A Global Evaluation of Voriconazole Activity Against Recent Clinical Isolates of *Candida* spp.

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D Diekema[1,2], D Sheehan[5], P Hogan[5], S Messer[2], R Hollis[2], L Boyken[2], S Tendolkar[2], J Kroeger[2], RN Jones[4] and MA Pfaller[2,3]

Departments of [1]Internal Medicine, [2]Pathology and [3]Epidemiology, University of Iowa, Iowa City, IA; [4]Jones Microbiology Institute, North Liberty, IA; [5]Pfizer, Inc., New York, NY

Corresponding Author:
Daniel J. Diekema, MD
C 606 General Hospital
200 Hawkins Drive
Iowa City, IA 52242
daniel-diekema@uiowa.edu
Ph: 319-356-8615
Fax: 319-356-4916

ABSTRACT

Approved by the FDA in 2002, voriconazole has been widely used for prevention and treatment of invasive fungal infections. As a result, prospective surveillance for emergence of in vitro resistance to voriconazole is indicated. From January 2004-December 2006, we collected 5615 invasive (blood or sterile site) clinical isolates of *Candida* from 78 centers in 30 countries worldwide (by region: 772 from Asia-Pacific, 1736 Europe, 1118 Latin America, 1657 North America). In vitro susceptibility to voriconazole was performed at a central lab using the CLSI M27-A2 method and CLSI breakpoints to classify organisms as susceptible (S), susceptible dose-dependent (SDD) or resistant (R). We present results overall, by species, by country and region. In addition, we compare in vitro activity of this recent collection with that of 5866 isolates collected during global surveillance from 1997-2001, prior to the availability or use of voriconazole. Species distribution was: 54% *C. albicans*, 14% *C. glabrata*, 14% *C. parapsilosis*, 11% *C. tropicalis*, and 3% *C. krusei*. Voriconazole was very active overall: MIC_{50}/MIC_{90}, 0.008/0.25 mg/L; 98% S. Of the 96 isolates not S to voriconazole (n=66 R, 30 SDD), 87 (91%) were *C. glabrata*, primarily from centers in North America and Europe. Finland had the highest rate of voriconazole resistance (10% R, 8% SDD, 82% S among 73 total isolates). In all other countries, ≥=97% of *Candida* were S to voriconazole. No temporal trends in species or MIC distribution were noted over the 3 years of surveillance. In comparison to 5866 *Candida* isolates collected during global surveillance from 1997-2001, there were no changes in mean MIC or MIC distribution (mean MIC=0.125, MIC_{90}, 0.25 mg/L for both collections). Voriconazole is highly active in vitro against a global collection of recent invasive *Candida* isolates, and there is no evidence for emerging resistance or "MIC creep" compared with isolates collected prior to voriconazole availability. Voriconazole SDD and voriconazole R isolates were primarily *C. glabrata* isolates from centers in North America and Europe.

INTRODUCTION

Voriconazole is a triazole antifungal agent with broad-spectrum activity against *Candida* spp., *Aspergillus* spp., and other opportunistic fungal pathogens (4, 7, 8, 10, 13). Voriconazole has been approved by the United States Food and Drug Administration for the treatment of invasive aspergillosis, invasive candidiasis in nonneutropenic patients, esophageal candidiasis, and serious infections caused by *Fusarium* and *Scedosporium* spp. in patients who are intolerant of or refractory to other antifungal agents (2, 4, 5, 8).

Although voriconazole has been demonstrated to be effective for both primary (5) and salvage (8) therapy of invasive candidiasis, we have previously demonstrated that cross-resistance between fluconazole and voriconazole exists among clinical isolates of *Candida* (10, 12, 13). In addition, breakthrough candidemia among voriconazole recipients has been reported, in some cases due to voriconazole-resistant *Candida* spp. (1, 3).

Given the widespread use of azole antifungal agents, given that decreased susceptibility to fluconazole may predict decreased susceptibility to voriconazole (10, 12, 13), and given prior reports of emergence of *C. glabrata* in the U.S. during the 1990s (14), there is a need for ongoing surveillance to monitor for the emergence of voriconazole resistance among *Candida*. In addition to monitoring for categorical resistance using established breakpoints (9), such surveillance should allow for monitoring of the entire MIC distribution over time, in order to capture subtle changes (i.e. "MIC creep") that may not be reflected using existing breakpoints.

