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In Vitro Activities of Fluconazole and Voriconazole against Clinical Isolates of *Candida* spp. Determined by Disk Diffusion Testing in Turin, Italy[▽]

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The in vitro activities of fluconazole and voriconazole against 1,024 clinical isolates of *Candida* spp. were determined by the agar disk diffusion test using the Clinical and Laboratory Standards Institute (CLSI) M44-A guidelines. The results of this investigation demonstrated the broad-spectrum in vitro activity of voriconazole, relative to that of fluconazole, against yeasts tested, in particular fluconazole-resistant isolates, such as *Candida krusei* that showed high susceptibility to voriconazole. The situation in Turin, Italy, is quite similar to that of the rest of Italy, reflecting the worldwide trend.

Candida species represent the most common cause of fungal infections. *Candida albicans* remains the predominant agent of candidemia and is usually susceptible to azoles, such as fluconazole. Recently, the incidence of invasive candidiasis and bloodstream infections by *C. albicans* decreased, while those due to non-*C. albicans* strains markedly increased. Routine prophylactic and therapeutic use of fluconazole in hospitalized and immunocompromised patients can be associated with a shift of species and resistance patterns. Reduced fluconazole susceptibility was observed for *C. glabrata*, and *C. krusei* appeared to be intrinsically resistant to fluconazole (4, 14, 16, 18). The increased antifungal resistance to antifungal agents most commonly used underscores the need for new antifungal agents. The more recent triazoles, such as voriconazole, ravuconazole, and posaconazole, and the echinocandins, such as caspofungin, are new drugs that broaden the available therapeutic armamentarium for the treatment of invasive fungal infections. In particular, voriconazole shows broad-spectrum activity with antifungal activity against clinically relevant yeasts and molds. Its key property is a potential activity against some fluconazole-resistant isolates, and it can be considered a salvage treatment for patients with refractory candidiasis (11, 18).

With the reference laboratory in Turin, Italy, part of the ARTEMIS Global Antifungal Surveillance Group, we evaluated the in vitro activity of fluconazole and voriconazole against clinical yeasts collected during the period of 2002 to 2006. The assessment of the susceptibility and resistance to these agents was based on the use of the standard disk diffusion method, approved by the Clinical and Laboratory Standards Institute (CLSI) in association with the BIOMIC system (Giles Scientific Inc., Santa Barbara, CA) (8). The BIOMIC system provides a cost-effective means to electronically read and interpret test plates and to collect and transfer data; moreover, the BIOMIC system provides improved intra- and interlaboratory reading consistency (5, 7, 13, 19).

A total of 1,024 yeast isolates were collected from various specimens (blood, normally sterile body fluids, deep tissue, genital tract, gastrointestinal tract, and respiratory tract) from three participating hospitals in Turin and Biella, Italy, between January 2002 and December 2006. All strains were identified by ID32C panels (bioMérieux, Rome, Italy).

Fluconazole and voriconazole susceptibility testing was performed in accordance with CLSI document M44-A for yeasts (8). For quality control, *C. albicans* ATCC 90029 was used in accordance with CLSI document M44-A (8). Fluconazole (25 μg) and voriconazole (1 μg) disks were manufactured and supplied by Becton Dickinson, Sparks, MD. Zone diameter endpoints were automatically read at 80% growth inhibition and interpreted using the BIOMIC image analysis plate reader system (Giles Scientific, Inc.) (5, 9, 13). The interpretive criteria for fluconazole and voriconazole disk diffusion tests were performed in accordance with CLSI (2, 8). The MICs were determined by the BIOMIC system that uses species-specific linear regression analysis to correlate all zone diameters from the disk diffusion test with corresponding MICs from the reference dilution method (12). The corresponding MIC breakpoints were those of CLSI (2, 8). An excellent correlation between the MIC and the zone diameter has been observed in previous studies (5, 12). Hence, fluconazole and voriconazole MIC_{50}s and MIC_{90}s were calculated and reported.

