Mycopathologia (2014) 178:1–9
DOI 10.1007/s11046-014-9772-2

Isavuconazole and Nine Comparator Antifungal Susceptibility Profiles for Common and Uncommon *Candida* Species Collected in 2012: Application of New CLSI Clinical Breakpoints and Epidemiological Cutoff Values

Mariana Castanheira · Shawn A. Messer · Paul R. Rhomberg · Rachel R. Dietrich · Ronald N. Jones · Michael A. Pfaller

Received: 30 April 2014 / Accepted: 11 June 2014 / Published online: 21 June 2014

Abstract The in vitro activity of isavuconazole and nine antifungal comparator agents was assessed using reference broth microdilution methods against 1,421 common and uncommon species of *Candida* from a 2012 global survey. Isolates were identified using CHROMagar, biochemical methods and sequencing of ITS and/or 28S regions. *Candida* spp. were classified as either susceptible or resistant and as wild type (WT) or non-WT using CLSI clinical breakpoints or epidemiological cutoff values, respectively, for the antifungal agents. Isolates included 1,421 organisms from 21 different species of *Candida*. Among *Candida* spp., resistance to all 10 tested antifungal agents was low (0.0–7.9 %). The vast majority of each species of *Candida*, with the exception of *Candida glabrata*, *Candida krusei*, and *Candida guilliermondii* (modal MICs of 0.5 μg/ml), were inhibited by ≤0.12 μg/ml of isavuconazole (99.0 %; range 94.3 % [*Candida tropicalis*] to 100.0 % [*Candida lusitaniae* and *Candida dubliniensis*]). *C. glabrata, C. krusei,* and *C. guilliermondii* were largely inhibited by ≤1 μg/ml of isavuconazole (89.7, 96.9 and 92.8 %, respectively). Decreased susceptibility to isavuconazole was most prominent with *C. glabrata* where the modal MIC for isavuconazole was 0.5 μg/ml for those strains that were SDD to fluconazole or WT to voriconazole, and was 4 μg/ml for those that were either resistant or non-WT to fluconazole or voriconazole, respectively. In conclusion, these data document the activity of isavuconazole and generally the low resistance levels to the available antifungal agents in a large, contemporary (2012), global collection of molecularly characterized species of *Candida*.

Keywords Isavuconazole · *Candida* · Surveillance · Susceptibility

M. Castanheira · S. A. Messer · P. R. Rhomberg · R. R. Dietrich · R. N. Jones · M. A. Pfaller (✉)
JMI Laboratories, 345 Beaver Kreek Centre, Suite A, North Liberty, IA 52317, USA
e-mail: mike-pfaller@jmilabs.com; michael-pfaller@jmilabs.com

Introduction

The systemically active antifungal armamentarium currently includes the polyenes, flucytosine, fluconazole, the extended-spectrum triazoles (itraconazole, posaconazole, and voriconazole), and the echinocandins [1–6]. Despite the fact that in total, these agents cover the vast majority of opportunistic fungal pathogens and are increasingly employed in either a prophylactic or preemptive treatment strategy [2, 7–10], breakthrough invasive fungal infections (bIFI) continue to be reported and increasingly involve yeasts and/or molds that are relatively uncommon and tend to exhibit decreased susceptibility to the available antifungal agents [4, 7, 11–19]. These observations underscore the need for continued

surveillance efforts designed to provide accurate identification and resistance detection in these emerging pathogens, as well as the development and introduction of new antifungal agents to address the present need for broad-spectrum antifungal coverage [4, 12, 20, 21]. The recent development of species-specific clinical breakpoints (CBPs) and epidemiological cutoff values (ECVs) for in vitro susceptibility testing of *Candida* spp. against both triazoles and echinocandins further emphasize the need for accurate identification of fungal pathogens [13, 22]. Indeed, erroneous identification of fungal species will now have potentially important effects on the interpretation of MIC data and appropriateness of therapeutic decisions [13, 22–25].

Isavuconazole is an investigational triazole that is presently in late-stage clinical development for the treatment of invasive candidiasis, invasive aspergillosis, and infections due to non-*Aspergillus* molds [26]. Isavuconazole may be administered orally or parenterally and exhibits broad antifungal activity against common and uncommon fungal pathogens, including *Candida*, *Aspergillus*, non-*Candida* yeasts, and non-*Aspergillus* molds [27–35].

Previously, we demonstrated a high level of concordance between isavuconazole MIC results produced with the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) broth microdilution (BMD) methods in testing *Candida* spp., suggesting that either method could be employed for the purpose of resistance surveillance [31]. In the present study, we use the database from the global SENTRY Antifungal Surveillance Program (2012) to further document the in vitro activity of isavuconazole as determined by CLSI BMD methods against both common and uncommon species of *Candida* causing IFIs. We compare the activity of isavuconazole with that of the established triazole and echinocandin antifungal agents and examine the extent of decreased susceptibility to isavuconazole among isolates classified as susceptible dose dependent (SDD), resistant (R), or non-wild type (non-WT) to fluconazole and voriconazole. Importantly, we employ DNA sequence analysis to confirm the identification of uncommon species of *Candida* to ensure an accurate assessment of the antifungal MIC phenotype for each species.

