JOURNAL OF CLINICAL MICROBIOLOGY, July 2011, p. 2404–2410 Vol. 49, No. 7
0095-1137/11/$12.00 doi:10.1128/JCM.02474-10

Comparison of In Vitro Susceptibility Characteristics of *Candida* Species from Cases of Invasive Candidiasis in Solid Organ and Stem Cell Transplant Recipients: Transplant-Associated Infections Surveillance Network (TRANSNET), 2001 to 2006[▽]

Shawn R. Lockhart,[1]* Debra Wagner,[1] Naureen Iqbal,[1] Peter G. Pappas,[2] David R. Andes,[3] Carol A. Kauffman,[4] Lisa M. Brumble,[5] Susan Hadley,[6] Randall Walker,[7] James I. Ito,[8] John W. Baddley,[2,9] Tom Chiller,[1] and Benjamin J. Park[1]

Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, Georgia[1]; Department of Medicine, Division of Infectious Diseases and Preventive Medicine, University of Alabama at Birmingham, Birmingham, Alabama[2]; University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin[3]; University of Michigan and Veterans Affairs Ann Arbor Healthcare System, Ann Arbor, Michigan[4]; Mayo Clinic, Jacksonville, Florida[5]; Tufts Medical Center, Boston, Massachusetts[6]; Mayo Clinic and Mayo Clinic College of Medicine, Rochester, Minnesota[7]; City of Hope National Medical Center, Duarte, California[8]; and Department of Medicine, Division of Infectious Diseases, Birmingham Veterans Affairs Medical Center, Birmingham, Alabama[9]

Received 7 December 2010/Returned for modification 29 December 2010/Accepted 29 April 2011

Invasive fungal infections (IFI) are a major cause of morbidity and mortality among both solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) recipients. *Candida* is the most common cause of IFI in SOT recipients and the second most common cause of IFI in HSCT recipients. We determined susceptibilities to fluconazole, voriconazole, itraconazole, posaconazole, amphotericin B, and caspofungin for 383 invasive *Candida* sp. isolates from SOT and HSCT recipients enrolled in the Transplant-Associated Infection Surveillance Network and correlated these results to clinical data. Fluconazole resistance in *C. albicans*, *C. tropicalis*, and *C. parapsilosis* isolates was low (1%), but the high percentage of *C. glabrata* and *C. krusei* isolates within this group of patients increased the overall percentage of fluconazole resistance to 16%. Voriconazole resistance was 3% overall but was 8% among *C. glabrata* isolates. On multivariable analysis, among HSCT recipients fluconazole nonsusceptibility was independently associated with *C. glabrata*, non-Hodgkin's lymphoma, cytomegalovirus (CMV) antigenemia, diabetes active at the time of the IFI, and any prior amphotericin B use; among SOT recipients, fluconazole nonsusceptibility was independently associated with any fluconazole use in the 3 months prior to the IFI, *C. glabrata*, ganciclovir use in the 3 months prior to the IFI, diabetes acquired since the transplant, and gender.

Both solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) recipients are at high risk for invasive fungal infections (IFI) due to risk factors including long-term central venous catheter use, immunosuppression, and cytopenia, as well as therapy given to prevent graft-versus-host disease or rejection (5, 28). *Candida* species are the leading cause of invasive fungal infections in SOT patients, causing 53% of fungal infections, and the second leading cause of invasive fungal infections in HSCT patients, causing 28% of infections (13, 20).

In the United States, *Candida albicans* has been the most common cause of invasive candidiasis, and most isolates of *C. albicans* have been susceptible to azole antifungal drugs (21). Over the last 2 decades there has been a steady increase in the proportion of non-*C. albicans Candida* infections and a concomitant decrease in the proportion of infections caused by *C. albicans* (9, 12, 22, 30). This is a matter of concern because *Candida glabrata* and *Candida parapsilosis*, species with decreased *in vitro* susceptibilities to azole and echinocandin antifungal drugs, respectively, are increasing (22, 23, 25). Although the incidence rate of *Candida krusei* does not seem to be increasing, this fluconazole-resistant species seems to have been disproportionately represented among bone marrow transplant patients in several studies (1, 17, 24, 34).

The Transplant-Associated Infections Surveillance Network (TRANSNET), a consortium of 23 U.S. academic transplant centers, was established to conduct prospective surveillance to determine the burden of invasive fungal infections among transplant recipients (13, 20). It is important to monitor for the emergence of antifungal-resistant isolates and increases in resistant species within the vulnerable transplant population. In this study, we examined the antifungal susceptibility profiles of 383 incident invasive isolates of *Candida* from SOT and HSCT patients in the TRANSNET cohort and correlated clinical variables with isolate nonsusceptibility.

* Corresponding author. Mailing address: Centers for Disease Control and Prevention, 1600 Clifton Road, N.E., Mailstop G-11, Atlanta, GA 30333. Phone: (404) 639-2569. Fax: (404) 639-3546. E-mail: gyi2@cdc.gov.

▽ Published ahead of print on 11 May 2011.