The species distribution and the in vitro susceptibilities of 1,024 *Candida* isolates to fluconazole and voriconazole are summarized in Table 1. In agreement with the international literature (1, 5, 13), we found that in Turin, Italy, *C. albicans* was the most common yeast species but the *C. albicans* frequency (55.6%) was slightly lower than that reported by other

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TABLE 1. In vitro susceptibilities of *Candida* spp. to fluconazole and voriconazole as determined by CLSI disk diffusion method

Species	No. of isolates (%) tested	No. of isolates (%) susceptible to[a]:					
		Fluconazole			Voriconazole		
		S	SDD	R	S	SSD	R
C. albicans	597 (55.6)	587 (98)	5 (1)	5 (1)	596 (99.8)		1 (0.2)
C. glabrata	201 (18.7)	164 (82)	19 (9)	18 (9)	175 (87)	6 (3)	20 (11)
C. tropicalis	59 (5.5)	54 (92)	2 (3)	3 (5)	53 (90)	2 (3)	4 (7)
C. parapsilosis	51 (4.7)	49 (96)	2 (4)		51 (100)		
C. krusei	28 (2.6)	3 (11)[c]	6 (21)[c]	19 (68)	26 (92)	1 (4)	1 (4)
C. kefyr	24 (2.2)	23 (96)		1 (4)	23 (96)	1 (4)	
C. norvegensis	14 (1.3)		3 (21)	11 (79)	13 (93)	1 (7)	
C. dubliniensis	7 (0.6)	6		1	6		1
C. famata	7 (0.6)	7			7		
C. valida	7 (0.6)	1	1	5	5	2	2
C. inconspicua	5 (0.5)	3	1	1	5		
C. pulcherrima	4 (0.4)	4			4		
C. guilliermondii	3 (0.3)	2		1	2		1
C. sake	3 (0.3)	2	1		3		
Other *Candida* spp.[b]	14 (1.4)	13 (92.9)	1		14 (100)		
Total	1,024	918 (90)	41 (4)	65 (6)	983 (96)	13 (1)	30 (3)

[a] S, susceptible; SDD, susceptible dose dependent; R, resistant.
[b] Includes isolates of more unusual *Candida* species, such as *C. holmii*, *C. humicola*, *C. intermedia*, *C. lambica*, *C. lusitaniae*, *C. membranifaciens*, *C. rugosa*, and *C. utilis*.
[c] Despite these values of sensitivity, isolates are assumed to be intrinsically resistant to fluconazole and their inhibition zone diameters should not be interpreted using breakpoints established by CLSI (2, 8).

authors both in Italy (3, 19) and worldwide (5, 7, 12, 13). Four hundred twenty-seven strains (39.7%) were non-*C. albicans* spp. Among these, *C. glabrata* (18.7%) was the first most common yeast pathogen, followed by *C. tropicalis* (5.5%) and *C. parapsilosis* (4.7%). Our findings confirm the worldwide pattern (12, 19). The frequency of *C. krusei* (2.6%) in our study was also similar to the 1.8 to 2% reported by other authors (1, 5, 6, 12, 19), while the percentage of *C. kefyr* in our study was higher (2.2%) than that reported in previous studies in Italy and worldwide (0.6%) (5, 13, 19). *C. kefyr* may be considered an emerging pathogen (15); however, few data are available about this pathogen. The frequency of *C. norvegensis* and *C. valida* did not vary markedly over the 5-year study period (data not shown) (5, 13, 19). Unusual *Candida* species (*C. holmii*, *C. humicola*, *C. intermedia*, *C. lambica*, *C. lusitaniae*, *C. membranifaciens*, *C. rugosa*, and *C. utilis*) constituted only a small percentage (1.4%) (Table 1).

The results of our antifungal susceptibility testing in Turin, Italy, are generally consistent with the findings from other Italian studies (3, 19) and with other sites globally in the ARTEMIS Global Antifungal Surveillance Group (5, 12, 13). Despite the widespread use of fluconazole for more than a decade, we found no evidence that *C. albicans* (98% susceptible) has developed increased resistance to fluconazole. Fluconazole was most active against *C. parapsilosis* (96% susceptible), *C. tropicalis* (92% susceptible), and *C. kefyr* (96% susceptible). Decreased susceptibility to fluconazole was seen for *C. glabrata* (82% susceptible). *C. krusei* remains primarily a pathogen that is fluconazole resistant. The observation that 32% of *C. krusei* strains were susceptible and susceptible dose dependent to fluconazole was unexpected, but other investigators have reported similar findings (3, 7). As *C. krusei* isolates are assumed to be intrinsically resistant to fluconazole (6), their inhibition zone diameters and MICs should not be interpreted using breakpoints established by CLSI (2, 8). According to Sugita et al. (17), fluconazole resistance was also observed in *C. norvegensis*, *C. valida*, and *C. inconspicua*; these three opportunistic fungal pathogens are rarely isolated from patients but are considered to be emerging pathogens in humans. The uncommon *Candida* species were all susceptible to the drug (Table 1).