We have performed global antifungal surveillance to monitor trends in antifungal susceptibility of clinical isolates of *Candida* since January 1997 (11). We now report recent data from the ARTEMIS program describing voriconazole activity against contemporary clinical isolates of *Candida* spp. from invasive infections worldwide. In addition, we compare these data to the voriconazole MIC distribution from global surveillance between 1997-2001 (prior to the widespread availability and use of voriconazole).

MATERIALS AND METHODS

Organisms. A total of 5615 clinical isolates obtained from 78 medical centers in 30 countries from January 2004 to December 2006 were tested as part of the ARTEMIS global antifungal surveillance program (13). The collection included 3040 strains of *Candida albicans*, 783 of *C. parapsilosis*, 782 of *C. glabrata*, 639 of *C. tropicalis*, 146 of *C. krusei*, 64 of *C. guilliermondii*, 63 of *C. lusitaniae*, 40 of *C. kefyr*, 24 of *C. famata*, 11 of *C. pelliculosa*, 8 of *C. lipolytica*, 8 of *C. dubliniensis*, 3 of *C. rugosa*, 3 of *C. zeylanoides*, and 1 of *C. intermedia*. All isolates were obtained from blood or other normally sterile sites and represented individual infectious episodes. Sites included blood (65%), "tissue" (10%), abscess (9%), peritoneal fluid (6%), joint fluid (1%), cerebrospinal fluid (1%), catheter (1%), and "other" (7%). The prior (comparator) collection of 5866 invasive clinical isolates was collected between January 1997 and December 2001 from 112 centers in 37 countries as part of two antifungal surveillance programs, SENTRY and ARTEMIS (11). The isolates were collected at individual study sites and were sent to the University of Iowa (Iowa City, IA) for identification and susceptibility testing as described previously (10, 12, 13). The isolates were identified by standard methods and stored as water suspension until used in the study. Prior to testing, each isolate was passaged at least twice onto potato dextrose agar (Remel, Lenexa, KS) and CHROMagar™ Candida (Becton Dickinson, Sparks, MD).

Antifungal agents. Reference powder of voriconazole was obtained from Pfizer Inc., New York, NY. Stock standards were prepared in water, and serial twofold dilutions were made in RPMI 1640 (Sigma, St. Louis, MO) buffered to pH 7.0 with 0.165M morpholinepropanesulfomic acid (MOPS) buffer (Sigma).

Antifungal susceptibility testing. Broth microdilution testing was performed in accordance with the guidelines in the Clinical and Laboratory Standards Institute (CLSI) document M27-A2 (6) using RPMI 1640 medium, an inoculum of 0.5×10^3 to 2.5×10^3 cells/mL, and ambient air incubation at 35°C. MICs were determined visually after 48h of incubation as the lowest concentration of drug that caused a significant diminution (≥50%) of growth below control levels. We used the voriconazole susceptibility breakpoints adopted by the CLSI: susceptible, ≤1 mg/L; susceptible dose-dependent, 2 mg/L; and resistant, ≥4 mg/L (9).

Quality Control. Quality control was performed by testing CLSI-recommended strains *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

Statistical analysis. Changes in voriconazole MIC distributions by time period were examined using t-tests (to compare means) and the Wilcoxon rank sum test. Alpha was set at 0.05 and all p-values were two-tailed. SPSS version 13 (Chicago, IL) was used for all statistical analyses.

RESULTS

Table 1 displays the species distribution of the *Candida* spp. invasive isolates collected from 2004-2006 and 1997-2001. Voriconazole demonstrated excellent in vitro activity against this collection of isolates, with an overall MIC_{50} of 0.008 mg/L, MIC_{90} of 0.25 mg/L, and 98.3% susceptible using the CLSI breakpoint of ≤1 mg/L (see Table 2). Figure 1 shows the MIC frequency distribution for the 5 major species groups. Among these major species, *C. glabrata* demonstrated the highest MICs ($MIC_{50/90}$, 0.25/2.0 mg/L). Indeed, *C. glabrata* represented 83% (25/30) of the total number of isolates that were susceptible dose-dependent to voriconazole, and 94% (62/66) of the isolates that were resistant to voriconazole.

Figure 2 compares the MIC distribution of the isolates collected from 1997-2001 with the current collection from 2004-2006. As is evident, the MIC distributions were not significantly different (mean MIC=0.125 mg/L and MIC_{90}=0.25 mg/L for both collections, p=0.8).