Materials and Methods

Organisms and Sources

A total of 1,421 non-duplicate strains of *Candida* were collected prospectively from 75 medical centers located in North America (30 sites), Europe (24 sites), Latin America (10 sites), and the Asia–Pacific region (11 sites). These strains were recovered consecutively from patients with bloodstream infections (1,094 strains), from normally sterile body fluids, tissues, and abscesses (162 strains), and 165 were collected from non-specified infection sites.

Isolates were identified at participant institutions using methods routinely employed at the submitting laboratory including the use of Vitek, MicroScan, API, and AuxaColor systems supplemented by classical methods for yeast identification [36, 37]. Isolates were submitted to JMI Laboratories (North Liberty, Iowa, USA), where the identification was confirmed by morphological, biochemical, and molecular methods [21–23, 25, 31]. Yeast isolates were subcultured and screened using CHROMagar *Candida* (Becton–Dickinson, Sparks, Maryland, USA) to ensure purity and to differentiate *Candida albicans*/*Candida dubliniensis*, *Candida tropicalis*, and *Candida krusei*. Biochemical and physiological tests including Vitek 2 (bioMerieux, Hazelwood, Missouri, USA) testing, trehalose assimilation (*Candida glabrata*), and growth at 45 °C (*C. albicans/C. dubliniensis*) were also used to establish the identification of common (*C. albicans, C. glabrata, C. parapsilosis, C. tropicalis,* and *C. krusei*) *Candida* species. Molecular methods were used for common species of *Candida* that could not be definitively identified using phenotypic methods or that presented unusual phenotypic or biochemical profiles, as well as all uncommon species of *Candida*. *Candida* spp. were identified using sequence-based methods for the internal transcribed spacer (ITS) region and 28S ribosomal subunit (D1/D2) [22, 23, 25]. Nucleotide sequences were examined using Lasergene software (DNAStar, Madison, Wisconsin, USA) and then compared to database sequences using BLAST (http://www.nbeti.nlmnih.gov.blast). Results were considered acceptable if homology was >99.5 % with other entries in the databases used for comparison. Available sequences that were considerably different from the majority of entries for one species were considered outliers and

were discarded in the analysis. Additionally, if no match was found in the database, the ID was based on species complex (SC), genus, family, or order, according to the most current classification systems.

Among the isolates of *Candida* (1,421), there were 671 isolates of *C. albicans*, 291 of *C. glabrata*, 236 of *C. parapsilosis*, 122 of *C. tropicalis*, 32 of *C. krusei*, 24 of *Candida lusitaniae*, 13 of *C. dubliniensis*, and 32 of miscellaneous *Candida* species (1 *C. bracarensis,* 1 *C. catenulata*, 2 *C. fabianii*, 2 *C. fermentati*, 1 *C. fluviatilis*, 6 *Candida guilliermondii*, 1 *C. haemulonii*, 8 *C. kefyr*, 2 *C. lipolytica*, 2 *C. orthopsilosis*, 2 *C. pelliculosa*, 1 *C. rugosa*, 1 *C. thasaenensis*, 1 *C. thermophila,* and 1 *Candida* spp. not further identified).

In addition to these isolates, we included additional isolates of less-common species from the SENTRY Program (2011) database: 5 *C. fermentati*, 8 *C. guilliermondii*, 10 *C. kefyr*, and 2 *C. lipolytica*. The MIC results for these isolates were added to those from 2012 to augment the numbers of these unusual species.

Antifungal Susceptibility Testing

All yeast isolates were tested for in vitro susceptibility to amphotericin B, flucytosine, the triazoles (fluconazole, isavuconazole, itraconazole, posaconazole, and voriconazole), and the echinocandins (anidulafungin, caspofungin, and micafungin) using CLSI [38] BMD methods. The MIC results for all agents were read following 24 h of incubation at 35°C. MIC values were determined visually, as the lowest concentration of drug that caused complete (amphotericin B) or significant (≥50 %) growth diminution levels (all other agents) [38, 39].

