Diagnostic Microbiology and Infectious Disease 79 (2014) 280–286

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Association of clinical and demographic factors in invasive candidiasis caused by fluconazole-resistant *Candida* species: a study in 15 hospitals, Medellín, Colombia 2010–2011

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ARTICLE INFO

Article history:
Received 7 October 2013
Accepted 2 February 2014
Available online 22 February 2014

Keywords:
Candidiasis
Antifungal susceptibility
Fluconazole resistance
Risk factors

ABSTRACT

Candida is the most important agent of fungal infections. Several risk factors have been described associated with invasive infection by fluconazole-resistant *Candida* spp. A prospective cross-sectional study with case–control analysis was conducted. Case group patients with fluconazole-resistant *Candida* isolate were included; control group were patients with fluconazole-susceptible *Candida* spp. A multivariate logistic regression model was performed. Three hundred isolates of *Candida* spp. were analyzed. Most frequent species were *Candida albicans*/*Candida dubliniensis* (48.3%) and *Candida tropicalis* (22.3%). Posaconazole susceptibility was 93.7%; voriconazole, 84%; and fluconazole, 78.7%. Susceptibility to anidulafungin and caspofungin was 92.7% and 92.3%, respectively. Neutropenia (adjusted odds ratio [aOR] 6.5, 95% confidence interval [CI] 1.0–43.1), antifungal exposure (aOR 5.1, 95% CI 2.3–11.2), and antituberculosis therapy (aOR 7.7, 95% CI 1.4–43.2) were associated to fluconazole resistance. Susceptibility results are useful to guide the selection of empiric antifungal treatment and the design of local therapeutic guidelines. Previous antifungal exposure suggests possible resistance to fluconazole, pointing towards the selection of a different class of antifungal agents.

1. Introduction

Candida is considered the most important causative agent of opportunistic fungal infections and a rising problem worldwide. *Candida* spp. are one of the most common causes of bloodstream infection in the United States (Hidron et al., 2008) and one of the most frequent isolates from infected patients in intensive care units (ICUs) in many countries (Vincent et al., 2009). Mortality associated to these infections is near 40%, making candidiasis a major health problem in hospital settings (Gudlaugsson et al., 2003; Pappas et al., 2003; Wey et al., 1988).

Several studies on candidiasis have shown notable variations in the prevalence of *Candida* spp. involved and antifungal susceptibility patterns according to geographical location and study population (Nucci et al., 2010; Pfaller et al., 2009). The Global Antifungal Surveillance Program, ARTEMIS DISK, analyzed a total of 256,882 *Candida* isolates obtained from 142 medical centers in Asia, Latin America, Europe, Africa, and North America between 1997 and 2007, showing that the most common species globally was *C. albicans* (65.3%), followed by *Candida glabrata* (11.3%), *Candida tropicalis* (7.2), *Candida parapsilosis* (6.0%), and *Candida krusei* (2.4%). These 5 *Candida* spp. are important in the 5 geographical regions, but their frequency varied significantly according to the setting (Pfaller et al., 2010a). Local studies in patients with invasive candidiasis infections

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http://dx.doi.org/10.1016/j.diagmicrobio.2014.02.003
0732-8893/

hospitalized in the ICUs of Medellin, Colombia, showed that *C. albicans* was the most frequently isolated (43.6%) species, followed by *C. tropicalis* (23.4%) and *C. parapsilosis* (13.9%) (Zuluaga et al., 2010).

In recent decades, the prevalence of these infections has significantly increased in critically ill patients, particularly those undergoing prolonged ICU hospitalization or invasive diagnostic procedures such as catheterism, mechanical ventilation, parenteral nutrition, hemodialysis, blood transfusions, prolonged antibiotic, and immunosuppressive treatments (Del Palacio et al., 2006). In addition, other studies have shown an increased frequency of invasive infections caused by *Candida* spp. with resistance to azoles, first-line drugs for the treatment of these infections, posing a particular challenge for clinical management (Bassetti et al., 2006).

Studies worldwide have reported the association of clinical and demographic factors with invasive infection by azole-resistant *Candida* spp. Among these factors are the Acute Physiology and Chronic Health Evaluation score, neutropenia, chronic renal disease, previous exposure to fluconazole, previous specific antibiotic exposition to piperacillin-tazobactam and vancomycin, history of gastrointestinal surgery, hematologic transplant recipients, and neonatal age (Garnacho-Montero et al., 2010; Lee et al., 2009; Lin et al., 2005; Playford et al., 2008).

In the present study, we determined the frequency and antifungal susceptibility profiles of invasive *Candida* spp. infections in hospitalized patients from third-level hospitals in our area, in order to determine the factors associated with invasive infection by fluconazole-resistant *Candida* spp.

2. Materials and methods

2.1. Study design

We conducted a descriptive prospective, cross-sectional study with a case–control analysis to determine factors associated with the isolation of fluconazole-resistant *Candida* strains from sterile fluids or specimens obtained from closed tissue sites. In this study, the case group included patients with susceptible dose-dependent or fluconazole-resistant *Candida* strains, while the control group consisted of patients from whom a fluconazole-susceptible *Candida* isolate was obtained.

2.2. Study sites and patients

The study population consisted of hospitalized patients including all age groups who had a *Candida* spp. isolated from blood cultures, sterile fluids, or specimens obtained from closed tissue sites. Patients were recruited from 15 hospitals located within Metropolitan Area of Medellín, Colombia, during a period of 15 months, between August 2010 and November 2011. Of the 15 institutions, 14 had high complexity hospital services including neonatal, pediatric, and adult ICUs, as well as coronary care units.