Table 2 demonstrates the voriconazole susceptibility of invasive *Candida* isolates by region and by country. The lowest voriconazole activity was noted among the 73 isolates from Finland, where 82% were susceptible to voriconazole. This lower percentage susceptible was explained by the fact that 40% (30/73) of the *Candida* isolates submitted from Finland were *C. glabrata*, the highest proportion of *C. glabrata* isolates from any country. In all other countries, over 97% of *Candida* isolates were susceptible to voriconazole.

Figure 1. Voriconazole MIC distribution for top 5 species groups, from global *Candida* surveillance, 2004-2006

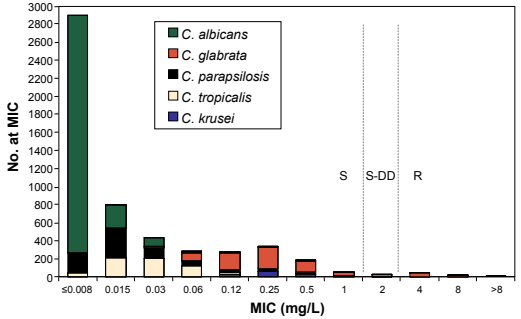

Figure 2. MIC distributions for global collections of clinical *Candida* isolates collected from 1997-2001 (n=5866) compared with 2004-2006 (n=5615)

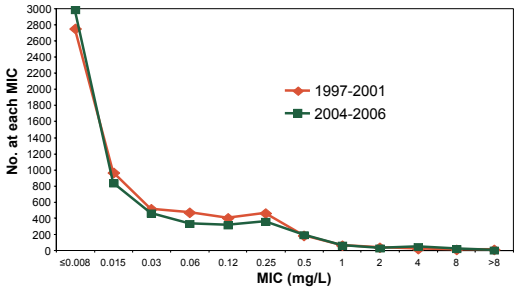

Table 1. Distribution of *Candida* spp. overall and by region, from the ARTEMIS global surveillance program

	% of total for each time period, stratified by region for recent collection (2004-06)					
Species	Overall 2004-2006 (n=5615)	North America (n=1657)	Latin America (n=1118)	Europe (n=1736)	Asia-Pacific (n=772)	Overall 1997-2001 (n=5866)
C. albicans	54.1	51.7	47.7	60.0	52.6	55.5
C. glabrata	13.9	22.8	5.5	13.9	9.6	15.8
C. parapsilosis	13.9	13.8	18.7	10.0	14.4	13.1
C. tropicalis	11.4	7.2	20.0	7.3	18.0	10.0
C. krusei	2.6	1.4	1.6	5.0	1.3	2.5
C. guilliermondii	1.1	0.4	3.7	0.5	1.0	0.8
C. lusitaniae	1.1	1.5	0.6	1.4	0.8	1.2
C. kefyr	0.7	0.5	0.7	1.3	0.1	0.2
C. famata	0.4	0.2	0.7	0.2	0.8	0.1
C. pelliculosa	0.2	0.1	0.4	0.1	0.5	0.3
Other	0.4*	0.4	0.3	0.3	0.9	0.5

*For 2004-06, includes *C. dubliniensis* (8), *C. intermedia* (1), *C. lipolytica* (8), *C. rugosa* (3), *C. zeylanoides* (3)

Table 2. Susceptibility of a recent global collection of invasive *Candida* spp. isolates (2004-06) to voriconazole, by region and country

		Percent by category*			
Region	Country	No. isolates	S	S-DD	R
North America	Canada	312	97.8	0.6	1.6
	United States	1345	97.0	0.7	2.4
	All North Am.	**1657**	**97.1**	**0.7**	**2.2**
Latin America	Argentina	136	98.5	0.7	0.7
	Brazil	198	100	0	0
	Chile	47	100	0	0
	Colombia	84	100	0	0
	Ecuador	176	100	0	0
	Mexico	165	100	0	0
	Peru	1	100	0	0
	Venezuela	311	99.7	0	0.3
	All Latin Am.	**1118**	**99.7**	**0.1**	**0.2**
Europe	Czech Republic	135	96.3	2.2	1.5
	Finland	73	82.2	8.2	9.6
	Hungary	163	99.4	0.6	0
	Italy	254	99.6	0	0.4
	Poland	182	97.3	1.1	1.6
	Portugal	79	100	0	0
	Russia	106	98.1	0	1.9
	Slovakia	253	98.0	0.8	1.2
	Spain	30	100	0	0
	Turkey	286	99.7	0.3	0
	United Kingdom	175	100	0	0
	All Europe	**1736**	**98.1**	**0.8**	**1.1**
Asia-Pacific	Australia	187	97.3	0.5	2.1
	China	137	98.5	0	1.5
	Korea	198	99.5	0	0.5
	Malaysia	139	100	0	0
	Taiwan	89	100	0	0
	Thailand	22	100	0	0
	All Asia-Pacific	**772**	**99.0**	**0.1**	**0.9**
Other	Israel	93	97.8	0	2.2
	South Africa	239	99.2	0.8	0
TOTAL	**All regions**	**5615**	**98.3**	**0.5**	**1.2**