MATERIALS AND METHODS

TRANSNET, a network of 23 U.S. transplant centers, conducted prospective surveillance for invasive fungal infections and has been described in detail elsewhere (13, 20). Briefly, participating centers enrolled and performed follow-up on 16,808 SOT and 15,820 HSCT recipients between March 2001 and March 2006. In all proven or probable cases of invasive fungal infection, as defined by modified European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria (4), clinical data were collected by trained personnel at each site. All proven or probable cases of *Candida* infection with available isolates were selected for this analysis.

Fungal isolates. Available isolates were sent to the Fungus Reference Unit at the Centers for Disease Control and Prevention (CDC), Atlanta, GA, for confirmation of species and antifungal susceptibility testing. Isolate purity was confirmed using CHROMagar Candida (BBL, Franklin Lakes, NJ). Species identification was confirmed using API 20C AUX (bioMérieux, Durham, NC), and when API did not give excellent or very good results, DNA sequencing of the D1/D2 region of the rDNA (15) was used. Only the first isolate from each patient was used for analysis.

Antifungal susceptibility testing. Prior to antifungal susceptibility testing, each isolate was subcultured twice on Sabouraud dextrose agar plates to ensure purity and optimal growth. One isolate per patient was tested. Broth microdilution MIC testing was performed as outlined in Clinical and Laboratory Standards Institute (CLSI) document M27-A2 (18) and as previously described (9) for fluconazole, itraconazole, and voriconazole. MICs of amphotericin B, caspofungin, and posaconazole were determined by Etest (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions. Plates were incubated at 35°C for 24 h for caspofungin or 48 h for amphotericin B and posaconazole. To confirm the Etest results for posaconazole, broth microdilution was repeated for any isolate with an initial posaconazole Etest MIC of ≥1 μg/ml and for all *C. glabrata* isolates. All final posaconazole MICs of ≥1 μg/ml were the values derived from broth microdilution. Quality control was performed on each day of testing using *C. krusei* strain ATCC 6258 and *C. parapsilosis* strain ATCC 22019.

Isolates were categorized as susceptible, susceptible-dose dependent, or resistant based on established MIC cutoff values for fluconazole, itraconazole, and voriconazole and as susceptible or nonsusceptible for caspofungin (6). Fluconazole nonsusceptibility in the bivariate and multivariate analyses was defined as an MIC of >8 μg/ml.

Factors associated with nonsusceptibility. For each transplant type, clinical information for case patients with susceptible isolates was compared to information for nonsusceptible isolates in order to identify factors associated with nonsusceptibility. Nine isolates were excluded from the modeling because no clinical information was available. Bivariate logistic regression was used to identify potential variables to evaluate for inclusion in a multivariate model for fluconazole nonsusceptibility.

For the multivariate model, variables with a P value of ≤0.2 were considered in addition to basic demographic variables. The 0.2 cutoff value was chosen to limit the number of potential variables for the multivariate model without being too restrictive in our selection criteria. Selected immunosuppressive medications, regardless of P value, were also considered for the model due to increased risk of infection with use. Antifungal medications, regardless of P value, were also considered for the model. Separate models were built for HSCT and SOT to reduce potential confounding due to transplant type. Logistic regression was used to build the multivariate models for fluconazole nonsusceptibility. The final multivariate model was chosen using the score selection method after evaluating for interaction and colinearity.

Data analysis. Differences between geometric means were calculated using Student's two-sample t test assuming unequal variances. Logistic regression for bivariate and multivariable analyses was conducted using SAS 9.2 (SAS Institute, Cary, NC).

RESULTS

A total of 915 proven and probable cases of invasive candidiasis were identified in TRANSNET (13, 20). *Candida albicans* was the most frequent species found (n = 350, 38% of cases), followed by *C. glabrata* (n = 250, 27%), *C. parapsilosis* (n = 94, 10%), *Candida tropicalis* (n = 51, 6%), and *C. krusei* (n = 30, 3%). Among HSCT patients, the most frequent species was *C. glabrata* (n = 92, 33% of cases), followed by *C. albicans* (n = 55, 20%), *C. parapsilosis* (n = 39, 14%), *C. tropicalis* (n = 23, 8%), and *C. krusei* (n = 17, 6%). In SOT recipients, *C. albicans* was the most frequent species (n = 295, 46%), followed by *C. glabrata* (n = 158, 25%), *C. parapsilosis* (n = 55, 9%), *C. tropicalis* (n = 28, 4%), and *C. krusei* (n = 13, 2%).

TABLE 1. Species distribution of *Candida* isolates available for susceptibility testing collected from solid organ or stem cell transplant recipients

Species[a]	No. (%) of isolates		
	Total (n = 383)	SOT (n = 264)	HSCT (n = 119)
C. albicans	154 (40)	131 (50)	23 (19)
C. glabrata	119 (31)	78 (30)	41 (34)
C. parapsilosis	48 (13)	23 (9)	25 (21)
C. krusei	32 (8)	14 (5)	18 (15)
C. tropicalis	21 (5)	12 (5)	9 (8)
C. lusitaniae	5 (1)	3 (1)	2 (2)
C. dubliniensis	2 (<1)	2 (<1)	0
C. guilliermondii	2 (<1)	1 (<1)	1 (<1)

[a] Species identification was confirmed at CDC.