Initial identification of the fungal isolates and, in some cases, susceptibility tests were performed at the microbiology laboratory of each participant hospital using either manual or automated procedures. Clinical and demographic information was collected from the medical record of the corresponding patient. The fungal isolates were transported using Culture Swab Amies transport medium with charcoal (BD BBL™ CultureSwab™), to the Medical and Experimental Mycology Laboratory at the Corporación para Investigaciones Biológicas (CIB), where the initial identification of *Candida* spp. was confirmed by phenotypic characteristics and complete antifungal susceptibility tests were performed. This study had the approval of the Ethics Committee of the CIB and included the review and approval of hospitals participating in the selection of patients. Informed consent of the patients was not required because the intervention in the study was limited to laboratory analysis of the *Candida* spp. isolates from hospitalized patients, obtained as part of their clinical care. No additional procedures were performed, and there were no risks associated to this research.

2.3. Microbiological methods

Identification of *Candida* spp. was performed on the microbiology laboratories either by standard procedures or biochemical analysis using Vitek 2 System Yeast identification cards (YST ID Card bioMerieux, Marcy-l'Étoile, France).

The isolates were referred to a central laboratory, and *Candida* isolates were cultured in ChromoAgar medium (bioMerieux) and incubated for 24 hours at 35 °C and identification was confirmed using API 20C AUX (bioMerieux). The identification of the isolates was not confirmed by molecular methods. Antifungal susceptibility was performed using the agar diffusion method with concentration gradients strips for fluconazole, itraconazole, voriconazole, posaconazole, amphotericin B, caspofungin, and anidulafungin (Etest; bioMerieux) following standard recommendations (Etest® Technical manual, AB bioMerieux, www.abbiodisk.com). After 24–48 hours, MIC was read by an experienced microbiologist, for each antifungal at the site where the growth inhibition ellipse intercepted the scale of antifungal concentrations. As fluconazole resistance was the outcome variable in this study, MICs were also determined for fluconazole by the reference broth microdilution method, following CLSI guidelines (CLSI, 2008). Discordant results between Etest and broth microdilution were excluded from epidemiological analysis.

The MICs breakpoints for fluconazole used were: for *C. albicans*/*C. dubliniensis*, *C. tropicalis*, and *C. parapsilosis* complex isolates with MIC ≤2 μg/mL were considered susceptible, those with MIC 4 μg/mL were considered susceptible dose-dependent, and those with MIC ≥8 were considered resistant; MIC >32 μg/mL were considered resistant for *C. glabrata*. Isolates of *C. krusei* were considered intrinsically resistant to fluconazole (Pfaller et al., 2010a).

For voriconazole, isolates of *C. albicans*/*C. dubliniensis*, *C. tropicalis*, and *C. parapsilosis* complex with MIC ≤0.125 μg/mL were considered susceptible and with MIC ≥1 μg/mL were considered resistant. For *C. glabrata* and *C. krusei*, MIC ≤0.5 μg/mL was considered susceptible, and MIC ≥2 μg/mL was considered resistant. For all species, MIC ≤1 μg/mL for posaconazole, MIC ≤0.125 μg/mL for itraconazole, and ≤1 μg/mL for amphotericin B were considered susceptible (Pfaller et al., 2011a, Sims et al., 2006).

The breakpoints for anidulafungin and caspofungin were: for *C. albicans*/*C. dubliniensis*, *C. tropicalis*, and *C. krusei* isolates with MIC ≤0.25 μg/mL were considered susceptible, and those with MIC ≥1 μg/mL were considered resistant; *C. parapsilosis* complex and *Candida guilliermondii* were considered susceptible with an MIC ≤2 μg/mL and resistant with an MIC ≥8 μg/ml. For *C. glabrata*, an MIC ≤0.12 μg/mL was susceptible, and MIC ≥0.5 μg/mL was considered resistant. Quality control was performed by testing *C. krusei* ATCC 6258 (Pfaller et al., 2011b).

2.4. Variables

Data were obtained from the patient's medical and laboratory records, according to a questionnaire that included demographic variables (age and sex), comorbidities (diabetes mellitus, malignancy, HIV infection or AIDS, tuberculosis, renal disease [defined as any disease that alters the renal function in an acute or chronic form], hepatic disease [defined as any disease that alters the hepatic function in an acute or chronic form], extensive burns, neutropenia, intravenous drug abuse, chemotherapy, autoimmune disease, prematurity, congenital malformations, organ or bone marrow transplant recipient), treatments prior to a positive culture (antifungal and antibiotic therapy, antituberculosis drugs, steroids, and other immunosuppressive treatments), invasive procedures prior to *Candida* spp. isolation (peripheral, central venous, or a urinary catheter; total parenteral

Table 1
Clinical specimens rendering positive cultures for *Candida* spp.

Specimens	No.	%
Blood	146	48.7
Abdominal fluid	112	37.3
Pleural fluid	16	5.3
Bile	7	2.3
Bone	7	2.3
Tissue (biopsy)	5	1.7
Amniotic fluid	2	0.7
Cerebrospinal fluid	2	0.7
Abscess	2	0.7
Lymph node	1	0.3

nutrition; mechanical ventilation; hemodialysis; transfusions; abdominal surgery), variables such as length of stay in the ICU, and main initial diagnosis. The period of time considered for drug exposition and invasive procedures went from the date of admission to the hospital to date of *Candida* spp. isolation in each patient.

2.5. Statistical analysis

The Whonet software (WHO Collaborating Centre for Surveillance of Antimicrobial Resistance) was used to determine the frequencies of *Candida* spp. causing invasive infections and their susceptibility to fluconazole, voriconazole, posaconazole, itraconazole, amphotericin B, caspofungin, and anidulafungin by Etest and broth microdilution. Mann–Whitney U-test was used to compare medians of quantitative variables without normal distribution; the Chi^2 test and Fisher's exact test were used to test the hypotheses in order to establish statistically significant differences in categorical variables between groups (cases and control). Variables with P value ≤0.25 on bivariate analysis were included in the multivariable logistic regression model. Adjusted odds ratios (aORs) and their respective confidence intervals (CIs) were calculated. The predictive power of the model was evaluated using the goodness-of-fit Hosmer-Lemeshow test, and Nagelkerke R square was used to determinate the percentage in which changes in dependent variable are explained by the independent variables. Variables with a P value ≤0.05 were retained in the final model. SPSS software 15.0 (SPSS®, Inc., Chicago, IL, USA) was used to perform the statistical analyses.