We used the recently revised CLSI clinical breakpoint (CBP) values to identify strains of the five most common species of *Candida* (*C. albicans, C. glabrata, C. parapsilosis, C. tropicalis,* and *C. krusei*) that were susceptible and resistant to the echinocandins as well as those that were susceptible and resistant to fluconazole and voriconazole [22, 39]: anidulafungin, caspofungin, and micafungin MIC values of ≤0.25 and ≥1 μg/ml were categorized as susceptible and resistant, respectively, for *C. albicans, C. tropicalis,* and *C. krusei*, and MIC results of ≤2 and ≥8 μg/ml were categorized as susceptible and resistant, respectively, for *C. parapsilosis*; anidulafungin and caspofungin MIC values of ≤0.12 and ≥0.5 μg/ml and micafungin MIC values of ≤0.06 and ≥0.25 μg/ml were considered susceptible and resistant, respectively, for *C. glabrata*; fluconazole MIC results of ≤2 and ≥8 μg/ml were defined as susceptible and resistant, respectively, for *C. albicans, C. parapsilosis,* and *C. tropicalis*, and MICs of ≤32 and ≥64 μg/ml were considered susceptible dose dependent (SDD) and resistant, respectively, for *C. glabrata*. All isolates of *C. krusei* were defined as resistant to fluconazole. The CLSI susceptible and resistant breakpoints for voriconazole are ≤0.12 and ≥1 μg/ml, respectively, for *C. albicans, C. parapsilosis,* and *C. tropicalis,* and MIC results of ≤0.5 and ≥2 μg/ml were categorized as susceptible and resistant, respectively, for *C. krusei*; CLSI has not assigned CBPs for voriconazole and *C. glabrata* and recommends the ECV of 0.5 μg/ml to be used to differentiate wild type (WT; MIC ≤ ECV) from non-WT (MIC > ECV) strains of this species [22, 39].

CBPs have not been established for amphotericin B, flucytosine, itraconazole, isavuconazole, or posaconazole and for any antifungal agent and the less-common species of *Candida*; however, ECVs have been established for amphotericin B, flucytosine, the triazoles (fluconazole, itraconazole, posaconazole, and voriconazole), and the echinocandins and six species of *Candida* that are encountered less frequently (*C. lusitaniae, C. guilliermondii, C. dubliniensis, C. kefyr, C. orthopsilosis,* and *C. pelliculosa*) [22].

Quality control was performed as recommended in CLSI document M27-A3 [38] using strains *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

Results and Discussion

Among the 21 species of Candida encountered in this survey, *C. albicans* was most common in Europe (56.2 %) and least common in North America (39.5 %), whereas *C. glabrata* was most common in North America (28.5 %) and least common in Latin America (6.9 %). *C. parapsilosis* was most common in Latin America (27.1 %), and *C. tropicalis* was most common in the Asia–Pacific region (17.2 %). *C. krusei* was most common in North America (2.7 %), as were other miscellaneous species of *Candida* (5.8 %) (data not shown).

Table 1 shows the MIC distributions for isavuconazole and the 11 most common species of *Candida*

referred from the 75 participating centers. Among these isolates, isavuconazole was most active against *C. albicans* (modal MIC, 0.008 μg/ml) and least active against *C. glabrata*, *C. krusei,* and *C. guilliermondii* (modal MICs, 0.5 μg/ml). The vast majority of each species, with the exception of *C. glabrata, C. krusei,* and *C. guilliermondii,* were inhibited by ≤0.12 μg/ml of isavuconazole [99.0 %; range 94.3 % (*C. tropicalis*) to 100.0 % (*C. lusitaniae and C. dubliniensis*)]. *C. glabrata, C. krusei,* and *C. guilliermondii* isolates were largely susceptible to isavuconazole at MIC values ≤1 μg/ml (89.7, 96.9, and 92.8 %, respectively).

The antifungal activity of isavuconazole and nine comparator antifungal agents against 1,352 isolates of *Candida* spp. as determined with CLSI BMD methods are shown in Table 2. The results are categorized using CLSI CBPs and/or ECVs as appropriate. The vast majority of these isolates represented WT strains as determined by the respective ECVs and very few (*C. glabrata* and *C. krusei)* were resistant to triazoles or echinocandins based on CBPs.

Among the azole antifungal agents, it is important to assess the issue of cross-resistance given the ubiquitous use of this antifungal class in various medical practice settings [3]. CBPs for isavuconazole and *Candida* have not yet been established; however, given that this agent is of the triazole class, it is reasonable to consider that it may be a substrate for CDR efflux pumps and also may be affected by quantitative or qualitative alterations in the lanosterol demethylase target enzyme [12, 20, 42, 44, 45]. Overexpression of *cdr1* and *cdr2* has been documented as the primary mechanism of resistance for *C. glabrata* versus fluconazole, itraconazole, posaconazole, and voriconazole [20, 46, 47]. As such, it is highly likely that isavuconazole can be pumped out of the cell as are the other members of this class. Thus, the discussion of potential cross-resistance is consistent with the available data. Having said this, it is true there are no CBPs to designate resistance to isavuconazole and so the discussion will focus on decreased susceptibility that may suggest cross-resistance, the impact of which must await data from ongoing clinical trials.