There were notable differences in the distribution of species between the SOT and HSCT populations. While 46% of isolates from SOT recipients were *C. albicans*, only 20% of isolates from HSCT recipients were *C. albicans*. In the HSCT population, 39% of isolates were *C. glabrata* and *C. krusei*, while only 27% of isolates from the SOT population were these species.

A total of 383 isolates were available from the 915 cases for susceptibility testing and species confirmation at CDC. Isolates were received from 20 of the 23 transplant centers. Four of the centers contributed a combined total of 58% of the isolates, with 17%, 17%, 13%, and 11% coming from those centers individually. The remaining isolates were spread somewhat evenly among the remaining 16 centers. The distribution of species among isolates received at CDC was not significantly different from the overall TRANSNET species distribution, with the exception that CDC received slightly more cases of *C. krusei*, with most coming from isolates which had no previous species identification performed (Table 1). Isolates were from blood (n = 238), peritoneal fluid (n = 41), abdominal fluid (n = 31), pleural fluid (n = 9), abscess (n = 6), liver (n = 6), ascites fluid (n = 6), lung (n = 5), bile (n = 5), tissue (n = 5), pancreas (n = 4), kidney (n = 3), bone (n = 3), mediastinal fluid (n = 2), wound fluid (n = 2), and other (n = 17).

The antifungal susceptibility testing results for all isolates are summarized in Table 2. All isolates were susceptible to caspofungin. Excluding isolates of *C. krusei*, 10% of isolates were resistant to fluconazole, including 23% of *C. glabrata* isolates (Table 2). When *C. krusei* isolates were added, the overall resistance was 16%. Among the *C. albicans*, *C. parapsilosis*, and *C. tropicalis* isolates (58% of the total isolates tested), resistance to fluconazole was only 1%. There was no association between any center and the proportion of fluconazole-resistant isolates received.

The overall resistance to itraconazole was 17%, again due primarily to the prevalence of *C. glabrata* isolates, and that among *C. albicans*, *C. parapsilosis*, and *C. tropicalis* was again around 1%. Voriconazole resistance was observed in 3% of isolates overall: two isolates of *C. albicans*, one isolate of *C.*

TABLE 2. *In vitro* susceptibilities of available TRANSNET *Candida* isolates

Species (no. of isolates)	Antifungal agent	MIC (μg/ml)			% Resistant[a]
		Range	MIC_{50}	MIC_{90}	
All (383)	Fluconazole	0.125–64	1	64	16
	Voriconazole	0.015–8	0.03	1	3
	Itraconazole	0.015–8	0.125	1	17
	Posaconazole	0.015–16	0.125	2	
	Caspofungin	0.012–2	0.094	0.38	
	Amphotericin B	0.038–6	0.38	2	
C. albicans (154)	Fluconazole	0.125–64	0.25	1	1
	Voriconazole	0.015–8	0.015	0.06	1
	Itraconazole	0.015–8	0.03	0.125	2
	Posaconazole	0.016–16	0.047	0.125	
	Caspofungin	0.012–0.25	0.047	0.125	
	Amphotericin B	0.038–1.0	0.25	0.38	
C. glabrata (119)	Fluconazole	0.25–64	8	64	23
	Voriconazole	0.015–4	0.5	2	8
	Itraconazole	0.125–8	1	16	52
	Posaconazole	0.125–4	1	2	
	Caspofungin	0.012–0.38	0.125	0.19	
	Amphotericin B	0.094–4	0.75	1	
C. parapsilosis (48)	Fluconazole	0.25–8	0.5	2	0
	Voriconazole	0.015–0.5	0.015	0.06	0
	Itraconazole	0.015–0.5	0.06	0.125	0
	Posaconazole	0.012–0.75	0.047	0.125	
	Caspofungin	0.064–1	0.38	0.5	
	Amphotericin B	0.038–2	0.5	1.5	
C. krusei (32)	Fluconazole	0.25–64	32	64	100
	Voriconazole	0.015–1	0.25	0.5	0
	Itraconazole	0.015–0.5	0.25	0.5	0
	Posaconazole	0.032–2	0.75	1.5	
	Caspofungin	0.047–2	0.38	0.5	
	Amphotericin B	0.25–6	2	4	
C. tropicalis (21)	Fluconazole	0.25–64	1	8	5
	Voriconazole	0.015–8	0.125	0.5	5
	Itraconazole	0.015–8	0.125	0.25	5
	Posaconazole	0.023–16	0.125	0.75	
	Caspofungin	0.032–1	0.064	0.19	
	Amphotericin B	0.19–1.5	0.75	1	

[a] Values assume intrinsic resistance of *C. krusei* regardless of MIC.

tropicalis, and nine *C. glabrata* isolates. Voriconazole resistance was lower than fluconazole resistance among *C. glabrata* isolates, with 8% resistant and 76% fully susceptible to voriconazole. All of the *C. glabrata* isolates that were resistant to voriconazole were also resistant to itraconazole and to fluconazole. They also had posaconazole MIC values at or above the average achievable serum levels at 400 mg twice a day (BID) (7). All of the *C. krusei* isolates had voriconazole MIC values of ≤1 μg/ml, which is interpreted as fully susceptible.