3. Results

During the study period, 300 isolates of *Candida* spp. from 260 patients were isolated from sterile fluids and closed tissue sites specimens. Two hundred isolates (66.7%) were from patients in the ICUs. Fifty-seven percent of the patients were male. The average age was 45.3 years (range 0–94 years); 11% of the patients were below 1 year of age; 7%, between 1 and 14 years; 28%, between 15 and 44 years; 19%, between 45 and 60 years; and 35% were older than 60 years. The average days of hospitalization prior to isolation of *Candida* were 28.8 (range 1–368 days). More frequent clinical specimens were blood (48.7%), abdominal fluid (37.3%), pleural fluid (5.3%), and bile (2.3%) (Table 1).

According to species, *C. albicans*/*C. dubliniensis* was predominant (48.3%), followed by *C. tropicalis* (22.3%), C. *parapsilosis* complex (15%), *C. glabrata* (6.7%), *C. guilliermondii* (4%), and *C. krusei* (2.7%). In addition, 2 isolates were identified as *Candida lusitaniae* (0.7%), and 1, as *Candida famata* (0.3%). No differences were observed in the distribution of *Candida* spp. isolated from patients hospitalized in ICUs as compared to other services.

Table 2 shows the distribution of MICs of fluconazole, voriconazole, itraconazole, and posaconazole by Etest. For the most frequent *Candida* spp. analyzed in this study, *C. albicans*/*C. dubliniensis*, *C. parapsilosis* complex, and *C. tropicalis*, representing all together 85% of the isolates, MICs distributions for itraconazole and posaconazole were similar. At least 90% of isolates of these 3 species had an MIC between 0.5 μg/mL

Table 2
In vitro susceptibilities of 300 isolates of *Candida* spp. to fluconazole, itraconazole, posaconazole, and voriconazole.

Organism	Agent	No. of isolates tested	Cumulative % of isolates susceptible at an MIC of											
			0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	≥64
C. albicans/*C. dubliniensis*	FL	144	9.8	16.1	45.2	71.6	81.3	**91**	92.4	92.4	93.8	95.2	95.9	100
	IT		9.8	20.9	47.9	81.3	**93.8**	95.9	96.6	97.3	98	98	100	
	POS		46	80.1	**93.3**	95.4	96.1	98.2	98.2	98.9	98.9	98.9	10	
	VO		87.5	**90.3**	91.7	93.1	94.5	95.9	95.9	97.3	97.3	97.3	98.7	100
C. parapsilosis complex	FL	45	11.1	17.7	28.8	35.5	55.5	66.6	75.5	79.9	88.7	**95.3**	100	
	IT		2.2	15.5	33.2	62.1	**93.2**	95.4	100					
	POS		37.7	59.9	88.8	**95.5**	100							
	VO		66.6	75.5	86.6	**93.2**	100							
C. tropicalis	FL	68			3	4.5	41.3	82.5	89.8	**92.8**	95.8	97.3	97.3	100
	IT		8.9	11.8	20.6	47.1	76.5	**95.6**	98.6	98.6	98.6	98.6	98.6	100
	POS		20.5	42.5	70.4	86.6	**98.4**	98.4	98.4	98.4	98.4	98.4	98.4	100
	VO		35.3	64.7	82.3	88.1	**91.1**	95.5	98.5	98.5	98.5	98.5	98.5	100
C. glabrata	FL	20					10	10	15	20	35	65	85	**100**
	IT				10	15	30	40	45	55	65	65	75	**100**
	POS		15	15	20	20	30	45	55	65	65	70	75	**100**
	VO		5	15	25	45	60	85	85	85	85	**90**	95	100
C. guilliermondii	FL	12		8.3	8.3	8.3	8.3	8.3	50	58.3	58.3	66.6	74.9	**100**
	IT					16.6	16.6	33.3	66.6	**91.6**	100			
	POS		16.6	16.6	24.9	24.9	66.6	**91.6**	100					
	VO		24.9	33.2	58.2	74.8	74.8	83.1	**100**					
C. krusei	FL	8											12.5	**100**
	IT						12.5	37.5	75	75	75	75	75	**100**
	POS					12.5	50	62.5	87.5	87.5	87.5	87.5	87.5	**100**
	VO					37.5	50	87.5	87.5	87.5	**100**			
Total	FL	300	6.3	10.6	26.9	41.6	58.2	73.8	79.5	81.5	85.1	89.5	**92.9**	100
	IT		6.9	14.9	33.2	60.9	79.9	87.5	**91.8**	93.8	95.5	95.5	97.2	100
	POS		34.7	59.7	77.4	83.4	**90.4**	93.7	95.4	96.4	96.4	96.7	97.7	100
	VO		62.1	72.4	80.4	86.7	**90.4**	95	96.3	97	97.3	97.6	98.6	100

MICs values at which an inhibition at least 90% of isolates was observed are shown in bold type.
FL = fluconazole; IT = itraconazole; POS = posaconazole; VO = voriconazole.

Table 3
In vitro susceptibilities of 300 isolates of *Candida* spp. to amphotericin B, anidulafungin, and caspofungin.