*CLSI breakpoints for voriconazole susceptibility were applied:
susceptible (S): ≤1 mg/L; susceptible-dose-dependent (S-DD): 2 mg/L; and resistant (R): ≥4 mg/L

SUMMARY

- **Voriconazole is highly active in vitro against a recent global collection of invasive *Candida* isolates.**
- **In comparison with a large global collection of invasive *Candida* isolates from prior to voriconazole availability (1997-2001), there is no evidence for emerging resistance or shifts in MIC distribution.**
- **The vast majority of *Candida* isolates not susceptible to voriconazole (MIC>1 mg/L) are *C. glabrata* from centers in Europe and North America.**

REFERENCES

1. Alexander, B.D., W.A Schell, et al. 2005. *Candida* glabrata fungemia in transplant patients receiving voriconazole after fluconazole. *Transplantation.* 80:868-71.
2. Herbrecht, R., D. W. Denning, et al. 2002. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N. Engl. J. Med.* 347:408-415.
3. Imhof A., S.A. Balajee, et al. 2004. Breakthrough fungal infections in stem cell transplant recipients receiving voriconazole. *Clin. Infect. Dis.* 39:743-46.
4. Johnson, L. B., and C. A. Kauffman. 2003. Voriconazole: a new triazole antifungal agent. *Clin. Infect. Dis.* 36:630-637.
5. Kullberg, B. J., J. D. Sobel, et al. 2005. Voriconazole versus a regimen of amphotericin B followed by fluconazole for candidemia in nonneutropenic patients: a randomized non-inferiority trial. *Lancet.* 366:1435-1442.
6. National Committee for Clinical Laboratory Standards. 2002. Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard, 2nd ed., M27-A2. National Committee for Clinical Laboratory Standards, Wayne, Pa.
7. Ostrosky-Zeichner, L., J. H. Rex, et al. 2003. Antifungal susceptibility survey of 2,000 bloodstream *Candida* isolates in the United States. *Antimicrob. Agents Chemother.* 47:3149-3154.
8. Perfect, J. D., K. A. Marr, et al. 2003. Voriconazole treatment for less-common, emerging, or refractory fungal infections. *Clin. Infect. Dis.* 36:1122-1131.
9. Pfaller, M. A., D. J. Diekema, et al. 2006. Correlation of MIC with outcome for *Candida* species tested against voriconazole: analysis and proposal for interpretive breakpoints. *J. Clin. Microbiol.* 44:819-826.
10. Pfaller, M. A., S. A. Messer, et al. 2002. In vitro activities of ravuconazole and voriconazole compared with those of four approved systemic antifungal agents against 6,970 clinical isolates of *Candida* spp. *Antimicrob. Agents Chemother.* 46:1723-1727.
11. Pfaller, M. A., and D. J. Diekema. 2002. Role of sentinel surveillance of candidemia: trends in species distribution and antifungal susceptibility. *J. Clin. Microbiol.* 40:3551-3557.
12. Pfaller, M.A., S. A. Messer, et al. 2007. 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.* 45:70-75.
13. Pfaller, M. A., S. A. Messer, et al. 2004. In vitro activities of voriconazole, posaconazole, and fluconazole against 4,169 clinical isolates of *Candida* spp. and *Cryptococcus neoformans* collected during 2001 and 2002 in the ARTEMIS global antifungal surveillance program. *Diagn. Microbiol. Infect. Dis.* 48:201-205.
14. Trick, W. E., S. K. Fridkin, et al, and the National Nosocomial Infections Surveillance System Hospitals. 2002. Secular trend of hospital-acquired candidemia among intensive care unit patients in the United States during 1989-1999. *Clin. Infect. Dis.* 35:627-630.

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