There are no established breakpoints for resistance to amphotericin B. The overall MIC_{90} was 2.0 μg/ml. Four isolates of *C. glabrata*, 1 isolate of *C. guilliermondii*, and 13 isolates of *C. krusei* displayed MIC values of ≥2 μg/ml, a value at which treatment with amphotericin B would not be recommended.

The geometric mean MIC values for HSCT cases were significantly higher than those for SOT cases for fluconazole (4.099 versus 1.206 μg/ml, $P < 0.001$), voriconazole (0.132 versus 0.000 μg/ml, $P = 0.002$), itraconazole (0.218 versus 0.115 μg/ml, $P = 0.007$), and caspofungin (0.150 versus 0.094 μg/ml, $P < 0.001$).

We also found higher geometric mean fluconazole MIC values among patients who had previously received fluconazole prophylaxis (Table 3). This association was seen among all 383 isolates from all transplant types (7.207 versus 1.238 μg/ml, $P < 0.0001$), among all 119 isolates from HSCT recipients (9.190 versus 2.403 μg/ml, $P = 0.0013$), and among all 264 isolates from SOT recipients (4.876 versus 1.020 μg/ml, $P = 0.0002$). Among the 119 *C. glabrata* cases, higher fluconazole MIC values were associated with having received fluconazole prophylaxis (geometric means of 19.870 versus 10.556 μg/ml, $P = 0.0133$).

On bivariate analysis, fluconazole nonsusceptibility (MIC of >8 μg/ml) was significantly associated with having received an HSCT compared to an SOT (odds ratio [OR], 4.02; 95% confidence interval [CI], 2.42 to 6.66). This strong association with

TABLE 3. Geometric mean MICs for fluconazole by species and fluconazole use

Transplant type and *Candida* species (*n*)	Geometric mean MIC (μg/ml)			*P* value
	All isolates	Fluconazole prophylaxis	No fluconazole prophylaxis	
All transplant types				
All species (383)	1.746	7.207	1.238	<0.001
C. albicans (154)	0.309	0.330	0.308	0.694
C. glabrata (119)	12.550	19.870	10.556	0.013
C. krusei (32)	19.027	20.950	16.812	0.717
HSCT only				
All species (119)	4.099	9.190	2.403	0.001
C. albicans (23)	0.224	0.250	0.220	0.383
C. glabrata (41)	23.209	29.748	18.730	0.219
C. krusei (18)	21.773	16.876	42.224	0.104
SOT only				
All species (264)	1.206	4.876	1.020	<0.001
C. albicans (131)	0.324	0.372	0.322	0.537
C. glabrata (78)	9.007	11.016	8.640	0.513
C. krusei (14)	16.000	36.758	10.079	0.110

transplant type necessitated separate analyses for each transplant type.

Bivariate analyses for nonsusceptibility among HSCT and SOT recipients are shown in Table 4. Among the HSCT recipients, non-Hodgkin's lymphoma (OR, 2.52; 95% CI, 0.93 to 6.77), cytomegalovirus (CMV) coinfection (OR, 2.81; 95% CI, 1.05 to 7.48), diabetes active at the time of IFI (OR, 3.65; 95% CI, 1.45 to 9.19), and prophylactic antifungal use (OR, 3.49; 95% CI, 1.49 to 8.19) were associated with fluconazole nonsusceptibility. Among SOT recipients, female gender (OR, 3.14; 95% CI, 1.51 to 6.54), diabetes acquired since the transplant (OR, 2.74; 95% CI, 1.29 to 5.84), and prophylactic antifungal use (OR, 3.58; 95%, CI 1.63 to 7.90) were significantly associated with fluconazole nonsusceptibility.

On multivariate modeling for fluconazole nonsusceptibility among HSCT recipients, prior fluconazole use trended toward statistical significance (adjusted odds ratio [aOR], 2.66; 95% CI, 0.93 to 7.62) (Table 5). Having *C. glabrata* was significantly associated with fluconazole nonsusceptibility (aOR, 10.47; 95% CI, 3.58 to 30.62). Other significant variables included non-Hodgkin's lymphoma, CMV antigenemia, diabetes active at the time of the *Candida* infection, and any amphotericin B use prior to *Candida* infection. The model was adjusted for gender, race, and age.

On multivariate modeling for fluconazole nonsusceptibility among SOT recipients, fluconazole use in the 3 months prior to the infection was significantly associated with fluconazole nonsusceptibility (aOR, 2.65; 95% CI, 1.17 to 5.99). *Candida glabrata* was again strongly associated with fluconazole nonsusceptibility (aOR, 4.70; 95% CI, 2.08 to 10.58). Other significant variables included ganciclovir use in the 3 months prior to the IFI, diabetes acquired posttransplant, and gender. The model was adjusted for race and age.

