

# Variation in Fluconazole Susceptibility of *Candida glabrata* Bloodstream Isolates in the United States, 2001-2007

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## ABSTRACT

**Background:** *Candida glabrata* is now the 2<sup>nd</sup> most common cause of candidemia in the U.S., and is often azole resistant (R). Trends in both the incidence and susceptibility of *C. glabrata* have important implications for empiric therapy for invasive candidiasis.

**Methods:** We examined trends in *C. glabrata* isolation and susceptibility using longitudinal surveillance in 24 U.S. hospitals from 2001-2007, comparing these findings with a prior study performed between 1992-2001. Antifungal susceptibility testing (AST) for fluconazole (FLU), voriconazole (VOR), caspofungin, andidulafungin and micafungin were performed by CLSI M27-A3 method, including use of CLSI susceptibility breakpoints. We grouped isolates by patient age and geographic location within the U.S. for comparison.

**Results:** From 2001-2007, a total of 2,536 *Candida* spp. isolates were submitted, 642 (25%) of which were *C. glabrata*. Comparing 2001-2007 with 1992-2001 data, there were increases both in the proportion of *Candida* BSI caused by *C. glabrata* (from 18% to 25%), and in the rate of FLU-R among *C. glabrata* (from 9% to 14%). FLU-R among *C. glabrata* was highest in the Northeast region (46% susceptible (S), 17% R) and was lowest in the West (70% S, 10% R). Whereas the frequency of *C. glabrata* increased with patient age, the rate of FLU-R among *C. glabrata* declined with age. The oldest age group (≥80 years) had the highest proportion of BSI isolates that were *C. glabrata* (32%) and the lowest rate of FLU-R (5%). By contrast, 18% of *C. glabrata* from those aged <59 yrs were FLU-R. All 8 echinocandins had good in vitro activity (99-100% S); VOR S varied from 85% (Northeast) to 91% (West), and correlated with FLU activity.

**Conclusions:** *C. glabrata* are increasing as a proportion of all *Candida* bloodstream isolates in U.S. hospitals, and rates of FLU-R among *C. glabrata* are also increasing. These data support IDSA recommendations for alternative agents for candidemia among the severely ill, or those with risk factors for *C. glabrata*.

## INTRODUCTION

Candidemia is without question the most important of the invasive mycoses (6, 33, 35, 61, 65, 68, 78, 86, 88). Treatment of candidemia over the past 20 years has been enhanced considerably by the introduction of fluconazole in 1990 (7, 10, 15, 28, 29, 31, 40, 56-58, 61, 86, 90). Because of its widespread usage, concern about the development of fluconazole resistance among *Candida* spp. abounds (2, 6, 14, 32, 47, 53, 55, 56, 59, 60, 62, 80, 86). Despite these concerns, fluconazole resistance is relatively uncommon among most species of *Candida* causing bloodstream infections (BSI) (5, 6, 22, 24, 33, 42, 54, 56, 65, 68, 71, 86). The exception is *Candida glabrata*, of which more than 10% of BSI isolates may be highly resistant (MIC ≥64 µg/ml) to fluconazole (6, 9, 15, 23, 30, 32, 36, 63-65, 71, 87, 91). Sub-optimal fluconazole dosing practices (low dose (<400 mg/day), poor indications) may lead to an increased frequency of isolation of *C. glabrata* as an etiological agent of candidemia (6, 17, 29, 32, 35, 41, 47, 55, 60, 68, 85), to increased fluconazole (and other azole) resistance secondary to induction of CDR efflux pumps (2, 11, 13, 16, 43, 47, 50, 55, 69, 77, 83, 84), and may adversely affect the survival of treated patients (2, 10, 29, 40, 59, 90). Among the various *Candida* species, *C. glabrata* alone has increased as a cause of BSI in intensive care units (ICU) since 1993 (99). Within the U.S., the proportion of fungemias due to *C. glabrata* has been shown to vary from 11% to 37% across the different regions of the country (63, 65) and from <10% to >30% within single institutions (9, 48). In addition to fluconazole exposure, the prevalence of *C. glabrata* as a cause of BSI may be affected by many disparate factors, including geographic characteristics (3, 63-65, 71, 88), patient age (5, 6, 25, 35, 41, 42, 48, 63, 82, 92), and other characteristics of the patient population studied (1, 32, 35, 51). Because *C. glabrata* is relatively resistant to fluconazole, the frequency with which it causes BSI has important implications for therapy (21, 29, 32, 40, 41, 45, 56, 57, 59, 80, 81, 86, 90).

