICs of ≥ 64 µg (fluconazole) and ≥ 4 µg (voriconazole).

Quality control (QC). QC was performed in accordance with CLSI document M44-S2 (15) by using *C. albicans* ATCC 90029 and *C. parapsilosis* ATCC 22019. A total of 15,413 and 14,987 QC results were obtained for fluconazole and voriconazole, respectively, more than 90% of which were within the acceptable limits. **Analysis of results.** All disk zone diameter results were read by electronic image analysis and interpreted and recorded with the BIOMEC plate reader system (Giles). Test results were sent by e-mail to Giles Scientific for electronics. The zone diameter, susceptibility category (S, S-DD, or R), and QC results were all recorded electronically. Patient and doctor names, duplicate test results (same patient, same species, and same biotype results), and uncontrolled results were automatically eliminated by the BIOMEC system prior to analysis. In the present study, the fluconazole and voriconazole S, S-DD, and R results for *C. glabrata* were stratified by year of collection, geographic region, and clinical specimen type. Temporal trends in fluconazole resistance were examined for 22 individual medical centers for the entire 7-year period of study.

Table 1. Variation in frequency of *C. glabrata* by geographic region

Region	Total no. of <i>Candida</i> species isolates	Total no. (%) of <i>C. glabrata</i> isolates
	N	%
Asia-Pacific	44,674	5,629 (12.6)
Africa and Middle East	8,259	728 (8.8)
Europe	109,643	12,439 (11.3)
Latin America	27,395	2,039 (7.4)
North America	11,682	2,470 (21.1)
Total	201,653	23,305 (11.6)

*Data were obtained from the ARTEMIS DISK Global Antifungal Surveillance Program (2001 to 2007). Isolates represent all incident isolates from all sites of infection in 133 institutions.

Table 2. Geographic variation in susceptibility of *C. glabrata* to fluconazole and voriconazole

Region or country	Fluconazole			Voriconazole		
	N	S	SDD	N	S	SDD
Asia-Pacific	5,629	66.0	21.0	13.0	53.5	82.3
Australia	374	67.1	19.0	13.9	37.4	83.4
China	2,411	70.0	15.4	14.5	24.09	81.9
Hong Kong	13	100.0	0.0	14	100.0	0.0
India	26	92.3	3.8	3.8	26	92.3
Indonesia	4	50.0	25.0	4	100.0	0.0
Malaysia	1,874	52.0	34.4	13.6	17.69	78.0
Singapore	19	100.0	0.0	19	89.5	10.5
South Korea	217	89.4	5.3	5.5	21.2	91.5
Taiwan	383	80.3	9.9	9.8	58.0	80.0
Thailand	108	74.1	20.4	5.6	108	93.5
Africa and Middle East	728	62.8	21.0	16.2	70.5	83.5
Israel	198	86.9	8.6	4.5	197	95.4
Saudi Arabia	84	84.5	8.3	7.1	84	89.3
South Africa	446	48.0	28.9	23.1	424	76.9
Europe	12,439	67.5	16.2	16.3	12,288	82.9
Belgium	677	53.0	16.5	30.4	672	88.2
Czech Republic	1,016	55.7	20.0	19.3	999	78.7
France	619	76.9	7.9	15.2	556	85.4
Germany	1,201	44.8	31.6	23.6	1,201	80.6
Greece	120	79.2	1.7	19.2	120	77.5
Hungary	2,535	63.5	21.0	15.5	2,497	81.7
Italy	936	82.8	7.6	9.6	936	90.3
The Netherlands	888	88.0	7.5	4.5	889	95.8
Norway	14	71.4	14.3	14.3	14	85.7
Poland	153	75.5	9.0	15.5	154	91.6
Portugal	341	84.5	9.4	6.2	341	94.1
Russia	562	60.7	15.7	23.7	562	74.2
Slovakia	432	65.0	18.1	16.9	439	79.5
Spain	617	65.2	13.9	20.9	617	81.5
Switzerland	378	83.3	6.9	9.8	378	87.8
Turkey	63	53.6	22.2	22.2	63	83.6
United Kingdom	1,885	74.5	11.5	14.1	1,852	84.0
Latin America	2,039	76.7	8.2	15.1	2,000	84.2
Argentina	906	76.6	8.8	14.6	889	86.1
Brazil	152	92.0	2.1	5.9	507	94.1
Ecuador	325	74.2	7.7	18.2	303	81.5
Mexico	69	62.3	15.9	21.7	69	72.5
Venezuela	186	43.0	21.0	36.0	191	55.0
North America	2,470	76.2	4.3	19.5	2,460	82.7
Canada	249	91.6	2.0	6.4	249	94.4
United States	2,221	74.5	4.5	21.0	2,211	81.4

*All isolates were tested by the disk diffusion method performed in accordance with CLSI standard M44-A. S, susceptible, with zone diameters of ≥ 19 mm for fluconazole and ≥ 17 mm for voriconazole; S-DD, susceptible dose dependent, with zone diameters of 15–18 mm for fluconazole and 11–16 mm for voriconazole; R, resistant, with zone diameters of ≤ 14 mm for fluconazole and ≤ 13 mm for voriconazole.

Table 3. Trends in *in vitro* resistance to fluconazole and voriconazole among *C. glabrata* as determined by CLSI disk diffusion testing over a 7-year period*

Region	Year	Fluconazole		Voriconazole	
		N	%R	N	%R
Asia-Pacific	2001	359	24.2	250	6.4
	2002	545	13.8	545	8.6
	2003	619	16.0	618	9.5
	2004	922	7.7	923	6.0
	2005	923	13.4	923	8.0
	2006	1,145	11.9	1,144	9.0
	2007	1,116	12.5	1,112	8.6
Africa and Middle East	2001	198	23.2	176	11.9
	2002	130	15.4	130	8.5
	2003	30	30.0	30	13.3
	2004	133	12.0	133	3.8
	2005	73	23.3	72	11.1
	2006	75	10.7	75	9.3
	2007	89	2.2	89	1.1
Europe	2001	1,337	19.3	1,186	12.0
	2002	1,334	15.1	1,323	8.1
	2003	2,291	15.7	2,293	10.5
	2004	1,892	14.2	1,896	9.7
	2005	1,792	14.7	1,798	9.2
	2006	1,707	15.0	1,708	9.1
	2007	2,086	19.9	2,084	10.0
Latin America	2001	266	7.1	241	3.3
	2002	390	14.9	390	7.9
	2003	284	16.5	279	12.2
	2004	286	10.5	286	9.8
	2005	246	21.5	238	15.5
	2006	292	20.5	292	18.8
	2007	275	14.9	274	12.0
North America	2001	271	13.3	270	7.4
	2002	286	14.8	237	11.4
	2003	709	20.7	717	15.7
	2004	671	25.6	666	20.0
	2005	159	17.6	154	13.0
	2006	196	15.3	194	13.9
	2007	688	15.7	688	7.7

*Includes all specimen types and all hospital locations in 133 institutions. Fluconazole (zone diameter ≥ 14 mm) and voriconazole (zone diameter ≥ 13 mm) disk diffusion testing was performed in accordance with CLSI document M44-A (2004).

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