DISCUSSION

In this analysis of the largest collection of invasive *Candida* isolates from transplant recipients to date, we demonstrate a high prevalence of fluconazole resistance (16%) when considering both resistant isolates and intrinsically resistant species.

The species distribution within TRANSNET reflects the changes in the epidemiology of candidiasis described over the past 2 decades, where the proportion of invasive *C. albicans* infections has declined consistent with an increase in the proportion of infections due to non-*C. albicans* species, especially *C. glabrata* (2, 9, 12, 22). In our study, the lower proportion of *C. albicans* was more evident among HSCT patients, with *C. glabrata* (34%) more common than *C. albicans* (19%). These data are consistent with PATH Alliance registry findings for U.S. HSCT patients (19), which showed that among the 20 invasive *Candida* isolates from HSCT patients in that study, *C. glabrata* was the most frequent (35%), followed by *C. parapsilosis* and *C. tropicalis* (30% each), *C. albicans* (25%), and *C. krusei* (10%).

We also found that the species distributions were markedly different for HSCT and SOT recipients. These differences in species distribution may be due to dissimilar antifungal use and prophylaxis practices, with a higher proportion of HSCT patients than of SOT patients in our study receiving antifungal prophylaxis (63% versus 15%). Multiple studies have suggested that the use of azoles can drive the species distribution within a population toward species with intrinsic resistance (1, 8, 10, 16, 26, 33, 34). Still, other factors may be contributing to this change in species distribution (27, 32), and a few studies have shown no increase in the incidence of azole-resistant *Candida* species following azole prophylaxis (14, 29). At least one report recorded an increase in *C. krusei* among immunocompromised patients who had not been treated with fluconazole (11).

Our data demonstrate statistically significantly higher MIC values for *C. glabrata* among patients who received fluconazole prophylaxis. Alexander and coworkers (3) found that five HSCT patients who had previous exposure to fluconazole developed infections with *C. glabrata* isolates with high MIC values to both fluconazole and voriconazole. Our results were similar. Of the 25 *C. glabrata* cases with MIC values of ≥2 μg/ml (susceptible-dose dependent to resistant) to voriconazole, 17 patients (68%) had received prior fluconazole prophylaxis. Of note is that any amphotericin B use prior to *Candida* infection was also associated with fluconazole nonsusceptibility in HSCT patients. This may be indicative of the underlying susceptibility of the patient to IFIs in general, and in an HSCT patient if the IFI was *Candida*, it was likely *C. glabrata*.

Our study provided further evidence that any prior fluconazole exposure was associated with fluconazole nonsusceptibility. In addition to higher geometric mean MICs among those with prior fluconazole exposure, prior fluconazole use in SOT patients was independently associated with fluconazole nonsusceptibility when analyzed by a multivariate model; this association was not as strong among HSCT patients. CMV antigenemia was associated with fluconazole nonsusceptibility among HSCT patients only, as has been noted previously (17). However, it is possible that some of these associations represent colinearity with other conditions that were not captured by our data collection form. For example, we found that diabetes was significantly associated with nonsusceptibility for both types of transplants. This relationship may reflect the higher risk of infection among transplant recipients receiving

TABLE 4. Risk factors associated with fluconazole-nonsusceptible *Candida* species from HSCT and SOT recipients after bivariate analysis