Organism	Agent	No. of isolates tested	Cumulative % of isolates susceptible at an MIC of										
			0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	>32
C. albicans/*C. dubliniensis*	AP	144	7.7	14	52.9	**93.1**	97.3	99.4	100					
	AND		**94.6**	96.7	97.4	98.1	98.1	98.1	98.8	98.8	98.8	98.8	99.5	100
	CS		34.9	60.6	**92.5**	98.1	99.5	100						
C. parapsilosis complex	AP	45	13.2	19.8	37.6	70.9	**93.1**	100						
	AND		4.4	4.4	6.6	11	22.1	48.7	70.9	77.5	**90.9**	90.9	95.3	100
	CS		4.4	4.4	6.6	19.9	62.1	86.5	**97.6**	97.6	97.6	97.6	100	
C. tropicalis	AP	68	4.5	8.9	14.8	35.4	**92.7**	100						
	AND		86.9	89.8	**94.2**	95.7	98.6	100						
	CS		11.7	35.2	88.1	**96.9**	96.9	100						
C. glabrata	AP	20		5	10	40	85	**95**	100					
	AND		**95**	95	100									
	CS		15	35	70	**100**								
C. guilliermondii	AP	12			25	66.7	75	**100**						
	AND		24.9	24.9	24.9	24.9	33.2	74.9	83.2	**100**				
	CS				8.3	58.3	83.3	**100**						
C. krusei	AP	8					25	75	**100**					
	AND		75	87.5	**100**									
	CS				12.5	50	87.5	**100**						
Total	AP	300	6.6	11.9	36.2	69.2	**91.5**	98.1	99.5	99.5	99.5	99.5	100	
	AND		75.6	77.9	80.2	81.6	84.2	**90.2**	94.2	95.9	97.9	97.9	98.9	100
	CS		20.9	39.9	70.9	82.6	**92.3**	98	99.6	99.6	99.6	99.6	100	

MICs values at which an inhibition at least 90% of isolates was observed are shown in bold type.
AP = amphotericin B; AND = anidulafungin; CS = caspofungin.

and 1 μg/mL for itraconazole and from 0.125 μg/mL to 0.5 μg/mL for posaconazole. A different distribution was observed for the same species for fluconazole, 91% of the isolates of *C. albicans*/*C. dubliniensis* were inhibited at 1 μg/mL MIC, whereas at the same concentration, only 82.5% of isolates of *C. tropicalis* and 66.6% of those of *C. parapsilosis* complex were inhibited. Isolates of *C. glabrata* and *C. krusei* were inhibited at higher concentrations by the 4 azole antifungals.

Distribution of MICs for amphotericin B and echinocandins are shown in Table 3. Ninety-one percent of isolates had an MIC of 0.5 μg/mL or less for amphotericin B. For *C. parapsilosis* complex, 97.6% of isolates had MICs ≤2 μg/mL for caspofungin, in contrast with 95.3% of isolates requiring ≤32 μg/mL of anidulafungin to be inhibited. For other *Candida* spp., except for *C. guilliermondii* that exhibited a similar behavior as *C. parapsilosis* complex, MICs distribution for anidulafungin were lower than those observed for caspofungin.

Results of interpretation of the MICs in susceptible, susceptible dose-dependent, and resistant categories are shown in Table 4. Ninety-eight percent of 300 isolates of *Candida* spp. were susceptible to amphotericin B; none of the resistant isolates were susceptible dose dependent. In general, disregarding the *Candida* spp., the most active azole was posaconazole with 93.7% of susceptibility, followed by voriconazole (84%) and fluconazole with 78.7% of isolates susceptible,

Table 4
Susceptibility patterns of 300 *Candida* spp. isolates obtained from invasive infections.

Species	No. of isolates	%	AP	AND	CS	FL	IT	POS	VO
C. albicans/*C. dubliniensis*	144	S	99.3	97.9	97.9	92.4	47.9	97.9	91.7
		SDD	0	0	1.4	0	45.8	0	2.1
		R	0.7	2.1	0.7	7.6	6.2	2.1	6.2
C. tropicalis	68	S	100	95.6	97.1	89.7	20.6	98.5	82.4
		SDD	0	2.9	0	2.9	55.9	0	8.8
		R	0	1.5	2.9	7.4	23.5	1.5	8.8
C. parapsilosis complex	45	S	100	71.1	97.8	75.6	33.3	100	86.7
		SDD	0	6.7	0	4.4	60	0	13.3
		R	0	22.2	2.2	20	6.7	0	0
C. glabrata	20	S	95	95	35	0	10	45	60
		SDD	0	5	65	85	20	10	0
		R	5	0	0	15	70	45	40
C. guilliermondii	12	S	100	83.3	100	58.3	0	91.7	83.3
		SDD	0	16.7	0	16.7	16.7	8.3	16.7
		R	0	0	0	25	83.3	0	0
C. krusei	8	S	75	100	50	0	0	62.5	50
		SDD	0	0	37.5	12.5	12.5	25	37.5
		R	25	0	12.5	87.5	87.5	12.5	12.5
C. lusitaniae	2	S	100	100	100	100	0	100	100
		SDD	0	0	0	0	100	0	0
		R	0	0	0	0	0	0	0
C. famata	1	S	0	100	100	0	0	100	100
		SDD	0	0	0	100	0	0	0
		R	100	0	0	0	100	0	0
Total of *Candida* spp.	300	S	98.3	92.7	92.3	78.7	33.3	93.7	84
		SDD	2	2.7	6	10	46.7	1.7	9.3
		R	1.7	4.7	1.7	11.3	20	4.7	6.7

S = susceptible; SDD = susceptible dose dependent; R = resistant; AP: amphotericin B; AND = anidulafungin; CS = caspofungin; FL = fluconazole; IT = itraconazole; POS = posaconazole; VO = voriconazole.

Table 5
Factors related to fluconazole-resistant *Candida* infection according to a bivariate and multivariate logistic regression analysis.