Previously, we examined cross-resistance among the triazoles (fluconazole, posaconazole, and voriconazole) using a large collection of *Candida* spp. from a

Table 1 Antifungal activity of isavuconazole against *Candida* spp. tested as part of the 2012 international surveillance program

Organism species/groups (no. tested)	No. (cumulative %) of isolates inhibited at MIC (μg/ml):										
	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8
Candida albicans (671)	306 (45.6)	289 (88.7)	66 (98.5)	5 (99.3)	4 (99.9)	0 (99.9)	1 (100.0)					
Candida glabrata (291)	0 (0.0)	2 (0.7)	0 (0.7)	5 (2.4)	31 (13.1)	48 (29.6)	117 (69.8)	58 (89.7)	16 (95.2)	12 (99.3)	2 (100.0)	
Candida parapsilosis (236)	24 (10.2)	46 (29.7)	91 (68.2)	49 (89.0)	23 (98.7)	3 (100.0)						
Candida tropicalis (122)	2 (1.6)	18 (16.4)	40 (49.2)	33 (76.2)	22 (94.3)	2 (95.9)	2 (97.5)	2 (99.2)	0 (99.2)	1 (100.0)		
Candida krusei (32)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (12.5)	6 (31.3)	19 (90.6)	2 (96.9)	1 (100.0)			
Candida lusitaniae (24)	3 (12.5)	3 (25.0)	11 (70.8)	6 (95.8)	1 (100.0)							
Candida dubliniensis (13)	5 (38.5)	6 (84.6)	1 (92.3)	1 (100.0)								
Candida kefyr (18)	3 (16.7)	13 (88.9)	2 (100.0)									
Candida guilliermondii (14)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (14.3)	3 (35.7)	5 (71.4)	3 (92.8)	0 (92.8)	1 (100.0)		
Candida fermentati (7)	0 (0.0)	0 (0.0)	1 (14.3)	0 (14.3)	2 (42.9)	3 (85.7)	0 (85.7)	1 (100.0)				
Candida lipolytica (4)	0 (0.0)	0 (0.0)	2 (50.0)	0 (50.0)	1 (75.0)	1 (100.0)						

Table 2 Antifungal activity of isavuconazole and comparator antifungal agents against key organism species/groups tested as part of the 2012 international surveillance program

Species (no. tested)	Antifungal agent	MIC/MEC (μg/ml)			% By category[a]			
					CLSI		ECV[b]	
		Range	50 %	90 %	%S	%R	%WT	%NWT
C. albicans (671)	Amphotericin B	≤0.12–2	1	1	–[b]	–	100.0	0.0
	Flucytosine	≤0.5 to >32	≤0.5	≤0.5	–	–	93.4	6.6
	Fluconazole	≤0.06–16	0.25	0.25	99.6	0.3	98.7	1.3
	Isavuconazole	≤0.008–0.5	0.015	0.03	–	–	–	–
	Itraconazole	≤0.008–1	0.03	0.06	99.1	0.1	99.1	0.9
	Posaconazole	≤0.008–0.5	0.03	0.06	–	–	94.9	5.1
	Voriconazole	≤0.008–0.5	≤0.008	0.015	99.7	0.0	99.6	0.4
	Anidulafungin	≤0.008–0.12	0.015	0.06	100.0	0.0	100.0	0.0
	Caspofungin	≤0.008–0.12	0.03	0.06	100.0	0.0	100.0	0.0
	Micafungin	≤0.008–0.06	0.015	0.03	100.0	0.0	99.7	0.3
C. glabrata (291)	Amphotericin B	0.25–2	1	1	–	–	100.0	0.0
	Flucytosine	≤0.5 to >32	≤0.5	≤0.5	–	–	98.6	1.4
	Fluconazole	0.25 to >128	8	32	(92.1)[c]	7.9	92.1	7.9
	Isavuconazole	0.015–8	0.5	2	–	–	–	–
	Itraconazole	0.06 to >8	1	2	–	–	94.2	5.8
	Posaconazole	0.03 to >8	1	2	–	–	96.2	3.8
	Voriconazole	≤0.008–4	0.25	0.5	–	–	90.4	9.6
	Anidulafungin	0.015–2	0.06	0.12	96.6	1.7	98.3	1.7
	Caspofungin	0.015–4	0.06	0.06	97.6	2.1	97.6	2.4
	Micafungin	0.015–2	0.015	0.03	97.9	1.7	97.2	2.8
C. parapsilosis (236)	Amphotericin B	0.25–2	1	1	–	–	100.0	0.0
	Flucytosine	≤0.5 to >32	≤0.5	≤0.5	–	–	98.3	1.7
	Fluconazole	0.12–64	1	2	93.2	3.8	93.2	6.8
	Isavuconazole	≤0.008–0.25	0.03	0.12	–	–	–	–
	Itraconazole	0.03–0.5	0.12	0.25	–	–	100.0	0.0
	Posaconazole	0.015–0.25	0.06	0.12	–	–	100.0	0.0
	Voriconazole	≤0.008–0.5	0.015	0.06	97.9	0.0	97.9	2.1
	Anidulafungin	0.12–4	2	4	86.4	0.0	100.0	0.0
	Caspofungin	0.06–2	0.5	0.5	100.0	0.0	99.2	0.8
	Micafungin	0.015–4	1	2	98.7	0.0	100.0	0.0
C. tropicalis (122)	Amphotericin B	0.25–2	1	1	–	–	100.0	0.0
	Flucytosine	≤0.5 to >32	≤0.5	≤0.5	–	–	94.3	5.7
	Fluconazole	0.12–64	0.5	0.5	95.9	2.5	95.9	4.1
	Isavuconazole	≤0.008–4	0.06	0.12	–	–	–	–
	Itraconazole	0.015–1	0.06	0.12	–	–	98.4	1.6
	Posaconazole	0.015–1	0.03	0.12	–	–	96.7	3.3
	Voriconazole	≤0.008–2	0.015	0.06	97.5	1.6	96.7	3.3
	Anidulafungin	≤0.008–0.12	0.015	0.03	100.0	0.0	100.0	0.0
	Caspofungin	0.015–0.12	0.03	0.06	100.0	0.0	100.0	0.0
	Micafungin	≤0.008–0.12	0.03	0.06	100.0	0.0	100.0	0.0