Transplant type and characteristic	*n* (%)		OR (95% CI)	*P* value
	Susceptible[a]	Not susceptible[b]		
HSCT				
Median age, yr (range)	44 (1–69)	45 (10–72)	1.24 (0.58–2.65)	0.576
Female	33 (51)	22 (48)	0.89 (0.42–1.89)	0.760
Caucasian	52 (80)	38 (83)	1.19 (0.45–3.15)	0.730
Hispanic	12 (19)	3 (7)	0.30 (0.08–1.14)	0.076
3-mo mortality	34 (52)	29 (63)	1.56 (0.72–3.37)	0.262
Underlying diseases and comorbid conditions				
Non-Hodgkin's lymphoma	8 (12)	12 (26)	2.52 (0.93–6.77)	0.068
Multiple myeloma	9 (14)	1 (2)	0.14 (0.02–1.13)	0.065
CMV antigenemia	8 (12)	13 (28)	2.81 (1.05–7.48)	0.039
Diabetes, acquired since transplant	8 (12)	15 (33)	3.45 (1.32–9.03)	0.012
Diabetes, active at time of IFI	9 (14)	17 (37)	3.65 (1.45–9.19)	0.006
Candida species				
C. glabrata	12 (18)	28 (61)	6.87 (2.90–16.27)	<0.001
C. krusei	2 (3)	15 (33)	15.24 (3.28–70.87)	<0.001
Medications used in prior 3 mo				
Inhaled amphotericin B	2 (3)	7 (15)	5.65 (1.12–28.61)	0.036
Any amphotericin B	12 (18)	16 (35)	2.36 (0.99–5.64)	0.054
Fluconazole	22 (34)	25 (54)	2.33 (1.07–5.05)	0.033
Any antifungal prophylaxis[c]	33 (51)	36 (78)	3.49 (1.49–8.19)	0.004
Granulocyte colony-stimulating factor	21 (32)	24 (52)	2.29 (1.05–4.98)	0.037
Foscarnet	5 (8)	10 (22)	3.33 (1.06–10.53)	0.040
SOT				
Median age, yr (range)	49 (1–77)	54 (2–80)	1.42 (0.70–2.89)	0.329
Female	73 (37)	24 (65)	3.14 (1.51–6.54)	0.002
Caucasian	153 (77)	32 (86)	1.88 (0.69–5.11)	0.215
Hispanic	11 (6)	2 (5)	0.97 (0.21–4.57)	0.971
3-mo mortality	55 (28)	10 (29)	1.03 (0.47–2.29)	0.937
Comorbid conditions/clinical presentation				
Diabetes, acquired since transplant	36 (18)	14 (38)	2.74 (1.29–5.84)	0.009
Diabetes, active at time of IFI	96 (48)	13 (35)	0.58 (0.28–1.20)	0.138
Hospitalized at time of IFI	127 (64)	31 (84)	2.89 (1.15–7.26)	0.024
Mental status changes within 7 days of IFI	16 (8)	8 (22)	3.14 (1.23–7.99)	0.017
Candida species				
C. albicans	111 (56)	3 (8)	0.07 (0.02–0.23)	<0.001
C. glabrata	40 (20)	21 (57)	5.18 (2.48–10.84)	<0.001
C. krusei	4 (2)	9 (24)	15.59 (4.50–54.01)	<0.001
Medications used in prior 3 mo				
Fluconazole	45 (23)	18 (49)	3.22 (1.56–6.65)	0.002
Any itraconazole	2 (1)	3 (8)	8.65 (1.40–53.68)	0.021
Any antifungal prophylaxis	26 (13)	13 (35)	3.58 (1.63–7.90)	0.002
Granulocyte colony-stimulating factor	8 (4)	4 (11)	2.88 (0.82–10.11)	0.099
Ganciclovir	61 (31)	20 (54)	2.64 (1.30–5.39)	0.008

[a] $n = 65$ for HSCT; $n = 198$ for SOT.
[b] $n = 46$ for HSCT; $n = 37$ for SOT.
[c] Any antifungal prophylaxis includes fluconazole, itraconazole, voriconazole, posaconazole, ravuconazole, amphotericin B, caspofungin, micafungin, anidulafungin, ketoconazole, and flucytosine.

higher doses of corticosteroids (and therefore corticosteroid-induced diabetes). However, diabetes has not been frequently described as a risk factor for resistant *Candida*, and this deserves further study.

Other studies have demonstrated higher *Candida* MIC values among patients receiving fluconazole prophylaxis. A recent study examined data from patients who underwent allogeneic HSCT or who had acute myelogenous leukemia and received prophylactic posaconazole, itraconazole, or fluconazole (16). The number of patients with *C. albicans* colonization decreased with antifungal therapy in all arms of the study, while the proportion of patients with *C. glabrata* colonization increased in the posaconazole and itraconazole arms and the proportion of patients with *C. krusei* colonization increased in the fluconazole arm. Perhaps their most insidious finding was that in 40% of the *C. glabrata*-colonized patients, the MICs for

TABLE 5. Multivariate models for fluconazole nonsusceptibility in *Candida* isolates from HSCT and SOT recipients

Transplant type and characteristic	OR (95% CI)	*P* value
HSCT		
Any fluconazole use prior to IFI	2.66 (0.93–7.62)	0.069
C. glabrata	10.47 (3.58–30.62)	<0.001
Non-Hodgkin's lymphoma	3.96 (1.00–15.59)	0.049
CMV antigenemia	5.02 (1.32–19.05)	0.018
Diabetes, currently active	5.25 (1.54–17.90)	0.008
Any amphotericin B use prior to IFI	5.69 (1.71–18.94)	0.005
Gender, female	0.77 (0.28–2.17)	0.625
Race, non-Caucasian	0.86 (0.22–3.37)	0.825
Median age	0.64 (0.21–1.92)	0.423
SOT		
Any fluconazole use prior to IFI	2.65 (1.17–5.99)	0.019
C. glabrata	4.70 (2.08–10.58)	<0.001
Ganciclovir use in 3 mo prior to IFI	2.19 (0.99–4.86)	0.053
Diabetes, acquired since transplant	2.73 (1.13–6.59)	0.025
Gender, female	2.33 (1.03–5.26)	0.042
Race, non-Caucasian	0.50 (0.16–1.57)	0.234
Median age	1.08 (0.48–2.44)	0.850

the isolates increased more than 4-fold during the course of prophylactic therapy. Similarly, Trifilio and colleagues (31) reported that of the six breakthrough *Candida* infections in HSCT patients receiving voriconazole prophylaxis, five of the isolates were *C. glabrata* and one was *C. krusei*. Although susceptibility testing was not performed, the authors noted that all of the infections occurred among inpatients with voriconazole trough levels of <2 μg/ml.