Previously, we examined the susceptibilities to fluconazole of 559 BSI isolates of *C. glabrata* and grouped the isolates by patient age and geographic location within the U.S. over the time period from 1992 to 2001 (63). In the current study we build upon this experience and report the fluconazole susceptibilities of 642 BSI isolates of *C. glabrata* collected from sentinel surveillance sites throughout the U.S. for the time period from 2001 through 2007 and stratify the results by geographic region and patient age. The activity of voriconazole and the echinocandins against this contemporary collection of *C. glabrata* isolates are also reported.

## MATERIALS and METHODS

**Organisms.** Between 2001 and 2007, a total of 2,536 bloodstream infection (BSI) isolates of *Candida* spp. from 24 sentinel surveillance sites in the U.S. were submitted to the University of Iowa College of Medicine (Iowa City) for identification and antifungal susceptibility testing with fluconazole, voriconazole, anidulafungin, caspofungin and micafungin. The isolates represent consecutive incident isolates from patients with candidemia treated at hospitals within the four major regions of the U.S. (Table 1). Patient ages were provided for 642 (89%) of the 718 BSI isolates of *C. glabrata*. These 642 isolates constitute the study set described herein.

All *C. glabrata* isolates were identified using Vitek and API products (BioMérieux, Durham, NC), the results of which were supplemented by conventional methods as required, and stored as yeast suspensions until they were used. Prior to testing, each isolate was passaged on potato dextrose agar (Remel, Lenexa, Kans) and CHROMagar (Becton Dickinson, Sparks, MD) to ensure purity and viability.

**Susceptibility test methods.** Fluconazole (Pfizer), voriconazole (Pfizer), anidulafungin (Pfizer), caspofungin (Merck), and micafungin (Astell) were all obtained from their respective manufacturers as reagent grade powders. Broth microdilution testing was performed exactly as described in Clinical and Laboratory Standards (CLSI) document M27-A3 (19). The interpretive criteria for each agent were those published by Pfaller et al (66, 67, 73) and in CLSI document M27-S3 (20) as follows: fluconazole, an isolate for which the MIC is ≤8 µg/ml is susceptible (S), an isolate for which the MIC is 16 to 32 µg/ml is susceptible dose dependent (SDD), and an isolate for which the MIC is ≥64 µg/ml is resistant (R); voriconazole, ≤1 µg/ml SDD, 2 µg/ml R; ≥4 µg/ml anidulafungin, caspofungin and micafungin, S ≤2 µg/ml; non-susceptible (NS), ≥2 µg/ml.

**Quality Control.** QC was accomplished by testing the following strains on each day of testing: *C. parapsilosis* ATCC 22019 and *C. Arusei* ATCC 6258 (20).

**Table 1.** Temporal and Geographic Trends in the Frequency of Isolation and Fluconazole Resistance Among Bloodstream Infection (BSI) Isolates of *Candida glabrata* in the United States

Region	Time Period	Total no. of <i>Candida</i> BSI isolates	<i>C. glabrata</i>	
			% of total	% resistant to fluconazole
West	1992-2001*	700	17	7
	2001-2007	61	34	10
Midwest	1992-2001*	678	23	7
	2001-2007	1,420	28	12
Northeast	1992-2001*	819	21	11
	2001-2007	897	19	17
South	1992-2001*	1,486	15	11
	2001-2007	619	21	11
Total	1992-2001*	3,683	18	9
	2001-2007	2,536	25 <sup>b</sup>	14 <sup>c</sup>

\*Data compiled from Pfaller et al (63)

<sup>b</sup>p<0.001 for increase in proportion of all *Candida* that were *C. glabrata* between 92-01 and 01-07.

<sup>c</sup>p=0.006 for increase in proportion of all *C. glabrata* that were fluconazole resistant, between 92-01 and 01-07.

**Table 2.** Regional Variation in Susceptibility of BSI Isolates of *Candida glabrata* to Azoles and Echinocandins, 2001-2007

Region	Antifungal agent	No. tested	MIC (µg/ml) <sup>a</sup>			% by category <sup>b</sup>
			Range	50%	90%	
			S	R		
West	Fluconazole	21	2->128	8	16	76
	Voriconazole	21	0.06-8	0.25	0.5	91
	Caspofungin	21	0.03-0.12	0.03	0.06	100
						0 <sup>c</sup>
Midwest	Fluconazole	368	1->128	8	64	61
	Voriconazole	368	0.015-8	0.25	2	90
	Anidulafungin	200	0.015-0.5	0.06	0.12	100
	Caspofungin	345	0.015-4	0.03	0.06	99
Northeast	Micafungin	178	0.007-1.2	0.015	0.015	100
	Fluconazole	162	0.5->128	16	128	46
	Voriconazole	161	0.015-8	0.25	2	85
	Anidulafungin	78	0.015-0.25	0.06	0.12	100
South	Caspofungin	160	0.015-0.5	0.03	0.06	100
	Micafungin	75	0.007-0.06	0.015	0.015	100
	Fluconazole	120	1->128	8	64	54
	Voriconazole	120	0.007-8	0.25	2	89
	Anidulafungin	45	0.03-0.25	0.06	0.12	100
	Caspofungin	118	0.015-0.5	0.03	0.25	100
	Micafungin	29	0.007-0.25	0.015	0.025	100