Covariate	No. (%) of patients[a]		Bivariate analysis		Multivariate analysis	
Co-morbidities	Fluconazole resistant *Candida* (n = 39)	Fluconazole susceptible *Candida* (n = 213)	OR (95% CI)	*P*	aOR (95% CI)	*P*
Male sex	25 (64.1)	121 (56.8)	1.358 (0.669–2.757)	0.396		
ICU stay	28 (71.8)	136 (63.8)	1.441 (0.680–3.055)	0.339		
HIV infection/AIDS[b]	2 (5.1)	3 (1.4)	3.730 (0.602–23.09)	0.176		
Diabetes mellitus	6 (15.4)	32 (15.2)	1.011 (0.392–2.609)	0.981		
Organ transplant recipient	1 (2.6)	2 (1.0)	2.737 (0.242–30.94)	0.401		
Cancer	10 (25.6)	47 (22.4)	1.196 (0.544–2.361)	0.656		
Autoimmune disease	2 (5.1)	4 (1.4)	2.784 (0.492–15.71)	0.238		
Chemotherapy	2 (5.1)	13 (6.2)	0.819 (0.177–3.781)	1.000		
Prematurity	3 (7.7)	12 (5.7)	1.375 (0.370–5.117)	0.712		
Congenital malformations	0 (0.0)	11 (5.2)	-	0.222		
Extensive burns	1 (2.6)	0 (0.0)	-	0.157		
Acute renal disease	1 (2.6)	3 (1.4)	1.816 (0.184–17.92)	0.496		
Neutropenia[b]	4 (10.3)	3 (1.4)	7.886 (1.692–36.75)	0.013	6.599 (1.009–43.135)	0.049
Intravenous drug use	1 (2.6)	6 (6.9)	0.895 (0.105–7.664)	1.000		
Tuberculosis[b]	4 (10.3)	3 (1.4)	7.886 (1.692–36.75)	0.013	7.792 (1.403–43.26)	0.019
Hepatic disease	1 (2.6)	6 (2.9)	0.895 (0.105–7.664)	1.000		
Antifungals exposure[b]	20 (51.3)	37 (17.9)	4.836 (2.356–9.952)	0.000	5.174 (2.381–11.24)	0.000
Antibiotics (yes/no)[b]	38 (97.4)	181 (88.3)	5.039 (0.661–38.39)	0.144		
β-Lactam/β-Lactam inhibitor	21 (53.8)	117 (56.8)	0.887 (0.446–1.765)	0.733		
Fluoroquinolones	6 (15.4)	21 (10.0)	1.628 (0.611–4.336)	0.398		
Carbapenems[b]	24 (61.5)	83 (39.7)	2.429 (1.204–4.901)	0.014		
Cephalosporins	5 (12.8)	37 (17.9)	0.676 (0.248–1.844)	0.442		
Aminoglycoside	5 (12.8)	21 (10.2)	1.296 (0.457–3.671)	0.578		
Linezolid	4 (10.3)	11 (5.3)	2.026 (0.610–6.724)	0.269		
Clindamycin	2 (5.1)	8 (3.9)	1.338 (0.273–6.652)	0.663		
Vancomycin[b]	19 (48.7)	59 (28.6)	2.367 (1.179–4.751)	0.014		
Metronidazole	7 (17.9)	15 (7.3)	2.785 (1.054–7.363)	0.059		
Antituberculosis drugs[b]	4 (10.3)	3 (1.4)	7.886 (1.692–36.75)	0.013		
Steroids treatment[b]	13 (33.3)	29 (13.8)	3.121 (1.441–6.758)	0.003	2.610 (1.105–6.163)	0.029
Central venous catheter	28 (71.8)	149 (71)	10.42 (0.488–2.225)	0.915		
Peripheral catheter	28 (64.1)	123 (58.9)	1.249 (0.614–2.539)	0.539		
Urinary catheter	23 (59)	111 (53.1)	1.249 (0.634–2.539)	0.500		
Parenteral nutrition	13 (33.3)	58 (27.9)	1.239 (0.622–2.687)	0.490		
Mechanical ventilation	18 (46.2)	89 (42.8)	1.146 (0.577–2.278)	0.697		
Hemodialysis[b]	6 (15.4)	17 (8.2)	2.043 (0.750–5.560)	0.155		
Blood transfusions	19 (48.7)	84 (40.4)	1.402 (0.706–2.786)	0.333		
Abdominal surgery	20 (51.3)	106 (51)	1.013 (0.511–2.008)	0.971		
Surgical procedures	27 (69.2)	136 (65.4)	1.191 (0.570–2.491)	0.642		

[a] The median age of the patients with fluconazole-resistant infection was 45 years (interquartile range [IQR]: 20–70) and that in the control was 53 years (IQR: 25–70). The median length of stay of the patients with fluconazole-resistant infection was 17 days (IQR: 7–38) and in the control group was 13 days (IQR: 6–25). There were no significant differences between cases and control in median age ($P = 0.340$) or in length of stay ($P = 0.142$).

[b] Variables with $P \leq 0.25$ on bivariate analysis were included in the multivariable logistic regression model. Only significant variables are shown.

10% of them susceptible dose dependent. A similar susceptibility to both echinocandins tested was observed, being 92.7%–92.3% for anidulafungin and caspofungin, respectively; however, important differences in susceptibility were found according to the species of *Candida* under consideration (Table 4).

We found a concordance of 96.92% between the fluconazole susceptibility results obtained by Etest and broth microdilution methods. Eight discordant isolates were excluded, and epidemiological analysis included 252 patients. Of these, 213 patients (84.5%) had a fluconazole-susceptible *Candida*, while 39 patients (15.5%) had a fluconazole-resistant or susceptible dose-dependent isolate. Of these 39 isolates, the most frequent species was *C. glabrata* (43.6%), followed by *C. parapsilosis* complex (12.8%), *C. albicans/C. dubliniensis* (12.8%), *C. krusei* (both 12.8%), *C. guilliermondii* and *C. tropicalis* (both species with 7.7% of isolates), and *C. famata* (2.6%).

No difference between case and control groups were observed by gender ($P = 0.396$) and age ($P = 0.340$). The median length of stay was higher in the cases (17 days) than in controls (13 days), but the difference was not significant ($P = 0.142$). Among the case groups, there was a greater proportion of patients with neutropenia (odds ratio [OR] 7.8; 95% CI, 1.6–36.7; $P = 0.013$) and coinfection with tuberculosis (OR 7.8; 95% CI, 1.6–36.7; $P = 0.013$). In addition, a greater proportion of cases than controls received antifungal therapy prior to a positive culture (OR 4.8; 95% CI, 2.3–9.9; $P \leq 0.001$), antituberculosis drugs (OR 7.8; 95% CI, 1.6–36.7; $P = 0.013$), and steroids treatment (OR 3.1; 95% CI, 1.4–6.7; $P = 0.003$).