This study had several limitations. While TRANSNET captured 10 to 20% of all transplants occurring in the United States during the surveillance period, it may not be representative of the entire U.S. transplant population. Additionally, not all of the participating centers sent isolates to CDC in a proportional manner. While the isolates studied at CDC did not largely differ from the overall cohort with respect to transplant type and antifungal use, the species identification was not reported in a substantial number of cases, so an exact correlation cannot be obtained and there is a possibility of selection bias. Another limitation is the risk of a spurious significant finding from the multivariate modeling due to the small sample size and a large number of variables considered in the model selection process. A number of nonsignificant variables from the bivariate analysis were included in the multivariate modeling to control for factors believed to influence antifungal susceptibility, increasing the number of variables considered during the model selection process. Finally, Etest is a nonreference method for susceptibility testing.

Fluconazole nonsusceptibility does seem to be prevalent in the overall transplant population. However, the problem relates not to individual isolates of multiple species demonstrating resistance but rather to the high frequency of *C. glabrata* and *C. krusei*. Based on the results of this study, susceptibility testing of *C. glabrata* isolates would be recommended prior to treatment with azoles. The rates of resistance to fluconazole and voriconazole for *C. albicans* and *C. parapsilosis* were exceedingly low (1%) despite widespread use of antifungal prophylaxis, and therefore routine antifungal susceptibility testing of these two species is not recommended. However, susceptibility testing should still be considered for breakthrough during prophylaxis or for other instances when clinical judgment would dictate usefulness, regardless of the species. Our results also suggest that susceptibility testing of isolates from patients responding to caspofungin therapy may not be warranted at this time.

ACKNOWLEDGMENTS

We thank Mary Brandt for her extremely helpful critical reading of the manuscript and Kathleen Wannemuehler for help with the data collection.

TRANSNET was sponsored by Astellas, Pfizer, Merck, and Schering-Plough.

Potential conflicts of interest are as follows: P.G.P. receives research support from and is an *ad hoc* advisor for Merck, Pfizer, and Astellas; J.W.B. received research support from Pfizer and is a consultant/advisor for Merck and Pfizer; J.I.I. received honoraria for speaker activities from Astellas, Cubist, and Pfizer; D.R.A. has received grants from and is a consultant for Merck, Astellas, and Pfizer; and C.A.K. receives research support from Merck and chairs a data adjudication committee for Pfizer. All other authors have no conflicts.

The findings and conclusions of this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

REFERENCES

1. **Abi-Said, D., et al.** 1997. The epidemiology of hematogenous candidiasis caused by different *Candida* species. Clin. Infect. Dis. **24:**1122–1128.
2. **Ahlquist, A., et al.** 2009. Epidemiology of candidemia in metropolitan Atlanta and Baltimore city and county: preliminary results of population-based active, laboratory surveillance—2008–2010, abst. M-1241. Abstr. 49th Intersci. Conf. Antimicrob. Agents Chemother., San Francisco, CA.
3. **Alexander, B. D., W. A. Schell, J. L. Miller, G. D. Long, and J. R. Perfect.** 2005. *Candida glabrata* fungemia in transplant patients receiving voriconazole after fluconazole. Transplantation **80:**868–871.
4. **Ascioglu, S., et al.** 2002. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin. Infect. Dis. **34:**7–14.
5. **Bhatti, Z., A. Shaukat, N. G. Almyroudis, and B. H. Segal.** 2006. Review of epidemiology, diagnosis, and treatment of invasive mould infections in allogeneic hematopoietic stem cell transplant recipients. Mycopathologia **162:** 1–15.
6. **Clinical and Laboratory Standards Institute.** 2008. M27–S3 reference method for broth dilution antifungal susceptibility testing of yeasts, third informational supplement. Clinical and Laboratory Standards Institute, Wayne, PA.
7. **Courtney, R., S. Pai, M. Laughlin, J. Lim, and V. Batra.** 2003. Pharmacokinetics, safety, and tolerability of oral posaconazole administered in single and multiple doses in healthy adults. Antimicrob. Agents Chemother. **47:** 2788–2795.
8. **Goodman, J. L., et al.** 1992. A controlled trial of fluconazole to prevent fungal infections in patients undergoing bone marrow transplantation. N. Engl. J. Med. **326:**845–851.
9. **Hajjeh, R. A., et al.** 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.
10. **Hope, W., A. Morton, and D. P. Eisen.** 2002. Increase in prevalence of nosocomial non-*Candida albicans* candidaemia and the association of *Candida krusei* with fluconazole use. J. Hosp. Infect. **50:**56–65.
11. **Iwen, P. C., D. M. Kelly, E. C. Reed, and S. H. Hinrichs.** 1995. Invasive infection due to *Candida krusei* in immunocompromised patients not treated with fluconazole. Clin. Infect. Dis. **20:**342–347.
12. **Kao, A. S., et al.** 1999. The epidemiology of candidemia in two United States cities: results of a population-based active surveillance. Clin. Infect. Dis. **29:**1164–1170.
13. **Kontoyiannis, D. P., et al.** 2010. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001–2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) database. Clin. Infect. Dis. **50:**1091–1100.
14. **Kunova, A., et al.** 1997. Eight-year surveillance of non-*albicans Candida* spp. in an oncology department prior to and after fluconazole had been introduced into antifungal prophylaxis. Microb. Drug Resist. **3:**283–287.
15. **Linton, C. J., et al.** 2007. Molecular identification of unusual pathogenic yeast isolates by large ribosomal subunit gene sequencing: 2 years of experience at the United Kingdom mycology reference laboratory. J. Clin. Microbiol. **45:**1152–1158.