<sup>a</sup>50% and 90%, MICs encompassing 50% and 90% of isolates tested.

<sup>b</sup>S, susceptible; R, resistant. Breakpoints from CLSI document M27-S3.

<sup>c</sup>Isolates with echinocandin MICs >2 µg/ml are considered non-susceptible.

**Table 3.** Frequency of Isolation and Fluconazole Resistance of BSI Isolates of *Candida glabrata* by Patient Age Group

Patient age group (yrs)	Total no. of <i>Candida</i> BSI isolates (%) <sup>a</sup>	<i>C. glabrata</i>	
		No. tested (% of total) <sup>b</sup>	% resistant to fluconazole
<1	21 (0.8)	1 (5)	0
1-9	103 (4.1)	4 (4)	2.5
10-19	70 (2.8)	6 (9)	3.3
20-29	128 (5.0)	25 (20)	2.8
30-39	184 (7.3)	32 (17)	2.2
40-49	372 (14.7)	80 (22)	2.3
50-59	483 (19.0)	146 (30)	1.4
60-69	481 (18.9)	141 (29)	9
70-79	449 (17.7)	128 (29)	8
≥80	245 (9.7)	79 (32)	5
All ages	2,536 (100)	642 (25)	1.4

<sup>a</sup>% of total BSI isolates.

<sup>b</sup>% of BSI isolates in each age group.

## RESULTS and DISCUSSION, continued

Overall, *C. glabrata* accounted for 25% of all *Candida* spp. BSI isolates and was the second most common species isolated. The frequency of *C. glabrata* as a cause of candidemia in the U.S. ranged from 19% in the Northeast to 34% in the West (Table 1). By comparison with our previous survey encompassing the years 1992 to 2001 (63), the proportion of *Candida* spp. BSI isolates that were *C. glabrata* increased in three of the four regions and decreased only slightly in the Northeast (from 21% to 19%).

As seen in our previous survey, the fluconazole susceptibilities of *C. glabrata* BSI isolates varied by region (Table 1). Notably, the rate of fluconazole resistance among the *C. glabrata* isolates from 2001-2007 increased when compared to those from 1992-2001 in all regions except for the South, where it was unchanged. Furthermore, the region with the highest prevalence of *C. glabrata* (West, 34%) had the lowest frequency of resistance (10%). Overall, 14% of the 2001-2007 U.S. *C. glabrata* isolates were resistant to fluconazole compared with only 9% in 1992-2001.

Given the increasing resistance to fluconazole among U.S. BSI isolates of *C. glabrata*, it is important to examine the activity of possible alternatives to fluconazole in the treatment of these infections. Voriconazole and the echinocandins are now available for the treatment of candidemia and other forms of invasive candidiasis (26, 56, 57, 61, 70-72, 86). All three echinocandins showed excellent activity against *C. glabrata* isolates from all four regions with >99% of isolates susceptible at the CLSI breakpoint of ≤2 µg/ml (Table 2). Although 85% to 91% of isolates were susceptible to voriconazole (Table 2), it is notable that the region with the lowest susceptibility to fluconazole (Northeast, 46%) also had the lowest susceptibility to voriconazole, a pattern consistent with previously determined cross-resistance (70, 71).

Consistent with previous observations (25, 48, 63), very few BSI due to *C. glabrata* were reported from the pediatric and adolescent age groups (5-19 years) (Table 3). Only 11 *C. glabrata* BSI isolates were submitted from patients <19 years of age. In contrast to that observed in 1992-2001 for isolates from this age group, 27% of the current isolates were resistant to fluconazole compared to only 7% of isolates from the earlier time period (63). This increased resistance may reflect the increased use of fluconazole prophylaxis and treatment in these young patients (12, 27, 49, 57, 58).