Table 2 continued

Species (no. tested)	Antifungal agent	MIC/MEC (μg/ml)			% By category[a]			
					CLSI		ECV[b]	
		Range	50 %	90 %	%S	%R	%WT	%NWT
C. krusei (32)	Amphotericin B	1–2	1	2	–	–	100.0	0.0
	Flucytosine	8–32	16	16	–	–	100.0	0.0
	Isavuconazole	0.12–2	0.5	0.5	–	–	–	–
	Itraconazole	0.25–4	0.25	0.5	–	–	96.9	3.1
	Posaconazole	0.12–2	0.25	0.5	–	–	93.8	6.3
	Voriconazole	0.12–4	0.25	0.25	96.9	3.1	96.9	3.1
	Anidulafungin	0.03–1	0.06	0.12	96.9	3.1	96.9	3.1
	Caspofungin	0.06–1	0.12	0.25	96.9	3.1	96.9	3.1
	Micafungin	0.015–0.12	0.12	0.12	100.0	0.0	100.0	0.0

[a] *MIC* minimum inhibitory concentration, *MEC* minimum effective concentration, *MIC/MEC* 50/90 concentration encompassing 50 and 90 % of isolates tested, respectively, *S* susceptible, *R* resistant, *WT* wild type, *non-WT* non-wild type, *CLSI* Clinical and Laboratory Standards Institute, *ECV* epidemiological cutoff value

[b] Interpretive criteria as defined by Pfaller and Diekema (2012)

[c] Results for fluconazole and *C. glabrata* are categorized as susceptible dose dependent (SDD) and R: 92.1 % SDD

global surveillance program [40, 41]. These studies provided strong support for the concerns of several investigators regarding the issue of cross-resistance among these agents that share both a common mechanism of action and certain mechanisms of resistance [7, 40–45]. Most importantly, these studies focused attention on *C. glabrata* as the species most likely to demonstrate cross-resistance among the three triazoles by virtue of expression of CDR efflux pumps as the primary mechanism of azole resistance [46, 47]. In the present study, we examine the potential for cross-resistance between fluconazole and isavuconazole (Table 3), and between voriconazole and isavuconazole (Table 4) using more than 1,300 isolates of *Candida* spp. from the 2012 surveillance study. One of the limitations of this analysis stems from the fact that the surveillance protocol specifies that only incident isolates be submitted for the study. Whereas this ensures that the population of each species in the surveillance collection represents a WT population, it also reduces the number of strains with resistance or decreased susceptibility to the various antifungal agents. Given this limitation, it is clear from the data in Tables 3 and 4 that isolates classified as susceptible to either fluconazole or voriconazole, with the exception of *C. glabrata* and *C. krusei*, represent WT strains with respect to isavuconazole and show MIC values that are almost all (99.7 %) ≤0.12 μg/ml. The higher MIC values of fluconazole and voriconazole observed with strains of *C. glabrata* classified as SDD or WT, respectively, are also reflected in those of isavuconazole where the modal MIC for isavuconazole was 0.5 μg/ml and 97 % of strains that were SDD to fluconazole and WT to voriconazole exhibited isavuconazole MIC values of ≤1 μg/ml. Although there were few strains of *Candida* classified as SDD, resistant, or non-WT to fluconazole and voriconazole, most showed elevated MIC results for isavuconazole as well. The exception to this statement was *C. parapsilosis*, where 98.7 % of 236 isolates were inhibited by ≤0.12 μg/ml of isavuconazole irrespective of their susceptibility to fluconazole or voriconazole. The most clear evidence of potential cross-resistance between isavuconazole and fluconazole or voriconazole may be seen with *C. glabrata* where the modal MIC for isavuconazole was 4 μg/ml for those strains that were either resistant or non-WT to fluconazole and voriconazole, respectively.