16. **Mann, P. A., et al.** 2009. Impact of antifungal prophylaxis on colonization and azole susceptibility of *Candida* species. Antimicrob. Agents Chemother. **53:**5026–5034.
17. **Marr, K. A., K. Seidel, T. C. White, and R. A. Bowden.** 2000. Candidemia in allogeneic blood and marrow transplant recipients: evolution of risk factors after the adoption of prophylactic fluconazole. J. Infect. Dis. **181:**309–316.
18. **National Committee for Clinical Laboratory Standards.** 2002. M27–A2 reference method for broth dilution antifungal susceptibility testing of yeasts: Approved standard, 2nd ed. National Committee for Clinical Laboratory Standards, Wayne, PA.
19. **Neofytos, D., et al.** 2009. Epidemiology and outcome of invasive fungal infection in adult hematopoietic stem cell transplant recipients: analysis of Multicenter Prospective Antifungal Therapy (PATH) Alliance registry. Clin. Infect. Dis. **48:**265–273.
20. **Pappas, P. G., et al.** 2010. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clin. Infect. Dis. **50:**1101–1111.
21. **Pfaller, M. A., et al.** 2007. Results from the ARTEMIS DISK Global Antifungal Surveillance study, 1997 to 2005: an 8.5-year analysis of susceptibilities of *Candida* species and other yeast species to fluconazole and voriconazole determined by CLSI standardized disk diffusion testing. J. Clin. Microbiol. **45:**1735–1745.
22. **Pfaller, M. A., et al.** 2010. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-year analysis of susceptibilities of *Candida* species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. J. Clin. Microbiol. **48:**1366–1377.
23. **Pfaller, M. A., et al.** 2008. Correlation of MIC with outcome for *Candida* species tested against caspofungin, anidulafungin, and micafungin: analysis and proposal for interpretive MIC breakpoints. J. Clin. Microbiol. **46:**2620–2629.
24. **Pfaller, M. A., et al.** 2008. *Candida krusei*, a multidrug-resistant opportunistic fungal pathogen: geographic and temporal trends from the ARTEMIS DISK Antifungal Surveillance Program, 2001 to 2005. J. Clin. Microbiol. **46:**515–521.
25. **Pfaller, M. A., et al.** 2009. Variation in susceptibility of bloodstream isolates of *Candida glabrata* to fluconazole according to patient age and geographic location in the United States in 2001 to 2007. J. Clin. Microbiol. **47:**3185–3190.
26. **Safdar, A., F. van Rhee, J. P. Henslee-Downey, S. Singhal, and J. Mehta.** 2001. *Candida glabrata* and *Candida krusei* fungemia after high-risk allogeneic marrow transplantation: no adverse effect of low-dose fluconazole prophylaxis on incidence and outcome. Bone Marrow Transpl. **28:**873–878.
27. **Sanglard, D., and F. C. Odds.** 2002. Resistance of *Candida* species to antifungal agents: molecular mechanisms and clinical consequences. Lancet Infect. Dis. **2:**73–85.
28. **Silveira, F. P., and S. Husain.** 2007. Fungal infections in solid organ transplantation. Med. Mycol. **45:**305–320.
29. **Slavin, M. A., et al.** 2010. Candidaemia in adult cancer patients: risks for fluconazole-resistant isolates and death. J. Antimicrob. Chemother. **65:**1042–1051.
30. **Trick, W. E., S. K. Fridkin, J. R. Edwards, R. A. Hajjeh, R. P. Gaynes, and National Nosocomial Infections Surveillance System Hospitals.** 2002. Secular trend of hospital-acquired candidemia among intensive care unit patients in the United States during 1989–1999. Clin. Infect. Dis. **35:**627–630.
31. **Trifilio, S., et al.** 2007. Breakthrough fungal infections after allogeneic hematopoietic stem cell transplantation in patients on prophylactic voriconazole. Bone Marrow Transpl. **40:**451–466.
32. **White, M. H.** 1997. The contribution of fluconazole to the changing epidemiology of invasive candidal infections. Clin. Infect. Dis. **24:**1129–1130.
33. **Wingard, J. R., et al.** 1993. Association of *Torulopsis glabrata* infections with fluconazole prophylaxis in neutropenic bone marrow transplant patients. Antimicrob. Agents Chemother. **37:**1847–1849.
34. **Wingard, J. R., et al.** 1991. Increase in *Candida krusei* infection among patients with bone marrow transplantation and neutropenia treated prophylactically with fluconazole. N. Engl. J. Med. **325:**1274–1277.