There were also differences in the proportion of patients exposed to carbapenems (OR 2.4; 95% CI, 1.2–4.9; $P = 0.014$), vancomycin (OR 2.3; 95% CI, 1.1–4.7; $P = 0.014$), and metronidazol (OR 2.7; 95% CI, 1.0–7.3; $P = 0.059$), being significantly higher in the case group. The multivariate analysis showed antifungal therapy prior to positive culture, coinfection whit tuberculosis, neutropenia, and steroids treatment as variables independently associated with infection by a fluconazole-resistant *Candida* (Table 5).

4. Discussion

In recent decades, *Candida* infections in critically ill patients have increased significantly according to reports around the world (Arendrup et al., 2011; Bassetti et al., 2006; Pfaller and Diekema, 2007; Zilberberg et al., 2008). This situation has been associated with surgical interventions, intensive care treatment (Tortorano et al., 2004), metabolic disorders, extremes of age, and neutrophil dysfunction, among others (Pfaller and Diekema, 2007). In Medellín and its Metropolitan area situated in the northern part of Colombia, the "Grupo para el Estudio de la Resistencia a Antibióticos de Medellín", a

network in charge of periodical surveillance of more common pathogens and their resistance to antimicrobials in hospitalized patients, reported that, in 2011, *Candida* spp. represented the 6th most common organism isolated in ICUs, being 7% of the total isolates, and occupying the 8th place in other hospital services (www.grupogermen.org). The present study, which included *Candida* isolates obtained from sterile sites, showed that the most frequently isolated species was *C. albicans*/*C. dubliniensis* (48.3%), followed by *C. tropicalis* (22.3%) and *C. parapsilosis* complex (15.0%); these results are similar to those obtained by other studies in patients from ICUs located in our city (Zuluaga et al., 2010), as well as in studies conducted in other Colombian cities including Bogotá and Bucaramanga (Torres et al., 2009; Villar et al., 2004).

Worldwide, after a 10-year period of surveillance, these 3 *Candida* species, together with *C. glabrata* and *C. krusei*, were the most common fungal isolates in the 5 geographical regions (Asia, Latin America, Europe, Africa, and North America) according to the Global Antifungal Surveillance ARTEMIS DISK. The study, however, showed that the frequency of these species significantly varies, according to the geographical location (Pfaller et al., 2010b). These variations in the frequency of the species have been confirmed by additional studies around the world (Eggimann et al., 2011). While *C. albicans* is the most frequent species isolated from patients with invasive fungal infections, an increased prevalence of infections caused by *Candida* non-albicans spp. as *C. glabrata* and *C. krusei* have shown by several studies around the world (Falagas et al., 2010; Mikulska et al., 2011). Considering the reduced susceptibility to antifungals in these species makes more difficult the selection of an empiric therapy for the treatment of these infections.

The proportion of fluconazole-resistant (11.3%) and susceptible dose-dependent isolates (10%) in our study was similar to the rates reported previously in our city (Zuluaga et al., 2010) but was higher than the one reported by Pfaller et al., as results of the SENTRY Antimicrobial Surveillance Program (2008–2009). In the later study, fluconazole resistance ranged from 0.1% for *C. albicans* to 5.6% in *C. glabrata* (Pfaller et al., 2011c), showing that there is more fluconazole resistance in our area compared with data from Europe, Asia, North America, and other Latin America countries. In our study, posaconazole was the azole, which exhibited the best in vitro antifungal activity, followed by voriconazole, but as was found with fluconazole, there was a higher proportion of resistant and with decreased susceptibility isolates to both antifungals compared to data reported in the previously mentioned worldwide studies.

Overall susceptibility to echinocandins was above 90% for anidulafungin and caspofungin, but isolates analyzed in this study showed higher MICs for both antifungal in almost all species, except for *C. albicans*/*C. dubliniensis* and *C. glabrata* to anidulafungin, compared with those reported in previous studies that included isolates from North America, Asia-Pacific, Latin America, and Europe (Pfaller et al., 2008). In the same study, Pfaller et al. also reported that isolates of *C. parapsilosis* and *C. guilliermondii* showed MICs higher than those observed for other species of *Candida* spp. observation that was confirmed by our study.

For echinocandins, important differences in susceptibility patterns were found according to the *Candida* spp. and antifungal tested, especially in C. *parapsilosis* complex showing 22.2% of resistance to anidulafungin and 2.2% to caspofungin and *C. glabrata* with 5% of isolates that were susceptible dose dependent to anidulafungin and 65% to caspofungin, but none resistant isolates to echinocandins. These data are different to those reported by the SENTRY Surveillance Program, which revealed no resistant isolates to anidulafungin or micafungin for Latin America between 2008 and 2009, and highest resistance to anidulafungin was 3.2% in *C. glabrata* isolates from Europe and North America (Pfaller et al., 2011c).

Early initiation of appropriate antifungal therapy in patients with invasive *Candida* infections is essential for reducing morbidity and mortality rates (Garey et al., 2006). Failure to provide opportune and adequate diagnosis makes the identification of patients with demographic and clinical factors related to infection by fluconazole-resistant *Candida* isolates an important and valuable tool to early onset of effective treatment in order to reduce the mortality associated with these infections.

An important finding of this study according to multivariate analysis was prior exposure to antifungal therapy, specifically to fluconazole, as an independent risk factor for invasive infection caused by any *Candida* spp. with *in vitro* resistance to fluconazole. Prior exposure to this antifungal as a predictor of a fluconazole-resistant *Candida* infection has been demonstrated in other studies around the world as the one reported by Garnacho-Montero et al. (2010), Ben-Ami et al. (2012), and Lortholary et al. (2011).

Previous use of fluconazole (OR, 2.3 [1.3–4.2]; $P = .007$) has been also identified by Lee et al. (2009) as an independent risk factors for bloodstream infection by fluconazole-resistant *C. glabrata*, in addition Tumbarello et al. (2008) found that patients with prior fluconazole use were more likely to develop fungemia due to fluconazole less-susceptible *C. glabrata* isolate. Candidemia caused by potentially fluconazole-resistant *C. glabrata* as well as *C. krusei* in ICU patients has been also associated to a recent prior fluconazole exposure (OR, 5.47 [1.23–24.32]; $P < 0.05$) (Playford et al., 2008). Horn et al. (2009) analyzed clinical data from 2019 patients with candidemia extracted from the Prospective Antifungal Therapy Alliance database, a comprehensive register that collects information regarding invasive fungal infections, and found that candidemia by *C. krusei* was most commonly associated with prior use of antifungal agents.