Whereas the proportion of BSI isolates of *Candida* that were *C. glabrata* increased with patient age, the rate of fluconazole resistance declined (Table 3). Thirty percent of *Candida* BSI in patients ≥60 years of age were due to *C. glabrata*; however, only 8% were resistant to fluconazole compared to 18% for those isolates from age groups 20-59 years (Table 3). The oldest age group (≥80 years) had the highest proportion of BSI isolates that were *C. glabrata* (32%) and the lowest rate of fluconazole resistance (5%).

These results confirm and extend the previous findings that we and others have reported concerning the increasing prevalence of *C. glabrata* as a cause of BSI in the U.S. both over time and as a function of patient age (1, 2, 25, 32, 33, 35, 37, 41, 47, 48, 55, 63, 68, 89). The variation in frequency of *C. glabrata* as a cause of BSI across clinical sites has clearly been shown by Horn et al (35) and by Hachem et al (32). Horn et al (35) found that patients with *C. glabrata* fungemias were more likely than other patients with candidemia to be older and to have received a solid organ transplant, whereas Hachem et al (32) found that antifungal prophylaxis with fluconazole was a predisposing risk factor for *C. glabrata* BSI among cancer patients.

Important new findings in this survey are the apparent increase in fluconazole resistance among *C. glabrata* BSI isolates from pediatric and adolescent patients as well as the very low rate of fluconazole resistance among BSI isolates from older patients (Table 3). Although earlier studies of fluconazole prophylaxis in infant and pediatric patients have not shown emergence of fluconazole resistance, most were not conducted long enough to demonstrate such a change (39).

Population-based studies have shown that the highest incidence of *Candida* BSI occurs at the extremes of age (3, 6, 8, 33, 37, 74, 82, 93). Older individuals are not only at high risk of *Candida* BSI and associated mortality, but are also at higher risk of infection with *C. glabrata* (5, 6, 25, 30, 35, 41, 42, 48, 82). Importantly, in this study we show that despite a high frequency of *C. glabrata* BSI, those isolates infecting the older patient age groups are considerably less likely to exhibit resistance to fluconazole (Table 3). This may reflect the fact that although older individuals may have more frequent contact with the healthcare environment, they are less likely than younger individuals to undergo hematologic stem cell transplantation (HSCT) or solid organ transplantation (SOT) and thus less likely to receive fluconazole prophylaxis (38). Furthermore, it is now apparent that colonization with *C. glabrata* is much more common among older individuals irrespective of exposure to the health care environment (34, 44, 46, 76, 77). Such colonization likely reflects a change in the ecology of *Candida* colonization with age rather than selection by drug exposure (44, 46, 75). Older individuals are more likely to be fluconazole naive and thus less likely to have acquired resistance to fluconazole (69).

## RESULTS and DISCUSSION, continued

Perhaps one of the greatest values of surveys such as this is the demonstration of the continued and widespread emergence of this potentially azole-resistant species among patients of all age groups throughout the U.S. Similar surveillance efforts should also be undertaken within individual institutions in order to provide knowledge of local epidemiological trends when selecting initial empirical therapy for *Candida* BSI (7, 21, 29, 56, 59, 80). Delays in administering effective antifungal therapy (right drug, right dose) directly influence mortality (28, 29, 52, 59) and such local epidemiological data provides the best way to optimize early initial antifungal therapy (21, 26, 59, 80). In an institution with high rates of infection from fluconazole-resistant *C. glabrata*, an echinocandin should be recommended as the initial treatment of choice (Table 2) (56-58, 80). In institutions that have lower rates of infection with *C. glabrata*, or in patients for whom infection due to fluconazole-resistant *C. glabrata* is less likely, fluconazole may still be appropriate as initial therapy for patients who are not critically ill and do not have prior fluconazole exposure (56-58, 80). In such settings, however, it is important to pay strict attention to the appropriate utilization of fluconazole (10, 18, 29, 41, 59, 90).