Several important observations can be made from this global survey. First, we confirm the excellent spectrum and potency of isavuconazole against *Candida* spp. Second, we have used the recently published CBPs and ECVs to demonstrate the generally low levels of resistance to the available antifungal agents

Table 3 In vitro activity of isavuconazole against 1,357 clinical isolates of *Candida* stratified by fluconazole susceptibility category

Species	Fluconazole susceptibility category (no. tested)[a,b]	No. for which isavuconazole MIC (μg/ml) was										
		≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
C. albicans	S (668)	306	289	66	5	2						
	SDD (1)					1						
	R (2)					1		1				
C. glabrata	SDD (268)		2		5	31	48	117	56	9		
	R (23)								2	7	12	2
C. parapsilosis	S (220)	24	45	86	47	18						
	SDD (7)		1	2	1	2	1					
	R (9)			3	1	3	2					
C. tropicalis	S (117)	2	18	40	33	21	1	2				
	SDD (2)						1		1			
	R (3)					1			1		1	
C. lusitaniae	WT (24)	3	3	11	6	1						
	non-WT (0)											
C. dubliniensis	WT (13)	5	6	1	1							
	non-WT (0)											

[a] *S* susceptible, *SDD* susceptible dose dependent, *R* resistant. Categories according to CLSI (2012)

[b] *WT* wild type, *non-WT* non-wild type. Categories according to Pfaller and Diekema (2012)

Table 4 In vitro activity of isavuconazole against 1,389 clinical isolates of *Candida* stratified by voriconazole susceptibility category

Species	Voriconazole susceptibility category (no. tested)[a,b]	No. for which isavuconazole MIC (μg/ml) was										
		≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
C. albicans	S (669)	306	289	66	5	3						
	SDD (2)					1		1				
	R (0)											
C. glabrata	WT (263)		2		5	31	48	117	53	7		
	non-WT (28)								5	9	12	2
C. parapsilosis	S (231)	24	46	90	49	20	2					
	SDD (5)			1		3	1					
	R (0)											
C. tropicalis	S (119)	2	18	40	33	22	2	2				
	SDD (1)								1			
	R (2)								1		1	
C. krusei	S (31)					4	6	19	2			
	SDD (0)											
	R (1)									1		
C. lusitaniae	WT (24)	3	3	11	6	1						
	non-WT (0)											
C. dubliniensis	WT (13)	5	6	1	1							
	non-WT (0)											

[a] *S* susceptible, *SDD* susceptible dose dependent, *R* resistant. Categories according to CLSI (2012)

[b] *WT* wild type, *non-WT* non-wild type. Categories according to Pfaller and Diekema (2012)

in a large, contemporary (2012) global collection of molecularly characterized isolates. Finally, our results document decreased susceptibility to isavuconazole among isolates classified as R or non-WT to fluconazole and voriconazole, with the greatest emphasis on *C. glabrata*. This decreased susceptibility may suggest cross-resistance, the impact of which must be documented by data from clinical trials.

Acknowledgments We acknowledge the excellent technical assistance of S. Benning in the preparation of the manuscript and the valuable contributions of the participants in the SENTRY Program. The antifungal global surveillance program, which served as the source of data used in the development of this manuscript, was, in part, supported by Astellas Pharma Global Development, Inc., and Pfizer Inc., JMI Laboratories, Inc., and has received research and educational grants in 2010–2012 from—Achaogen, Aires, American Proficiency Institute (API), Anacor, Astellas, AstraZeneca, bioMerieux, Cempra, Cerexa, Contrafect, Cubist, Dipexium, Enanta, Furiex, GlaxoSmithKline, Johnson & Johnson, LegoChem Biosciences Inc., Meiji Seika Kaisha, Nabriva, Novartis, Pfizer, PPD Therapeutics, Premier Rearch Group, Rempex, Rib-X Pharmaceuticals, Seachaid, Shionogi, The Medicines Co., Theravance, ThermoFisher and some other corporations. Some JMI employees are advisors/consultants for Astellas, Cubist, Pfizer, Cempra, Cerexa-Forest, and Theravance. In regard to speakers, bureaus and stock options—none to declare.