Although in multivariate analysis, exposure to certain classes of antibiotics was not significant, in the case exhibited resistance to fluconazole, a higher proportion of previous exposure to vancomycin, metronidazole, and carbapenems was present, in agreement with results obtained by other studies, which have shown that previous exposure to these antibiotics is a factor associated with infection by fluconazole-resistant *Candida* (Lin et al., 2005; Ben-Ami et al., 2012).

We found a statistically significant relationship between the infection by fluconazole-resistant *Candida* and coinfection with tuberculosis or prior exposure to antituberculosis drugs. This association has been reported in a study in which treatment with antituberculosis drugs, previous tuberculosis, and fluconazole exposure were determinants for the development of oropharyngeal colonization or infection by fluconazole-resistant *Candida* strains in HIV-infected patients (Masiá Canuto et al., 2000). The drug-drug interaction between azole antifungals with antimicrobials including rifampin and its clinical impact is well known (Bates and Yung, 2003). This association, in settings where *Candida* infections and tuberculosis are common comorbidities, warrants further exploration.

Others factors reported as associated with fluconazole-resistant *Candida* spp. were neutropenia (Ben-Ami et al., 2012; Garnacho-Montero et al., 2010) and steroid treatment (Horn et al., 2009), both of which also proved to be significantly associated in our study. Meanwhile, other factors such as chronic renal disease (Garnacho-Montero et al., 2010), history of gastrointestinal surgery (Playford et al., 2008), diabetes, and central venous catheter (Tumbarello et al., 2008) have also been identified as important, but no association with infection by fluconazole-resistance strains was confirmed herein.

In conclusion, it is important to highlight 3 findings of this study that appear useful to improve the care of patients with invasive infections by *Candida* spp. First, previous knowledge of resistance patterns of the different *Candida* species is necessary to guide the selection of empiric antifungal treatment and to design local therapeutic guidelines. In addition, susceptibility results to echinocandins observed for *C. parapsilosis* complex and *C. glabrata* should guide the selection of antifungal therapy when there is an infection by these species. Lastly, the MIC levels observed in our study are above the levels reported for other studies, which may suggest, in our area, a

shift toward more resistance to azole and equinocandins among *Candida* spp. population.

Likewise, a suspected invasive infection by *Candida* spp. in patients with tuberculosis and antituberculosis therapy, as well as in patients who have received previous antifungals and those who are treated with steroids, and neutropenic patients, according to the results of this study, should suggest possible resistance to fluconazole, pointing towards the selection of another class of antifungal agents.

Acknowledgments

We thank all the microbiology staff of clinical laboratories in the 15 hospitals surveyed. This study was supported in part by research grant from Investigator Initiated Studies Program of Merck. The opinions expressed in this paper are those of the authors do not necessarily represent those of Merck.

References

Arendrup MC, Bruun B, Christensen JJ, Fuursted K, Johansen HK, Kjaeldgaard P, et al. National surveillance of fungemia in Denmark (2004 to 2009). J Clin Microbiol 2011;49(1):325–34.

Bassetti M, Righi E, Costa A, Fasce R, Molinari MP, Rosso R, et al. Epidemiological trends in nosocomial candidemia in intensive care. BMC Infect Dis 2006;6:21.

Bates DW, Yung DT. Clinical impact of drug-drug interactions with systemic azole antifungals. Drugs Today 2003;39:801–13.

Ben-Ami R, Olshtain-Pops K, Krieger M, Oren I, Bishara J, Dan M, et al. Antibiotic exposure as a risk factor for fluconazole-resistant *Candida* bloodstream infection. Antimicrob Agents Chemother 2012;56(5):2518–23.

Clinical and Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast. Third Informational Supplement. CLSI Document M27-S. Wayne, PA: CLSI; 2008.

Eggimann P, Bille J, Marchetti O. Diagnosis of invasive candidiasis in the ICU. Ann Intensive Care 2011;1:37.

Falagas ME, Roussos N, Vardakas KZ. Relative frequency of albicans and the various non-albicans *Candida* spp among candidemia isolates from inpatients in various parts of the world: a systematic review. Int J Infect Dis 2010;14(11):954–66.

Garey KW, Rege M, Pai MP, Mingo DE, Suda KJ, Turpin RS, et al. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. Clin Infect Dis 2006;43(1):25–31.

Garnacho-Montero J, Díaz-Martín A, García-Cabrera E, Ruiz Pérez de Pipaón M, Hernández-Caballero C, Aznar-Martín J, et al. Risk factors for fluconazole-resistant candidemia. Antimicrob Agents Chemother 2010;54(8):3149–54.

Gudlaugsson O, Gillespie S, Lee K, Vande Berg J, Hu J, Messer S, et al. Attributable mortality of nosocomial candidemia, revisited. Clin Infect Dis 2003;37(9):1172–7.

Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, Fridkin SK. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. Infect Control Hosp Epidemiol 2008;29(11):996–1011.

Horn DL, Neofytos D, Anaissie EJ, Fishman JA, Steinbach WJ, Olyaei AJ, et al. Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. Clin Infect Dis 2009;48(12):1695–703.

Lee I, Fishman NO, Zaoutis TE, Morales KH, Weiner MG, Synnestvedt M, et al. Risk factors for fluconazole-resistant *Candida glabrata* bloodstream infections. Arch Intern Med 2009;169(4):379–83.

Lin MY, Carmeli Y, Zumsteg J, Flores EL, Tolentino J, Sreeramoju P, et al. Prior antimicrobial therapy and risk for hospital-acquired *Candida glabrata* and *Candida krusei* fungemia: a case-case–control study. Antimicrob Agents Chemother 2005;49(11):4555–60.