Inappropriate antifungal therapy can occur due to omission of antifungal treatment, incorrect antifungal dosing, or administration of an antifungal agent to which the infecting organism was resistant (21, 29, 40, 41, 59, 85, 90). Previous Infectious Diseases Society of America (IDSA) guidelines recommend that if fluconazole is used in the treatment of *C. glabrata* BSI, a dose of 12 mg/kg/d (usually ≥800 mg/d) should be administered, while a dose of 8 mg/kg/d (usually 400 mg/d) is sufficient for *C. albicans*, *C. parapsilosis* and *C. tropicalis* (57, 58). Unfortunately, fluconazole has been shown to be the antifungal agent that is most likely to be used inappropriately (either wrong dose or resistant organism) (28, 29, 41, 59). Galey et al (29) has shown in a retrospective analysis that 78% of patients with *C. glabrata* BSI were treated with a dose of fluconazole less than that recommended by the IDSA. Likewise Klevay et al (41) found that in contrast to patients with *C. albicans* BSI, those with *C. glabrata* infection were less likely to receive an adequate dose of fluconazole as empiric therapy (12% vs 22%, p<0.05) and that time to receipt of adequate therapy was longer for patients infected with *C. glabrata* versus *C. albicans* (p<0.001). Although Wilson et al (90) found that fluconazole was a viable therapy for *C. glabrata* fungemia, they noted that higher doses of fluconazole (>400mg/d) were more likely to achieve fungemia eradication than lower doses (≤400mg/d) among patients who received only fluconazole (91% vs 50% respectively [p=0.042]). Finally, Sendik et al (85) found that the emergence of *C. glabrata* as a cause of BSI in a French University Hospital was linked to low dose (50-100 mg/d) fluconazole usage, whereas the prevalence of *C. glabrata* decreased with an institutional shift to higher doses (>200 mg/d) of fluconazole concomitant with the introduction of voriconazole and caspofungin. IDSA guidelines also suggest that once *Candida* infection is confirmed, species level identification is in most cases an effective method for prediction of antifungal susceptibility (56-58). However, as shown in the present study, resistance to fluconazole among isolates of *C. glabrata* is not predictable and therefore antifungal susceptibility testing is needed to ensure optimal antifungal treatment of *C. glabrata* BSI (7, 21, 29, 59). Indeed, several authors have now demonstrated the importance of the relationship between the daily dose of fluconazole and the organisms in vitro susceptibility to fluconazole as a predictor of therapeutic success or failure (4, 10, 18, 29). Baddeley et al (10) recently demonstrated that a fluconazole 4 mg/kg over the concentration curve (AUC) – to – MIC ratio of <11.5 or MIC of ≥64 µg/ml was associated with increased mortality among patients with candidemia treated with fluconazole. Parkins et al (59) reported that empirical therapy of candidemia with an adequate antifungal agent (isolate susceptible in vitro) was associated with a significant reduction in all-cause mortality (27% vs 46%, p=0.02). Notably, empirical therapy with fluconazole was more likely to be deemed inadequate. Thus, both accurate and timely identification of *Candida* BSI to species and MIC testing may be more important than previously recognized in the successful management of candidemia (7).

The rising cost of novel antifungals, in conjunction with an increase in available therapies, increasing fluconazole resistance, local variation in the prevalence of *C. glabrata*, and growing evidence that inappropriate or delayed therapy increases mortality associated with candidemia has made therapeutic decision-making more complex, requiring more mycological data and clinical expertise (7). Given the variability in incidence and fluconazole resistance among *C. glabrata*, the newly released IDSA guidelines recommend that patients with *C. glabrata* BSI be empirically treated with an echinocandin, and with de-escalation to fluconazole only after the patient is stable and the organism is known to be susceptible (21, 45, 56-58, 79). Collins et al (21) have shown that the timely use of fluconazole susceptibility testing of *C. glabrata* BSI isolates can facilitate de-escalation from a costly echinocandin to fluconazole, resulting in lower overall treatment costs in patients with documented *C. glabrata* fungemia. Increasingly, antifungal susceptibility testing appears to be a necessity in today's world of resistant organisms and expensive agents (21, 56-58, 79).

In summary, we provide further documentation of the emerging frequency of *C. glabrata* as a cause of BSI in patients of all ages throughout the U.S. The variable frequency of occurrence and resistance to fluconazole associated with this species underscores the need for both prompt identification and antifungal susceptibility testing of bloodstream isolates in order to optimize antifungal therapy. The overall decreased susceptibility of *C. glabrata* to fluconazole makes proper dosing of this agent essential to its optimal use in the treatment of *C. glabrata* and other candidal infections. Antifungal susceptibility testing can be used in an efficient and cost-effective manner in guiding de-escalation from costly echinocandins to fluconazole in the treatment of *C. glabrata* infections.

The relatively small number of isolates from certain regions and age groups limits this study. In addition we could not obtain complete data on several patient-related factors that might have influenced the risk of *C. glabrata* or the risk for fluconazole resistance (e.g., severity of illness, device use, underlying disease, and antifungal use). Regardless, the overall size of this collection of *C. glabrata* BSI isolates does provide useful descriptive information. Such information will continue to be useful as a basis for comparison for future studies regarding the prevalence and antifungal susceptibility of *C. glabrata* as a BSI pathogen in the U.S.

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A copy of this poster can be found at the following website:

<http://www.healthcare.uowa.edu/pathology/itw/research/ICAAAC.html>

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