References

1. Ha YE, Peck KR, Joo EJ, et al. Impact of first-line antifungal agents on the outcomes and costs of candidemia. Antimicrob Agents Chemother. 2012;56:3950–6.
2. Lepak A, Andes D. Fungal sepsis: optimizing antifungal therapy in the critical care setting. Crit Care Clin. 2011;27:123–47.
3. Pakyz AL, Gurgle HE, Oinonen MJ. Antifungal use in hospitalized adults in U.S. academic health centers. Am J Health Syst Pharm. 2011;68:415–8.
4. Posteraro B, Posteraro P, Sanguinetti M. Update on antifungal resistance and its clinical impact. Curr Fungal Infect Rep. 2013; (in press).
5. Sardi JC, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes Giannini MJ. Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Med Microbiol. 2013;62:10–24.
6. Wilke M. Treatment and prophylaxis of invasive candidiasis with anidulafungin, caspofungin and micafungin and its impact on use and costs: review of the literature. Eur J Med Res. 2011;16:180–6.
7. Auberger J, Lass-Florl C, Aigner M, Clausen J, Gastl G, Nachbaur D. Invasive fungal breakthrough infections, fungal colonization and emergence of resistant strains in high-risk patients receiving antifungal prophylaxis with posaconazole: real-life data from a single-centre institutional retrospective observational study. J Antimicrob Chemother. 2012;67:2268–73.
8. Cornely OA, Bassetti M, Calandra T, et al. ESCMID guideline for the diagnosis and management of *Candida* diseases 2012: non-neutropenic adult patients. Clin Microbiol Infect. 2012;18(Suppl 7):19–37.
9. Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis. 2009;48:503–35.
10. Ullmann AJ, Akova M, Herbrecht R, et al. ESCMID guideline for the diagnosis and management of *Candida* diseases 2012: adults with haematological malignancies and after haematopoietic stem cell transplantation (HCT). Clin Microbiol Infect. 2012;18(Suppl 7):53–67.
11. Alexander BD, Johnson MD, Pfeiffer CD, et al. Increasing echinocandin resistance in *Candida glabrata:* clinical failure correlates with presence of *FKS* mutations and elevated minimum inhibitory concentrations. Clin Infect Dis. 2013; 56:1724–32.
12. Arendrup MC, Dzajic E, Jensen RH, et al. Epidemiological changes with potential implication for antifungal prescription recommendations for fungaemia: data from a nationwide fungaemia surveillance programme. Clin Microbiol Infect. 2013;19:E343–53.
13. Borman AM, Szekely A, Palmer MD, Johnson EM. Assessment of accuracy of identification of pathogenic yeasts in microbiology laboratories in the United Kingdom. J Clin Microbiol. 2012;50:2639–44.
14. Miceli MH, Diaz JA, Lee SA. Emerging opportunistic yeast infections. Lancet Infect Dis. 2011;11:142–51.
15. Miceli MH, Lee SA. Emerging moulds: epidemiological trends and antifungal resistance. Mycoses. 2011;54:e666–78.
16. Park BJ, Pappas PG, Wannemuehler KA, et al. Invasive non-*Aspergillus* mold infections in transplant recipients, United States, 2001–2006. Emerg Infect Dis. 2011;17:1855–64.
17. Pfaller MA, Diekema DJ. Rare and emerging opportunistic fungal pathogens: concern for resistance beyond *Candida albicans* and *Aspergillus fumigatus*. J Clin Microbiol. 2004;42:4419–31.
18. Pfeiffer CD, Garcia-Effron G, Zaas AK, Perfect JR, Perlin DS, Alexander BD. Breakthrough invasive candidiasis in patients on micafungin. J Clin Microbiol. 2010;48:2373–80.
19. Sun HY, Singh N. Characterization of breakthrough invasive mycoses in echinocandin recipients: an evidence-based review. Int J Antimicrob Agents. 2010;35:211–8.
20. Fera MT, La Camera E, De Sarro A. New triazoles and echinocandins: mode of action, in vitro activity and mechanisms of resistance. Expert Rev Anti Infect Ther. 2009;7:981–98.
21. Pfaller MA, Messer SA, Woosley LN, Jones RN, Castanheira M. Echinocandin and triazole antifungal susceptibility profiles of opportunistic yeast and mould clinical isolates (2010–2011); Application of new CLSI clinical breakpoints and epidemiological cutoff values to characterize geographic and temporal trends of antifungal resistance. J Clin Microbiol. 2013; (in press).
22. Pfaller MA, Diekema DJ. Progress in antifungal susceptibility testing of Candida spp. by use of Clinical and Laboratory Standards Institute broth microdilution methods, to 2012. J Clin Microbiol. 2010;2012(50):2846–56.