Lortholary O, Desnos-Ollivier M, Sitbon K, Fontanet A, Bretagne S, Dromer F, et al. Recent exposure to caspofungin or fluconazole influences the epidemiology of candidemia: a prospective multicenter study involving 2,441 patients. Antimicrob Agents Chemother 2011;55(2):532–8.

Masiá Canuto M, Gutiérrez Rodero F, Ortiz de la Tabla Ducasse V, Hernández Aguado I, Martín González C, Sánchez Sevillano A, et al. Determinants for the development of oropharyngeal colonization or infection by fluconazole-resistant *Candida* strains in HIV-infected patients. Eur J Clin Microbiol Infect Dis 2000;19(8):593–601.

Mikulska M, Bassetti M, Ratto S, Viscoli C. Invasive candidiasis in non-hematological patients. Mediterr J Hematol Infect Dis 2011;3(1):e2011007.

Nucci M, Queiroz-Telles F, Tobón AM, Restrepo A, Colombo AL. Epidemiology of opportunistic fungal infections in Latin America. Clin Infect Dis 2010;51(5):561–70.

Del Palacio A, Alhambra A, Cuétara MS. Factores de riesgo de la candidiasis invasora: estratificación. Rev Iberoam Micol 2006;23(1):29–31.

Pappas PG, Rex JH, Lee J, Hamill RJ, Larsen RA, Powderly W, et al. A prospective observational study of candidemia: epidemiology, therapy, and influences on mortality in hospitalized adult and pediatric patients. Clin Infect Dis 2003;37(5):634–43.

Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev 2007;20(1):133–63.

Pfaller MA, Boyken L, Hollis RJ, Kroeger J, Messer SA, Tendolkar S, et al. In vitro susceptibility of invasive isolates of *Candida* spp. to anidulafungin, caspofungin, and micafungin: six years of global surveillance. J Clin Microbiol 2008;46(1):150–6.

Pfaller MA, Messer SA, Hollis RJ, Boyken L, Tendolkar S, Kroeger J, et al. 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 2009;47(10):3185–90.

Pfaller MA, Andes D, Diekema DJ, Espinel-Ingroff A, Sheehan D. CLSI Subcommittee for Antifungal Susceptibility Testing. Wild-type MIC distributions, epidemiological cutoff values and species-specific clinical breakpoints for Fluconazol and Candida time for harmonization of CLSI and EUCAST broth microdilution. Drug Resist Updat 2010a;13(6):180–95.

Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Ellis D, Tullio V. 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 2010b;48(4):1366–77.

Pfaller MA, Andes D, Arendrup MC, Diekema DJ, Espinel-Ingroff A, Alexander BD, et al. Clinical breakpoints for voriconazole and *Candida* spp. revisited: review of microbiologic, molecular, pharmacodynamic, and clinical data as they pertain to the development of species-specific interpretive criteria. Diagn Microbiol Infect Dis 2011a;70(3):330–43.

Pfaller MA, Diekema DJ, Andes D, Arendrup MC, Brown SD, Lockhart SR, et al. Clinical breakpoints for the echinocandins and *Candida* revisited: integration of molecular, clinical, and microbiological data to arrive at species-specific interpretive criteria. Drug Resist Updat 2011b;14(3):164–76.

Pfaller MA, Moet GJ, Messer SA, Jones RN, Castanheira M. Geographic variations in species distribution and echinocandin and azole antifungal resistance rates among *Candida* bloodstream infection isolates: report from the SENTRY Antimicrobial Surveillance Program (2008 to 2009). J Clin Microbiol 2011c;49(1):396–9.

Playford EG, Marriott D, Nguyen Q, Chen S, Ellis D, Slavin M, et al. Candidemia in nonneutropenic critically ill patients: risk factors for non-albicans *Candida* spp. Crit Care Med 2008;36(7):2034–9.

Sims CR, Paetznick VL, Rodriguez JR, Chen E, Ostrosky-Zeichner L. Correlation between microdilution, E-test, and disk diffusion methods for antifungal susceptibility testing of posaconazole against *Candida* spp. J Clin Microbiol 2006;44(6):2105–8.

Torres NA, Alvarez CA, Rondón MA. Evaluación mediante tres técnicas de susceptibilidad a fluconazol en especies de *Candida* aisladas en pacientes con infecciones invasoras. Bogotá – Colombia. Rev Chil Infect 2009;26(2):135–43.

Tortorano AM, Peman J, Bernhardt H, Klingspor L, Kibbler CC, Faure O, et al. 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 2004;23(4):317–22.

Tumbarello M, Sanguinetti M, Trecarichi EM, La Sorda M, Rossi M, de Carolis E, et al. Fungaemia caused by *Candida glabrata* with reduced susceptibility to fluconazole due to altered gene expression: risk factors, antifungal treatment and outcome. J Antimicrob Chemother 2008;62(6):1379–85.

Villar LA, Quijano FA, Cespedes JI, Torres A, De Bedout C. Prevalencia de patrones de sensibilidad al fluconazol de las especies de Candida aisladas de pacientes de unidades de cuidados intensivos de Bucaramanga-Colombia. Infectio 2004;8(3):185–93.

Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in intensive care units. JAMA 2009;302(21):2323–9.

Wey SB, Mori M, Pfaller MA, Woolson RF, Wenzel RP. Hospital acquired candidemia. The attributable mortality and excess length of stay. Arch Intern Med 1988;148(12):2642–5.

Zilberberg MD, Shorr AF, Kollef MH. Secular trends in candidemia-related hospitalization in the United States, 2000–2005. Infect Control Hosp Epidemiol 2008;29(10):978–80.

Zuluaga A, De Bedout C, Agudelo CA, Hurtado H, Arango M, Restrepo A, et al. Sensibilidad a fluconazol y voriconazol de especies de *Candida* aisladas de pacientes provenientes de unidades de cuidados intensivos en Medellín, Colombia (2001–2007). Rev Iberoam Micol 2010;27(3):125–9.