Compared to fluconazole, voriconazole showed an excellent in vitro potency and broad-spectrum activity against all tested species, including many yeasts that were found to be resistant to fluconazole, such as *C. krusei*, which showed high susceptibility to voriconazole (92%). Although voriconazole was highly active against *C. glabrata*, resistance to voriconazole was detected (Table 1), as confirmed by the worldwide data reported by Pfaller et al. (12, 13). This decreased susceptibility of *C. glabrata* to multiple azole drugs may be ascribed to the broad current usage of fluconazole and/or itraconazole for the treatment of infections due to *Candida* spp. and other opportunistic yeasts and yeastlike fungi (10). We found that voriconazole was highly active against most rare *Candida* species, such as *C. dubliniensis*, *C. famata*, *C. guilliermondii*, *C. holmii*, *C. humicola*, *C. intermedia*, *C. lusitaniae*, *C. membranifaciens*, *C. pulcherrima*, and *C. utilis*. We found that a high percentage of *C. valida* isolates were resistant to fluconazole but susceptible to voriconazole (Table 1); this species has been emerging in recent years (5, 13).

MIC ranges, MIC_{50}s, and MIC_{90}s for fluconazole and voriconazole are listed in Table 2. Voriconazole inhibited 90% of the isolates at a concentration six to eight times lower than that determined for fluconazole. *C. krusei* and *C. glabrata* fluconazole MICs were markedly higher than those for voriconazole (256 versus 2 μg/ml and 64 versus 4 μg/ml, respectively).

There was very little variation in the rates of resistance of *Candida* isolates to either fluconazole and voriconazole over the study period (2002 to 2006) (data not shown). Only *C. kefyr*

TABLE 2. Fluconazole and voriconazole MICs for *Candida* spp., calculated from disk zone diameter measurements

Species (no. of isolates tested)	Antifungal agent[a]	MIC (μg/ml)		
		Range	50%[b]	90%[b]
C. albicans (597)	FLU	0.125–>64	0.5	4
	VOR	0.008–8	0.06	0.12
C. glabrata (201)	FLU	0.125–>64	4	64
	VOR	0.008–8	0.25	4
C. tropicalis (59)	FLU	0.25–>64	2	16
	VOR	0.032–8	0.12	2
C. parapsilosis (51)	FLU	0.125–64	0.5	4
	VOR	0.008–2	0.06	0.12
C. krusei (28)	FLU[c]	0.5–256	128	256
	VOR	0.032–4	0.5	2
C. kefyr (24)	FLU	0.125–0.5	0.25	0.25
	VOR	0.008–0.06	0.01	0.06
C. norvegensis (14)	FLU	32–256	128	256
	VOR	0.064–8	0.25	1
Other *Candida* spp. (50)	FLU	0.125–256	2	128
	VOR	0.008–8	0.06	1

[a] FLU, fluconazole; VOR, voriconazole.
[b] 50% and 90%, MIC encompassing 50% and 90% of isolates tested, respectively.
[c] Despite these values of sensitivity, isolates are assumed to be intrinsically resistant to fluconazole, and MICs should not be interpreted using breakpoints established by CLSI (2, 8).

exhibited a trend of decreased fluconazole susceptibility over the study period, from 100% (2002 to 2005) to 86% in 2006 (data not shown).

In conclusion, our data revealed that the resistance to fluconazole among pathogenic yeasts is still a restricted phenomenon in Turin, Italy. We confirm that voriconazole is a promising antifungal triazole that appears to be more active against infections caused by yeasts intrinsically resistant to fluconazole and by many less-common species. The susceptibility patterns and trends observed in Turin are similar to those observed elsewhere in Italy, reflecting the worldwide patterns (7, 9, 12).

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