23. Castanheira M, Woosley LN, Diekema DJ, Jones RN, Pfaller MA. Candida guilliermondii and other species of Candida misidentified as Candida famata: assessment by Vitek2, ITS sequence analysis and Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry in two global surveillance programs. J Clin Microbiol. 2013;51:117–24.
24. Johnson EM. Rare and emerging *Candida* species. Curr Fungal Infect Rep. 2009;3:152–9.
25. Pfaller MA, Woosley LN, Messer SA, Jones RN, Castanheira M. Significance of molecular identification and antifungal susceptibility of clinically significant yeasts and moulds in a global antifungal surveillance program. Mycopathologia. 2012;174:259–71.
26. Thompson GR 3rd, Wiederhold NP. Isavuconazole: a comprehensive review of spectrum of activity of a new triazole. Mycopathologia. 2010;170:291–313.
27. Guinea J, Pelaez T, Recio S, Torres-Narbona M, Bouza E. In vitro antifungal activities of isavuconazole (BAL4815), voriconazole, and fluconazole against 1,007 isolates of zygomycete, *Candida, Aspergillus, Fusarium*, and *Scedosporium* species. Antimicrob Agents Chemother. 2008;52:1396–400.
28. Guinea J, Recio S, Escribano P, Pelaez T, Gama B, Bouza E. In vitro antifungal activities of isavuconazole and comparators against rare yeast pathogens. Antimicrob Agents Chemother. 2010;54:4012–5.
29. Illnait-Zaragozi MT, Martinez GF, Curfs-Breuker I, Fernandez CM, Boekhout T, Meis JF. In vitro activity of the new azole isavuconazole (BAL4815) compared with six other antifungal agents against 162 *Cryptococcus neoformans* isolates from Cuba. Antimicrob Agents Chemother. 2008;52:1580–2.
30. Perkhofer S, Lechner V, Lass-Florl C. In vitro activity of isavuconazole against *Aspergillus* species and zygomycetes according to the methodology of the European Committee on Antimicrobial Susceptibility Testing. Antimicrob Agents Chemother. 2009;53:1645–7.
31. Pfaller MA, Messer SA, Rhomberg PR, Jones RN, Castanheira M. In vitro activity of isavuconazole and comparator antifungal agents tested against a global collection of opportunistic yeasts and molds. J Clin Microbiol. 2013;51:2608–16.
32. Rudramurthy SM, Chakrabarti A, Geertsen E, Mouton JW, Meis JF. In vitro activity of isavuconazole against 208 *Aspergillus flavus* isolates in comparison with 7 other antifungal agents: assessment according to the methodology of the European Committee on Antimicrobial Susceptibility Testing. Diagn Microbiol Infect Dis. 2011;71:370–7.
33. Seifert H, Aurbach U, Stefanik D, Cornely O. In vitro activities of isavuconazole and other antifungal agents against *Candida* bloodstream isolates. Antimicrob Agents Chemother. 2007;51:1818–21.
34. Thompson GR 3rd, Wiederhold NP, Sutton DA, Fothergill A, Patterson TF. In vitro activity of isavuconazole against *Trichosporon, Rhodotorula, Geotrichum, Saccharomyces* and *Pichia* species. J Antimicrob Chemother. 2009;64:79–83.
35. Verweij PE, Howard SJ, Melchers WJ, Denning DW. Azole-resistance in *Aspergillus*: proposed nomenclature and breakpoints. Drug Resist Updat. 2009;12:141–7.
36. Howell SA, Hazen KC. *Candida, Cryptococcus,* and other yeasts of medical importance. In: Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW, editors. Manual of clinical microbiology. 10th ed. Washington: ASM Press; 2011. p. 1793–821.
37. Larone DH. Medically important fungi: a guide to identification. 4th ed. Washington: ASM Press; 2002.
38. CLSI. M27-A3. Reference method for broth dilution antifungal susceptibility testing of yeasts. 3rd ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
39. CLSI. M27-S4. Reference method for broth dilution antifungal susceptibility testing of yeasts: 4th informational supplement. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
40. Pfaller MA, Messer SA, Boyken L, et al. Use of fluconazole as a surrogate marker to predict susceptibility and resistance to voriconazole among 13,338 clinical isolates of *Candida* spp. tested by Clinical and Laboratory Standards Institute-recommended broth microdilution methods. J Clin Microbiol. 2007;45:70–5.
41. Pfaller MA, Messer SA, Boyken L, Tendolkar S, Hollis RJ, Diekema DJ. Selection of a surrogate agent (fluconazole or voriconazole) for initial susceptibility testing of posaconazole against *Candida* spp.: results from a global antifungal surveillance program. J Clin Microbiol. 2008;46:551–9.
42. Alexander BD, Schell WA, Miller JL, Long GD, Perfect JR. *Candida glabrata* fungemia in transplant patients receiving voriconazole after fluconazole. Transplantation. 2005;80:868–71.
43. Imhof A, Balajee SA, Fredricks DN, Englund JA, Marr KA. Breakthrough fungal infections in stem cell transplant recipients receiving voriconazole. Clin Infect Dis. 2004;39:743–6.
44. Magill SS, Shields C, Sears CL, Choti M, Merz WG. Triazole cross-resistance among *Candida* spp.: case report, occurrence among bloodstream isolates, and implications for antifungal therapy. J Clin Microbiol. 2006;44:529–35.
45. Panackal AA, Gribskov JL, Staab JF, Kirby KA, Rinaldi M, Marr KA. Clinical significance of azole antifungal drug cross-resistance in *Candida glabrata*. J Clin Microbiol. 2006;44:1740–3.
46. Sanglard D, Ischer F, Calabrese D, Majcherczyk PA, Bille J. The ATP binding cassette transporter gene *CgCDR1* from *Candida glabrata* is involved in the resistance of clinical isolates to azole antifungal agents. Antimicrob Agents Chemother. 1999;43:2753–65.
47. Sanguinetti M, Posteraro B, Fiori B, Ranno S, Torelli R, Fadda G. Mechanisms of azole resistance in clinical isolates of *Candida glabrata* collected during a hospital survey of antifungal resistance. Antimicrob Agents Chemother. 2005;49:668–79.